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Abstract Book

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M1 - COVID-19 among the asymptomatic health care workers in some tertiary care centers in the Southern regions of Saudi Arabia: risk

Presenting Author – *Abdelwahid Ali, King Khalid University, Saudi Arabia*

Author/s – *Ahmed Al-Hakami, Ali Al Bshabshe, Abdullah Alkahtani, Abdullah Alsabaani*

Abstract Content

Background: COVID-19 is the respiratory infection caused by an emerging coronavirus named severe acute respiratory syndrome coronavirus virus 2 (SARS-CoV-2). The virus was first reported from Wuhan city, China in late December of 2019. COVID-19 was firstly impacted China with a massive epidemic, then rapidly spread to cause global pandemic with high infectivity and morbidity rates among human populations worldwide. COVID-19 pandemic had sent an obnoxious waves of medical emergency all over the world. As the pandemic advances, it becomes necessary to screen the HCWs for COVID-19 as they may constitute potential sources for the disease transmission.

Objectives: This study was carried out to screen for the COVID-19 among asymptomatic HCWs in some tertiary care centers in the Southern regions of Saudi Arabia.

Methods: A cross-sectional, hospital-based study was conducted to determine the incidence of COVID-19 among 186 consented asymptomatic HCWs using RT-PCR, ELISA and rapid ICT.

Results: The total number of COVID-19 cases among the participants using the three tests was 34 (18.3%). Out of the total participants, 4.8%, 3.2%, 7%, 10.2%, and 11.8% of positive COVID-19 cases were detected using RT-PCR, rapid ICT for IgG, rapid ICT for IgM, ELISA for IgG and ELISA for IgM respectively. Significantly ($P < 0.05$) higher cases were observed among HCWs working in the ICU of Aseer Central Hospital. 100% of the medical students and the administrative staff, 40% of respiratory therapists, 31.8% of laboratory specialists, 22.7% of cleaners, 13.5% of the physicians, 12.2% of the nurses were positive to COVID-19.

M2 - Subgenomic viral RNA profile in SARS-CoV-2 infection with Omicron and non-Omicron variants

Presenting Author – *Paul Chan, University of Hong Kong, China*

Author/s – *Zigui Chen, Apple Yeung, Wendy Ho, Rita Ng, Grace Lui, Lowell Ling*

Abstract Content

Background: SARS-CoV-2 transcribes subgenomic RNAs (sgRNAs) essential for translation of structural and accessory proteins. Knowledge of sgRNA testing is limited.

Objectives: Delineate the profile of sgRNAs and examine their correlation with host characteristics and clinical outcome.

Methods and Results: Respiratory samples collected from 456 patients (118 Omicron BA.2 and 338 authentic D614G variants) were submitted for subgenomic RNA profiling using probe hybridization RNA-seg.

We found samples harbored a full-set of the 9 canonical sgRNAs ("sg9") showed a significant positive association with crude viral load (Ct: 19.0, 11.3 – 27.7) when compared with samples containing 8 or less canonical sgRNAs ("sg0"-"sg8") (Ct: 26.3, 15.9 – 32.5) ($p \leq 0.001$). sgORF7b was the least expressed and not detected in 96.7% of samples harboring partial canonical sgRNAs. Samples harboring a "sg9" pattern were collected earlier (63.3% of early vs 27.4% of late period). Changes in detection rates of the 9 canonical sgRNAs correlated with crude viral load and sampling day, but not with age, gender and pneumonia. The Receiver Operating Characteristic (ROC) curve analysis showed that the detection of a full-set of the 9 canonical sgRNAs (AUC = 0.91, 95% CI 0.88-0.94), sgRNA ORF7b (0.90, 0.87 – 0.93) and sgORF7a (0.89, 0.84 – 0.93) exhibited the best association with crude viral load for both BA.2 and D614G variants.

Conclusions: Subgenomic RNA profile correlates with the stage of infection and may assist clinical decision such as treatment and isolation

M3 - Inconclusive SARS-COV-2 PCR test: the follow up - our experience

Presenting Author – *Bojana Mohar Vitezic, Medical faculty Rijeka, Croatia*

Author/s – *Elena Štefančić, Davorka Repac Antić, Tanja Grubić Kezele, Maja Abram, Marina Bubonja Šonje*

Abstract Content

Background: Effective and accurate SARS-CoV-2 detection assays are crucial for hospital routines maintenance to identify infected hospital employees and patients prior hospital admission. Inconclusive PCR test results from potentially infectious borderline SARS-CoV-2 patients can delay appropriate infection control.

Objectives: In this study we analysed results of follow-up testing from borderline SARS-CoV-2 patients, tested at Department of Clinical Microbiology, during 8-months period. We aimed to determine the positivity conversion rate within seven days after inconclusive PCR test results.

Methods: RNA were extracted using Exiprep™ 96 Viral DNA/RNA Kit, with ExiPrep™ 96 Lite instrument and SARS-CoV-2 detection with AccuPower® SARS-CoV-2 Multiplex RT PCR Kit. Results were inconclusive if had no detected E gene or amplified with Ct > 36 and amplified RdRp-N with Ct >36 and < 40. Our laboratory manages the inconclusive PCR results with resampling within three days. Patient status differences (converted to positive or negative) are based on the same extraction/detection method but performed from the second sample.

Results: Of 25 552 analyses, 23 851 tests were found negative (93.34 %), 1 454 were found positive (5.69 %), and 247 were inconclusive (0.97%). Of 247 borderline patients, resampled and retested in our laboratory, 60 patients (29,4%) showed conversion of the borderline viral load (inconclusive RT PCR test) to a positive one.

Our results highlight the need for re-sampling and re-testing of borderline patients with inconclusive SARS-CoV-2 results. Identification of additional positive patients reduces the potential risk of intrahospital transmission.

M4 - Peste des petits ruminants virus (PPRV) non-structural proteins adversely affect the pro-inflammatory cytokine gene expression b

Presenting Author – *Juhi Jain, University of Delhi, India*

Author/s – *Rajeev Kaul*

Abstract Content

Background: Peste des petits ruminants virus causes an economically valuable contagious disease of sheep and goats with extreme morbidity and mortality rate. The Office International des Epizooties launched an eradication program to eradicate the virus by 2030.The viral non-structural proteins execute a critical role in evading the immune response.

Objective: To decipher the role of PPRV non-structural proteins in immune modulation.

Methods: To identify the host cellular protein interaction with the PPRV C and V protein, LCMS was carried out. Co-immunoprecipitation and GST-Pull Down assays were performed to validate the interaction with NF-kB p65 protein. The effect of viral proteins on NF-kB p65 translocalization was monitored via immunofluorescence assay. Moreover, to study the viral protein's impact on NF-kB transcriptional activation and interferon induction, a Dual-luciferase reporter assay was performed. At last, NF-kB mediated pro-inflammatory cytokine gene expression was analyzed by RT- qPCR.

Results: Our data has shown that the PPRV C and V proteins directly interact with the immune and inflammatory response regulator NF-kB p65 (Rel A) protein. The PPRV V protein inhibit its translocalization into the nucleus and significantly downregulate its activation by 40% while PPRV C interacts with the TAD domain of p65 and hence suppresses the activation by 30%. PPRV C and PPRV V significantly inhibit the ISRE activity by 50% and 89%, respectively. Our RT-qPCR data showed the significant downregulation of NF-kB mediated pro-inflammatory cytokine gene expression. Our study illustrate that PPRV non-structural proteins disrupt the NF-kB signaling and hence altering the immune response.

M6 - Investigation of polyomaviruses in wild rat populations across Europe and West Asia

Presenting Author – *Emilija Vasiliūnaitė, Vilnius University Life Sciences Centre, Lithuania*

Author/s – *Emilija Vasiliūnaitė, Rainer G. Ulrich, Alma Gedvilaitė*

Abstract Content

Background: Polyomaviruses (PyVs) are double-stranded DNA viruses that are prevalent in human populations and are generally considered to have low pathogenicity, slow evolution rate and high host specificity. Nevertheless, the mechanisms of the virus-host relationship are yet unclear. At present, only 16 of the 117 known PyVs have been identified in rodent species, including laboratory animals. Further investigation into PyVs in closely related wild rodent species could provide greater insight into the relationship between PyVs and their hosts.

Objectives: Investigate wild rat (*Rattus norvegicus* and *Rattus rattus*) samples collected in Europe and West Asia for polyomaviruses.

Methods: Diagnostic PCR for previously known *Rattus norvegicus* infecting PyVs 1 and 2 (RnorPyV1 and RnorPyV2) as well as broad-range nested PCR were used for PyV DNA identification. Whole genome PyV sequences were amplified, and Sanger sequenced. Virus-like particles (VLPs) of sequenced PyVs' VP1 proteins were synthesized in yeast and used for virus seroprevalence determination.

Results: RnorPyV2-like sequences were identified in both *Rattus norvegicus* and *Rattus rattus* samples. However, RnorPyV1-like sequences were only found in *R. norvegicus* samples, while an investigation of *Rattus rattus* samples resulted in the discovery of a presumably novel PyV with an 8 % genomic divergence from RnorPyV1. PyV DNA prevalence as well as seroprevalence using virus-specific VP1-VLPs were determined for the three viruses identified.

M7 - Anti-human papillomavirus-16 &-18 and circulating cell-free DNA status among sexually active females in Abuja, Nigeria

Presenting Author – *Idris Nasir Abdullahi, Ahmadu Bello University, Nigeria*

Author/s – *Nicholas Bamlong, Amos Dangana, Sanusi Musa, Balkisu Sule, Yusuf Mohammed Sabo, Musa Ismail*

Abstract Content

Background: There have not been widely adopted human papillomavirus (HPV) vaccination programs in Nigeria (a highly heterogeneous country). It is important to understand the state of seroprevalence on the high-risk HPV (hr-HPV) genotypes in women residing in the Suburbs, prior to vaccination uptake.

Objectives: To determine the seroprevalence and cell-free DNA of HPV-16 & -18, and risk factors for prior exposure in women residing in an Abuja Suburb, Nigeria.

Methods: After ethical approval, a community-based cross-sectional study was undertaken. Blood samples were collected, and structured questionnaires were collated from one hundred and eighty-two (182) consented women. The blood samples were processed and analysed for the anti-HPV 16-&-18 E6 oncoprotein levels using commercially available enzyme-linked immunosorbent assay (ELISA). Circulating cell-free DNA (ccfDNA) were extracted from the blood of seropositive participants and assayed for ccfHPV16 and ccfHPV18 DNA by PCR.

Results: The mean±SD age of the participants was 25±6.8 years and none of the participants had a history of HPV vaccination. The seroprevalence of HPV-16 and -18 were 15.4% and 21.9%, respectively. Marital status (OR = 3.24, 95%CI: 1.78–9.23]), age (OR = 5.21, 95%CI: 2.43–10.97]), history of urinary tract infection (OR = 3.08, 95% CI: 1.04–5.65]), the status of circumcision (OR = 3.24, 95%CI: 1.92–9.17), and marital status (OR = 1.65, 95%CI: 1.09–6.72), were significant risk factors of HPV-18 seroprevalence (p < 0.05). However, education status (OR = 2.36, 95%CI: 1.25–4.99]), family type (OR = 3.25, 95% CI: 2.03–4.56]), monthly income (OR = 1.93, 95%CI: 1.05–3.52) were significant risk factors of HPV-18 seroprevalence (p < 0.05). Of all the HPV-16 and -18 seropositive participants, 7.1% and 7.5%, respectively had highly reactive titers (≥1/320), suggesting ongoing infection. Of the seropositive samples, only 3 and 2 had ccfHPV-18 and -16 DNA, respectively.

M8 - Post-COVID-19 Neuropsychiatric Sequelae of Omicron and non-Omicron Variant Infections in Hong Kong

Presenting Author – *Ching Sze Ho, The Chinese University of Hong Kong, Hong Kong*

Author/s – *Wendy CS Ho, Apple CM Yeung, Dora PT Kwok, Arthur DP Mak, Rita WY Ng, Grace CY Lui, Lowell Ling, Paul KS Chan*

Abstract Content

Background: We investigated the morbidity of depression, anxiety, post-traumatic stress disorder (PTSD), subjective cognitive impairment (SCI) and fatigue among persons infected with SARS-CoV-2 in Hong Kong.

Methods and Results: The standardized self-administered COVID-19 Mental Health Impact Survey derived from the World Mental Health (WMH) Survey was used. Altogether, 318 adults aged 18-75 (mean: 49.8) years hospitalized for COVID-19 participated. 66.4% were female, 84% had mild infections (without pneumonia) and 11.1% had moderate infections (pneumonia), 22.6% infected with Omicron variants and the remaining infected with the earlier authentic strain and D614G variant.

Participants completed the survey on average 276 days post-symptom onset. 22.2% were screened to have at least one mental disorders. Depression (PHQ-9: ≥10) was the most prevalent found in 15.4%, followed by post-traumatic stress disorder (PTSD: ≥7, 13.8%) and general anxiety disorder (GAD-7: ≥10, 8.8%).

Furthermore, 37.5% reported major fatigue (CFQ-11: ≥4) and 13.1% had moderately severe somatic symptoms (PHQ-15: ≥10). Subjective cognitive impairment (AMIC: ≥3) was found in 37.4%

Non-Omicron infections (early authentic strain and D614G variants) showed significantly greater severity in PTSD (p < .001) compared to Omicron infections. However, the prevalence of depression, anxiety, fatigue, somatic symptoms and cognitive impairment did not differ.

Conclusions: A substantial proportion of persons with mild to moderate COVID-19 have features suggestive of mental disorders, and one-third reported fatigue and subjective cognitive impairment. Infection with Omicron variants appears to have a lower degree of post-traumatic stress compared to non-Omicron variants.

M9 - Comparison of bacteriophage ϕ6 and SARS-CoV-2 in antimicrobial surface tests

Presenting Author – *Sabine Pölzl, Medical University Of Graz, Austria*

Author/s – *Sabine Pölzl, Clemens Kittinger, Julia Rieger, Kurt Zatloukal, Andreas Hinterer, Maximilian Stummer*

Abstract Content

Due to the COVID-19 pandemic, researchers have focused in recent years on new preventive measures to limit the spread of SARS-CoV-2. One promising application would be the usage of antimicrobial materials on often-touched surfaces to reduce the load of infectious virus particles quickly.

Since tests against SARS-CoV-2 are limited to a Biosafety Level 3 (BSL-3) laboratory with high cost and time expense, experiments against an appropriate surrogate like bacteriophage ϕ6 are preferred in most studies. Therefore, we generated a comparable setup for both virus types to investigate Polyethyleneterephthalate (PET) foils without (reference) and with different copper coatings. Viral suspensions were pipetted on the surfaces and were incubated for different incubation periods (0 h, 1 h and 24 h). To investigate the still infectious viral particles a plaques assay for ϕ6 was performed, whereas SARS-CoV-2 was examined by RT-qPCR.

The results of the antiviral surface tests showed similar activity against ϕ6 and SARS-CoV-2. After an incubation of 24 h, both types of virus particles were no longer infectious. A high amount of copper loading on the specimen led also to a complete reduction after a shorter incubation period. Instead, the inactivation of both SARS-CoV-2 and ϕ6 on the uncoated reference was negligible for these short incubation times. The generated results by the adapted protocols showed a good comparability of the two different virus types. Therefore, our data confirmed that bacteriophage ϕ6 is an adequate substitute for SARS-CoV-2 to test this type of antimicrobial surface coating.

M10 - Detection of Tomato Brown Rugose Fruit Virus (ToBRFV) in Quebec and development of means of control

Presenting Author – *Emilien DI ROSA, Inrs, Canada*

Author/s – *Emilien Di Rosa, Audrey-Anne Durand, Caroline Provost, Philippe Constant*

Abstract Content

Tomato brown rugose fruit virus (ToBRFV) is an emerging plant pathogen first detected in Israel in 2014, and has since spread worldwide. The primary hosts of this virus are tomato (*Solanum lycopersicum* L.) and bell pepper (*Capsicum annuum* L.). ToBRFV causes significant economic losses in greenhouse production due to its rapid transmission and long stability on surfaces and materials. The main symptoms that were observed on fruits are, but are not limited to, a discoloration or browning but also a deformation and necrosis. Here, we present detection and quantification methods for ToBRFV. Those methods were applied for an extensive survey of ToBRFV in commercial greenhouses distributed in the province of Quebec (Canada). The aim of this approach is to measure the severity of the situation, but also to identify parameters specific to each production and that could explain the virus prevalence. The survey is completed with a series of experiments to evaluate the disinfection efficiency of several chemicals applied on tomato seeds and soil. Preliminary results regarding both research objectives will be presented with research perspective to reduce the propagation of ToBRFV in commercial greenhouses.

M11 - Generation of chimera Japanese encephalitis virus genotype 1 using reverse genetics and approach to a vaccine

Presenting Author – *Hee-Jung Lee, Konkuk University, Korea, Republic of*

Author/s – *Hee-Jung Lee, Sehyun Kim, Eumji Lee, Young Bong Kim*

Abstract Content

Background: Japanese encephalitis (JE) is a mosquito-borne zoonotic disease affecting approximately 68,000 clinical cases of JE worldwide yearly and 13,600 to 20,400 deaths. The prevalent JE virus (JEV) genotype in Korea was genotype 3 (G3), but since the 1990s, JEV-G1 has been dominant. Vaccination of humans is the most effective means of preventing JE, and there are three types of inactivated vaccines and one type of live attenuated vaccine currently used worldwide.

Objectives: Since all live attenuated and inactivated JEV vaccines are derived from G3 strains, it is necessary to evaluate their immunogenicity against JEV genotype I induced by vaccination.

Methods: To develop a novel JE vaccine, a reverse genetics system was used to construct an infectious full-size clone of vaccine strain SA14-14-2 and replace its envelope region with the env gene from JEV-G1 to generate a chimeric virus.

Results: The infectious cDNA clones were constructed by linking her T7 promoter to the 5' end of the pACYC184 vector and transforming the poly(A) signal to the 3' end. To generate the chimeric virus, JEV-G1 was transfected into BHK21 cells in which the T7 RNA polymerase gene is stably expressed. Furthermore, we characterized and compared the recombinant JEV in vitro and in vivo. This recombinant virus has the potential to greatly accelerate the understanding of the development of JE vaccines that cover diverse genotypes. Here, we propose a recombinant virus using a reverse genetics system to design an effective and safe JE vaccine.

M12 - SARS-CoV-2 detection by RT-PCR and SEM in aerosols from COVID-19 patient rooms

Presenting Author – *Noelia Gómez Sánchez, Miguel Hernandez University, Spain*

Author/s – *Violeta Esteban Ronda, Eduardo Yubero Funes, Jaime Javier Crespo Mira, Eusebi Chiner Vives, María Francisca Colom Valiente, Consuelo Ferrer Rodríguez*

Abstract Content

More than two years after the first positive cases of COVID-19 caused by SARS-CoV-2 and despite the vaccines and treatments developed, most of the world still faces serious risks caused by rapid transmission of this virus. However, there are still no firm conclusions about its airborne spread. Therefore, the aim of the study was to demonstrate the presence of SARS-CoV-2 in airborne particles (aerosols) of less than 10 µm in diameter, as evidence of its possible transmission in hospitalised or housebound COVID-19-positive patient settings. The study was conducted from January to July 2022 with COVID-19-positive patients diagnosed by RT-PCR. Low-flow air samplers (4L/min) with glass fiber filters were placed at different distances (1 and 2 meters) in patient rooms and left for an average of 24 hours. After exposure, the filters were processed for nucleic acid extraction and viral load quantification by RT-PCR. Positive filters were processed for visualisation of viral particles by scanning electron microscopy (SEM). A total of 28 patients and 41 aerosol samples were studied, of which 31 were positive for SARS-CoV-2 RNA (75.60 %) and viral particles of 60-80 nm in diameter were observed in SEM images. The results indicate that in the closed rooms, where patients with COVID-19 are housed, SARS-CoV-2 can be detected at different distances and for a long time by RT-PCR and SEM. We found no significant differences between viral loads obtained on filters and viral load in patients, neither for the different distances at which the air sampling equipment was placed.

M13 - The Connection between Yeast dsRNA Viruses and Transposons

Presenting Author – *Gerda Skinderytė, Vilnius University Life Sciences Centre, Lithuania*

Author/s – *Aleksandras Konovalovas, Saulius Serva*

Abstract Content

RNA viruses and viral elements like LTR-retrotransposons are common in *Saccharomyces* yeast. Recent findings suggest that dsRNA viruses may play a major role in shaping host response to the environment and even the evolution of genome. However, yeast viruses are commonly overlooked in research as they don't noticeable change yeast growth during standard lab cultivation.

Our analysis of yeast RNA viruses is focused on functional relationship with host transposons by raising question whether yeast endogenous elements may influence each other's life cycle. A recent study developed an original in vivo fluorescent transposition assay. It was used to test whether transposition efficiency is dependent on different RNA viruses and their satellites present in the host, and impact of other factors such as yeast strain, mating type and ploidy.

The results of the study demonstrated that Totivirus and their satellites indeed modulate the transposon activity in yeast. In some cases, the elimination of the virus resulted in a reduction of transposon activity. This study highlights the potential interplay between the Totivirus dsRNA viruses and yeast transposons.

M14 - Current incidence of HPV-driven oropharyngeal cancer and the possible role of liquid biopsies in recurrence monitoring

Presenting Author – *Ondrej Bouska, Palacký University Olomouc, Czech Republic*

Author/s – *Vladimira Koudelakova, Zuzana Horakova, Hana Jaworek, Marian Hajduch*

Abstract Content

Background: High-risk human papillomaviruses (HPVs) are etiological agents of several human malignancies, including oropharyngeal squamous cell carcinomas (OPSCCs). HPV-driven OPSCCs are newly recognized as a distinct subgroup with unique epidemiological and clinical profile. Although HPV-driven OPSCCs are more treatment responsive and have favorable prognosis, a certain subgroup of HPV-positive OPSCCs retains a higher risk of later recurrence. Liquid biopsies represent a promising strategy for early detection and post-treatment monitoring of disease recurrence.

Objectives: This study aims to evaluate liquid biopsy collection methods and monitoring of HPV persistence in OSPCC patients for early diagnosis and recurrence risk stratification.

Methods: In this study, newly diagnosed OPSCC patients and patients in remission were enrolled. HPV tumor status was determined using AnyPlex™ II HPV28 Detection (Seegene) and p16 immunohistochemistry. Pre & post-treatment HPV testing in gargle lavage (GL), oropharyngeal swabs (OPS), and plasma samples was performed, followed by regular testing according to the standard follow-up protocol.

Results: In total, 46 OPSCC patients have been enrolled. In a prospective cohort, 13/17 (76.5%) of newly diagnosed OPSCC patients were HPV positive. Positive agreement between HPV testing in tumor tissue compared to OPS and GL samples in newly diagnosed OPSCC was 100% and 75%, respectively. In retrospective cohort, 6/29 enrolled patients had detectable post-treatment HPV infection. HPV16 genotype was found in 100% of newly diagnosed HPV(+) OPSCC. In conclusion, these preliminary data show a predominant incidence of HPV-positive OPSCCs compared to HPV-negative OPSCCs. At the time of diagnosis, HPV testing in OPS showed higher sensitivity.

M16 - Reliable approach in assessment of protective humoral immune response to SARS-CoV-2

Presenting Author – *Ina Belskaya, The Republican Research And Practical Center For Epidemiology And Microbiology, Belarus, Belarus*

Author/s – *Ina Belskaya, Tamara Amvrosieva, Zoya Bohush, Natallia Paklonskaya, Tatsiana Yudziankova*

Abstract Content

Background: Despite the high seroprevalence of SARS-CoV-2 among population COVID-19 remains a global health emergency associated with a rapid appearance of new viral variants. The simple detection of antiviral IgG becomes uninformative in the estimation of protective immunity.

Objectives: Our research focuses on studying of the humoral immunity indicators and anti-SARS-CoV-2 IgG avidity index (AI) determination as one the parameters reflecting the strength of antigen-antibody bond.

Methods: Quantitative detection of SARS-CoV-2 anti-RBD IgG (10-500 BAU/ml) was performed by ELISA 3-4 months after first-time exposure to viral antigen. The determination of AI was based on dissociation of the antigen-antibody complex with 5M urea. Statistical analysis: Spearman correlation, Mann–Whitney U test.

Results: The primary immune response (fully vaccinated (n = 82) / COVID-19 patients (n = 138)) resulted in production of low avidity IgG to RBD SARS-CoV-2 (average AI – 32-33% (median 25%-27%) while additional stimulation of the mature B-lymphocytes proliferation in individuals with a secondary immune response (n = 68) was accompanied by increased production of high avidity IgG (65% (median 64%)). At the same time sufficiently high antiviral IgG concentrations were recorded in all the examined groups (COVID-19 patients: 363.5 BAU/ml (median – 476 BAU/ml), individuals with hybrid immunity: 438 BAU/ml (median – 500 BAU/ml)) excluding the vaccinated patients (p<0.001). A strong positive correlation (0.62) between AI and anti-RBD IgG concentration was observed only in individuals with hybrid immunity which showed the disadvantages of the IgG concentration parameter and indicated the importance of using AI in a comprehensive assessment of SARS-CoV-2 immunity.

M17 - Prophages of clinical *Pseudomonas aeruginosa*: insights into their role through their activity, abundance and persistence

Presenting Author – *Ifigeneia Kyrkou, Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Denmark*

Author/s – *Jennifer Bartell, Cédric Lood, Rob Lavigne, Helle Krogh Johansen, Søren Molin*

Abstract Content

It remains unclear how much the accessory genome contributes in the complex processes of establishment and virulence of bacterial infections. *P. aeruginosa* is one of the most common opportunistic human pathogens and can establish difficult-to-eradicate infections. Genome-integrated viruses, known as prophages, are frequent elements of this bacterium's large accessory genome and can contribute to the virulence of *P. aeruginosa*. However, systematic interpretations of the contributing role of prophages in the evolution and fitness of the ubiquitous P. aeruginosa in its diverse niches are pending. This study provides insights into these roles by exploring the activity, abundance and persistence of prophages belonging to P. aeruginosa from the cystic fibrosis (CF) lung. We selected a cohort of 12 young CF patients with a high-resolution history of difficult-to-eradicate *P. aeruginosa* infections. Nanopore technology was used to sequence high-contiguity genomes of one early isolate per CF patient. Subsequently, we applied a strategy that combined bioinformatics, antibiotic-assisted inductions, lysate sequencing and genomics to identify complete prophages in the host genomes and assess their long-term survival in follow-up isolates. From these data, we observed that CF P. aeruginosa genomes harbor a high abundance of intact prophages which are often self-induced. A wide prophage genomic diversity was unraveled and more than half of the prophages identified were found to persist long-term in follow-up isolates. In addition to elucidating the role of prophages in *P. aeruginosa*, we expect our findings to assist in developing novel diagnostics and phage-based therapies for *P. aeruginosa* infections.

M18 - A common strategy for virus adaptation to low host density at different temperatures

Presenting Author – *Mara Laguna-Castro, Center For Astrobiology, Spain*

Author/s – *Maria Laguna-Castro, Alicia Rodríguez-Moreno, Ester Lázaro*

Abstract Content

Microbes, as well as their viruses, can be found in most environments on our planet, including some presenting very harsh conditions for life. In the same way as the formers are able to get adapted to adverse conditions, viruses have developed mechanisms to optimize the infection of their hosts. However, the molecular basis of the adaptive strategies can be difficult to identify. Experimental evolutionary studies carried out under controlled conditions can be of great help in this cases.

We have used bacteriophage Qβ, an RNA phage that replicates optimally at 37°C, to carry out evolution experiments at suboptimal host density, both at optimal temperature (37ºC) and at a suboptimal one (43ºC). Qβ adaptation to low host density at optimal temperature took place through a mutation in the minor capsid protein, whose location and function are not well defined. In contrast, Qβ adaptation to low host density at 43ºC took place through a different mutation, located in the receptor binding protein, which also acts as a promoter of bacterial lysis. Surprisingly, both mutations have the same effect; they enhance virus capacity to entry into the cell, revealing a common strategy for adaptation to low host density. However, mutation selected at 43°C has a strong fitness cost at 37°C, manifested in a great increase on the length of the latent period. Our results show how the interaction between adaptive advantages and potential fitness costs define viral adaptive pathways depending on the environment.

M19 - *Haloferax gibbonsii* LR2-5 is susceptible to many viruses

Presenting Author – *Zaloa Aguirre Sourrouille, University Of Groningen, Netherlands*

Author/s – *Sabine Schwarzer, Sari Korhonen, Sebastian Lequime, Hanna M. Oksanen, Tessa E.F. Quax*

Abstract Content

Despite viruses being able to infect members across all three domains of life, the life cycle of archaeal viruses is still not well understood. Additionally, most of the characterized archaeal viruses are known to infect extremophilic hosts. A recent study suggested that host range determinants are unique to each virus, as closely related viruses have diverse host ranges1. To enhance our understanding of the host range determinants and interactions of haloarchaeal viruses with their hosts, we employed several species of the halophilic euryarchaeon *Haloferax* as a model system. Our findings indicate that the *Haloferax* strain *Haloferax gibbonsii* LR2-5 is susceptible to infection by 10 virus isolates and the factors determining host range specificity might include adhesins, viral egress proteins and restriction-modification systems2.

M20 - Mechanisms of superinfection exclusion in haloarchaea

Presenting Author – *Emine Rabia Sensevdi, University Of Groningen, Netherlands*

Abstract Content

Viruses are an essential part of life and the main predator of microbes, as they are found in all kinds of environments. They are able to maintain different types of relationships with their host. These can form a continuum ranging from a parasitic to a symbiotic or even beneficial one. To date, most studies on the interplay between viruses and hosts have been carried out on viruses infecting bacteria and eukaryotes, while little is known about the interplay in archaea.

The relatively high number of viruses in different habitats implies that it is very likely that a new virus will encounter an already infected cell, which can lead to competition for cell resources. To circumvent this, some viruses block infection of their host cell by another virus, known as superinfection exclusion (SIE). Arguably, this could even be a beneficial relationship that secures resources for the virus and protects the host from infection by a lytic virus. However, apart from a few well-studied model bacteriophages, knowledge of this topic is rather scarce. Some cases of SIE have also been found in studies with viruses that infect archaea.

This work aims to provide a first insight into the requirements of SIE in haloarchaeal viruses infecting different halophilic Euryarchaea. To achieve this goal, a combinatory approach of adsorption assays, qRT-PCR and microscopy is applied, with which it is aimed to gain deeper understanding of the conditions under which SIE can be observed in archaea.

M21 - Host adaptive radiation is associated with rapid virus diversification and cross-species transmission in *African cichlids*

Presenting Author – *Vincenzo Costa, The University Of Sydney, Australia*

Author/s – *Jonathon Mifsud, Fabrizia Ronco, Walter Salzburger, Erin Harvey, Edward Holmes*

Abstract Content

Adaptive radiations are generated through a complex interplay between a series of biotic and abiotic factors. Although they have been widely studied in the context of animal and plant evolution, little is known about how they might impact the diversity and evolution of viruses that infect these hosts, which in turn may provide important insights into the drivers of disease emergence. Here, we examine the relationship between host adaptive radiation and virus evolution using 74 *African cichlid* fishes of Lake Tanganyika, that have rapidly diversified over the last 10 million years, as a model system. Using a meta-transcriptomic approach, we identified 121 novel vertebrate-associated viruses that fell within 13 RNA and 4 DNA virus groups. Reconciliation analysis of virus and host phylogenies revealed that cross-species transmission was the most common event among virus-host associations, particularly within the Astroviridae, Metahepadnavirus, Nackednavirus, Picornaviridae, and Hepacivirus groups. We assembled a time-calibrated phylogeny of cichlid hepaciviruses and show that hepacivirus evolution was not constant throughout the cichlid radiation, but seemingly accelerated 2-3 million years ago, coinciding with a period of rapid cichlid diversification in Lake Tanganyika and hence more closely related hosts available for infection. These data show that the fish species within this adaptive radiation contain a complex interacting pool of virus diversity, likely reflecting their close genetic relationships that enables frequent cross-species virus transmission.

M22 - Viral infections in phytoplankton communities and *Ostreococcus* sp. from the Western Baltic Sea

Presenting Author – *Luisa Listmann, Institute for Marine Ecosystem and Fisheries Science, University of Hamburg, Germany*

Author/s – *Luisa Listmann, Jana Hinners, Elisa Schaum*

Abstract Content

Viruses represent an important cause for (marine) phytoplankton mortality and can thereby influence biogeochemical cycling of carbon and other nutrients. Given that viruses are highly abundant in the ocean and play an important role it is pertinent that we begin understand how viruses affect ecological and evolutionary dynamics in ecosystems. In recent years, an understanding of the potential importance of phytoplankton-targeting viruses on ecosystem dynamics has emerged, but a broadscale investigation of host-virus interactions is still scarce.

Over the last years, we investigated phytoplankton communities and the picoplankton species complex of *Ostreococcus* sp. from four regions of the Western Baltic Sea that differ mainly in temperature and salinity. Here, we present two experiments investigating i) the top-down controls of viruses and zooplankton on phytoplankton communities over 7 months and ii) infection dynamics of *Ostreococcus* sp and its viruses over more than two years. For the first experiment, we used modified dilution experiments and found that top-down control on phytoplankton communities varies strongly between seasons and less so between the different geographical regions. For the second experiment, we used freshly isolated strains of both *Ostreococcus* and its viruses in a cross-infection set up and explicitly confirm species and strain specificity in *Ostreococcus* sp. from the Baltic Sea. Moreover, we found the timing of virus-host co-existence, was driver of infection patterns as well. In combination, these findings prove that host-virus co-evolution can be rapid in natural systems.

M23 - High-Quality database of SARS-CoV-2 genome sequences and variants

Presenting Author – *Yeeun Jo, Korea Research Institute Of Bioscience And Biotechnology (kribb), Korea, Republic of*

Author/s – *Yeeun Jo, Kiwon Jang*

Abstract Content

Since the outbreak of SARS-CoV-2, various variants have emerged as it spread throughout the world. Most variants do not cause significant changes to the virus, but certain variants increase its transmission and fatality rate. National authorities and research institutes have been continuously monitoring these variants and closely examining whether they may have crucial for public health. Various nomenclatures are utilized for tracking variants of SARS-CoV-2, with particular attention given to variants such as Delta or Omicron. Recently, as Omicron has spread among many people, numerous Omicron recombination variants, in which genes from different viruses, have occurred.

To find and track these variants, a large amount of sequences are required. Fortunately, public databases such as Global Initiative on Sharing All Influenza Data (GISAID) and National Center for Biotechnology Information (NCBI) provide virus sequences from around the world. We collected about 20 million genome sequences of SARS-CoV-2 since December 2019. Then, we developed QC pipeline to improve the reliability and validity of these sequences.

We filtered out low quality sequence data, including duplicates. Then we analyzed the variants of each sequence and classified the sequence data phylogenetically. As a result, we have produced a high-quality dataset of purified SARS-CoV-2 sequences that can serve as a foundation for vaccine and therapeutic development, as well as be used by researchers in various fields.

M24 - The effect of immediate-early expression of ILTV glycoprotein D on superinfection exclusion and genomic recombination

Presenting Author – *Paola Vaz, The University of Melbourne, Australia*

Author/s – *Turgut Aktepe, Carol Hartley, Joanne Devlin*

Abstract Content

Background: Genomic recombination is a major contributor to viral evolution, and in Gallid alphaherpesvirus 1 (ILTV) recombination of live-attenuated vaccines have resulted in the generation of in-field progeny with increased virulence. Recombination requires coinfection of a cell, which likely occurs through superinfection. Superinfection exclusion has been demonstrated in other herpesvirus species, and the early protein glycoprotein D (gD) was implicated to play a role. However in ILTV, superinfection exclusion has not been demonstrated and the role of gD is unknown.

Objective: In this study we investigate superinfection exclusion rates in ILTV and the impact of enhancing gD expression on superinfection exclusion and recombination.

Methods: A gD-enhanced virus was constructed by introducing a second gD into the ILTV genome with a cytomegalovirus immediate early promotor thereby shifting gD expression to the immediate early phase of viral replication. The ability of wild-type and gD-enhanced ILTV to inhibit superinfection was compared through superinfection exclusion assays and the subsequent impact on genomic recombination was assessed through in vitro coinfection assays.

Results: Both wild type ILTV and gD enhanced ILTV exhibited superinfection exclusion, where the virus with enhanced gD production showed the more efficient SIE than wild type. Although fewer recombinants were produced by coinfection with gD-enhanced virus, earlier expression of gD did not significantly reduce genomic recombination compared to parent.

M25 - Deciphering the tail fibre complexes of a crassvirales phage

Presenting Author – *Ciara Tobin, University College Cork, Ireland*

Author/s – *Ciara A Tobin, Christian Cambillau, Bianca Govi, Lorraine A Draper, R. Paul Ross, Andrey N Shkoporov, Colin Hill*

Abstract Content

Background: Bacteriophages of the order crassvirales are prolific and widespread in the human gut. They can account for over 86% of gut viral genomes in some individuals and are a core component of the gut virome in healthy adults. First discovered in silico in 2014, only five crassvirales have been isolated with their hosts thus far. While a number of intriguing genomic features have been observed in silico, many of their biological and structural characteristics remain unknown. Recently, reconstruction of crassvirales phage CrAss001 through the use of cryogenic electron microscopy has resolved the structure of most of the proteins of this phage. One tail fibre protein, gp22, was shown to be interacting with the virion. The remaining tail fibres could not be resolved to a high enough resolution because they are flexible relative to the main phage structure.

Objectives: The aim of this research is to provide a structural understanding of CrAss001 tail fibres, which will aid in completing the first structural overview of a crassvirales phage.

Methods: Structural predictions of individual tail fibres and complexes were made using the structure prediction software, AlphaFold. Pull-down assays to confirm predicted protein complexes were carried out using affinity chromatography.

Results: AlphaFold predicted trimeric structures for all five proteins (gp22-gp26). Gp24 and gp25 and gp24 and gp26 were both predicted to form complexes and this was confirmed by pull-down assays. In addition, pull-down assays demonstrated that gp22 and gp23 interact. All three complexes fit well into the electron density map of CrAss001.

M28 - Approaches in vaccination strategies to limit herpesvirus recombination

Presenting Author – *Carol Hartley, The University of Melbourne, Australia*

Author/s – *Turgut Aktepe, Paola Vaz, Marzieh Armat, Joanne Devlin*

Abstract Content

Background: Vaccines are used to control severe disease caused by herpesviruses in animals, but some vaccination approaches can drive the evolution and spread of herpesviruses with increased virulence and improved fitness. Our model is an avian alphaherpesvirus (infectious laryngotracheitis virus, ILTV) in the natural host (poultry), where recombination between two attenuated vaccine strains has resulted in more virulent recombinant progeny now dominating outbreaks in poultry flocks.

Objective: Our studies explore (i) the potential for safer vaccines candidates through designing viruses with reduced capacity for recombination and (ii) how the use of existing commercial vaccines influences the recombination profiles of viruses that spread between individuals in flocks.

Methods: (i) Viruses were constructed using codon bias deoptimization to reduce expression of genes (ICP8 and UL12) associated with viral replication and recombination and (ii) the recombination profiles of viruses transmitted to in-contact birds were compared between unvaccinated or birds vaccinated with an existing commercial vaccine.

Results: Reducing expression of ICP8 but not UL12 reduced recombination after in vitro coinfection studies and requires further investigation *in vivo*. *In vivo* studies have been performed with existing commercial vaccines, and these showed that vaccination shifts the recombinant profile of viruses transmitted to in-contact birds. Studies are ongoing to characterise the diversity, virulence and fitness of the altered recombinant progeny. The results suggest that a dual approach involving both the generation of safer vaccines, and the more prudent use of vaccines in flocks, may be the best way to minimise ILTV recombination and optimise vaccine safety.

M29 - Antimicrobial agents induced disruption of gut microbiome leads to depression and behavioral changes in mice

Presenting Author – *Azza Alahmed, Rak Medical University, United Arab Emirates*

Author/s – *Ashfak Hussain, Godfred Menezes, Hafiz Ahmed, Michael Menezes, Ashfaque Hossain*

Abstract Content

Background: Gut microbiome plays an important role in humans and other animals by influencing various health and disease states through modulation cell functions in local and distant organs, including the central nervous system. The bidirectional communication between the gut microbiome and the brain is referred to as the "Gut–brain axis", links alterations in gut microbiota with brain functions in various neurological conditions including behavioral changes. Here, we used a mouse model of antibiotic induced disruption gut microbiome and investigated the resultant behavioral alternation in the animals.

Methods: Mice were subjected to oral administration of a cocktail of antimicrobial agents containing vancomycin (0.5 g/L), muropenem (1.0 g/L), neomycin (1.0 g/L) and metronidazole (1.0 g/L) for 7 days. Disruption / elimination of gut microbiota was monitored by determining bacterial counts and 16S rRNA gene sequencing. The behavioral change in mice was monitored using elevated maize system.

Results: Treatment with antimicrobial agents significantly reduced gut microbial load as determined by viable counts. Decrease in microbial diversity was noted in 16S rRNA gene sequencing data with significant decrease Bacteroidetes and increase in Firmicutes, which occurs when gut mcirobiome shifts toward dysbiotic state. In Elevate maize experiments, antimicrobial agents treed mice spent 3 times time in dark tunnels in comparison to the lighted tunnels, indicating stress and depression.

Conclusion: The data obtained in this study indicate that antimicrobial agents can disrupt the gut microbiota in mice and establish a dysbiotic state, which is accompanied by stress / depression and behavioral changes in mice.

M30 - Olive pomace paste spontaneous fermentation as a valorisation tool

Presenting Author – *Helena Ferreira - University of Porto - Faculty of Pharmacy, Research Unit on Applied Molecular Biosciences - UCIBIO, Portugal*

Author/s – *Débora Araújo, Catarina C. Mota, Rita C. Alves, M. Beatriz P. P. Oliveira, Helena Maria Neto Ferreira*

Abstract Content

Olive-oil is an important Mediterranean-food which consumption are increasing worldwide. Along with its production, several by-products are generated, including olive-pomace(OP). OP is a by-product with high-levels of polyphenols with recognized antioxidant-activity. Remotion of OP stone-pieces originates olive-pomace paste(OPP). Our study evaluated the potential of the spontaneous-fermentation(SF) of OPP as a process of valorisation of OPP as a new-food-ingredient.

Were collected 20kg of OPP in Nov/2022 in an olive-oil company from Portugal. SF was promoted in three different temperatures (4°C/RT–Room temperature/37°C), 3 bottles for each temperature, during 32 days(d). At 0d/2d/4d/8d/16d/32d total-microorganism count(TMC) was evaluated, through surface-spread of OPP and dilutions in PCA. The TMC was done in triplicate and incubated at 37°C/48h.

The results show that the SF of OPP promotes the growth of natural-microbiota. The time evaluation shows that 2d are necessary to observe a change in the natural-flora, with highlight to RT with TMC growing from 9.2x104 to 1.35x107, followed by 37°C with 9.2x104 to 4.9x106. For both temperatures the population decreased after 2d. For 4°C, the growing was slow and the population increased until 8d. The fermented-OPP shows a functional-food potential. An exploratory-approach of the organoleptic-features was done showing that fermentation improves the aroma/taste of OPP.

The results of SF of OPP show that this by-product has the potential to be transformed into a new food-ingredient, with short or long fermentation time-depending on temperature-conditions. This represents a new-approach for by-product valorisation, relevant in terms of food-security and environmental-sustainability.

M31 - Life cycle assessment of *Alicyclobacillus acidoterrestris*: dual approach to properly delineate an in vitro antimicrobial effect

Presenting Author – *Jasmine Hadj Saadoun, University of Parma, Italy*

Author/s – *Elena Bancalari, Annalisa Ricci, Valentina Bernini, Erasmo Neviani, Monica Gatti, Camilla Lazzi*

Abstract Content

Background: Spore-forming microorganisms represent a current issue for food industries as they resist to different treatments becoming responsible for spoilage. *Alicyclobacillus acidoterrestris* mainly affect microbiological quality, it often contaminates fruit juices and beverages causing important economic losses for industries. To take effective actions and increase the efficacy of industrial treatments, the understanding of the life cycle of the microorganisms could be useful to define the optimal condition to apply the treatment.

Objectives: Knowledge of germination, duplication, and sporulation onset would provide a starting point for evaluating the use of an antimicrobial and its effectiveness. This work aims to investigate the life cycle of *A. acidoterrestris*, to identify the physiological state in which the addition of a natural antimicrobial could maximize the inactivation.

Methods: An integrated approach including impedance analysis and fluorescence microscopy, compared with the traditional agar plate count method, was used to study the *A. acidoterrestris* growth kinetics under optimal condition and in presence of antimicrobial obtained from fermented by-products.

Results: The results of impedometric analysis showed changes in conductivity in the medium during sporulation cycle highlighting the effect of the presence of the antimicrobial. Combining these results with those of fluorescence microscopy analysis, the effect of the antimicrobial on spores germination has been clearly observed. By comparing the results obtained with the integrated approach, the limit of the traditional plate counts methods has been identified.

M32 - *Vibrio* sp. as a vehicle for the spread of hidden antimicrobial resistances encoded on plasmids transmissible to Enterobacteria

Presenting Author – *Jens Andre Hammerl, German Federal Institute For Risk Assessment, Germany*

Abstract Content

Antimicrobial resistance (AMR) is on a rise and challenges global One Health increasingly. The emergence of different antimicrobial-/biocide resistances in the individual One Health compartments (environment, animals and humans) is usually associated with an adaption of the bacteria against prevailing selection pressures (antimicrobials, residues or biocides). Mobile genetic elements (MGEs) carrying transmissible resistance determinants are common and are widely spread among bacteria. Because of their localization on plasmids, bacteriophages and insertion sequences, they can also be transmitted between bacteria by different mechanisms during horizontal gene transfer. Investigation and monitoring on the emergence of transmissible resistances is important, but sometimes their phenotypic development is masked by their hosts. Recently, a *V. parahaemolyticus* isolate was notified to carry a carbapenemase-producing plasmid, which only leads to slightly increased MIC values for carbapenems in *Vibrio* spp. Nevertheless, the location of the resistances on a self-transmissible plasmid results in a high resistance phenotype against different carbapenems after natural transmission into a broad range of Enterobacteriaceae isolates. The properties and the genome of the plasmid associated with this hidden resistance phenotype as well as the genotypic and phenotypic features of the *V. parahaemolyticus* isolate will be presented and discussed. The data clearly showed that some bacteria can acquire and mask resistance plasmids, which were further spread to clinically relevant genera associated with severe nosocomial infection in human.

M33 - Inhibition performance of Caseicin FS against *Listeria monocytogenes* in soft cheese

Presenting Author – *Lucilla Iacumin, University Of Udine, Italy*

Author/s – *Francesco Salini, Giuseppe Comi, Andrea Colautti, Lucilla Iacumin*

Abstract Content

Caseicin FS is a newly discovered bacteriocin belonging to class IIa with a promising in vitro activity anti-Listeria monocytogenes, a foodborne pathogen of particular concern for human health. The in silico analysis of *Lacticaseibacillus casei* UD 2202 genome revealed unknown pediocin-like biosynthetic gene clusters, which was named CAS-X. Additionally, the manual annotation of these areas of interest revealed a gene collection having an unambiguous relation with the class IIa operons systems. Due to the failure of the antimicrobial capability of the strain, the heterologous protein production was optimized and its antimicrobial effectiveness was tested both *in vitro* (using agar well diffusion assay) and *in vivo* (soft cheese). Among the different tested *Listeria monocytogenes* strains, the more resistant to Caseicin FS in vitro was used as target pathogen for food trials. In particular, the effectiveness of the bacteriocin was tested against the pathogen on purpose inoculated in soft cheese and conserved both under refrigerated conditions and thermal abuse, simulating the conditions of storage during the shelf life of these kind of products. Results confirmed the effectiveness of Caseicin FS in reducing the pathogen concentration at zero tolerance level. In fact, already after 2 days from the inoculation, *Listeria monocytogenes* resulted absent/25 g of product.

M34 - Competitive interactions between *Zygomycetes* and *Aspergillus* originated from Korean traditional soybean-fermented brick, meju

Presenting Author – *Inhyung Lee, Kookmin University, Korea, Republic of*

Author/s – *So Hyun Kim*

Abstract Content

In the early stage of Korean traditional soybean-fermented brick, meju, fermentation, *Zygomycetes* are dominant although various fungi including *Aspergillus* exist in the environment. Interactions among fungi may determine the dominance of certain fungi during meju fermentation in addition to intrinsic and extrinsic factors. To understand the effects of fungal interactions on the fungal flora dynamics during meju fermentation, interactions between *Zygomycetes* and *Aspergillus* were analyzed. During co-culture of *Zygomycetes* and *Aspergillus*, competitive interactions were observed, resulting in decreased growth of both fungi. Morphological alterations such as hyphal swelling and fragmentation, and tip lysis were observed in both fungi, however, the degree of hyphal damage was more obvious in *Aspergillus* than in *Zygomycetes*. Cell-free broth of *Zygomycetes* also inhibited the growth of *Aspergillus* significantly, however, its effect was greatly reduced with heat-treated cell-free broth. Therefore, enzymes and/or chemicals produced by *Zygomycetes* might affect the growth and morphology of *Aspergillus*. Interestingly, the hyphal alterations were observed in the treatment of both heat-treated and untreated cell-free broths; swelling or protoplast degeneration were mainly observed with heat-treated cell-free broth, whereas tip lysis was observed with heat-untreated cell-free broth. Heat-liable enzymes such as extracellular chitinase and β-1,3-glucanase, etc. may be associated with hyphal damage such as tip lysis. Therefore, the antagonistic interactions of *Zygomycetes* against *Aspergillus* can be an important factor to determine the fungal dominance at the early stage of meju fermentation.

M36 - Antibacterial and Antibiofilm Activities of Alpha-Mangostin against *Staphylococcus* and *Corynebacterium* Associated with Mastitis

Presenting Author – *Thararat Chitov, Chiang Mai University, Thailand*

Author/s – *Supanida Choomjai, Hataichanok Pandit, Yingmanee Tragoolpua, Sakunnee Bovonsombut, Thararat Chitov*

Abstract Content

Bovine mastitis is one of the major problems faced by dairy farmers and industry, affecting both animal health and milk production and quality. Most mastitis cases are caused by infection with bacteria from the farm environment. Biofilm formation by mastitis-causing bacteria is understood to be associated with the persistence and spread of the disease. The purpose of this research was to investigate the antibacterial and antibiofilm activities of alpha-mangostin, the main xanthone obtained from mangosteen pericarp, against biofilm-forming *Staphylococcus* and *Corynebacterium* species associated with bovine mastitis. These genera have been known to increasingly develop resistance to antimicrobial agents and antibiotics. The results showed that alpha-mangostin inhibited all (100%) of the isolates tested, with the highest inhibitory effect against *Corynebacterium lactis*. The minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of alpha-mangostin against the isolates were in the ranges of 0.001–1.0 (MIC) and 0.002–2.0 (MBC) µg/ml, respectively. Alpha-mangostin also showed antibiofilm effects by inhibiting cell attachment, with the percentages of inhibition of biofilm formation ranging from 49–100%. The substance also had destructive effects on preformed biofilms produced by some isolates (-64–99%), although the biofilm destruction mechanism was less effective than the antibiofilm formation mechanism. The results from this study showed that alpha-mangostin can be considered a potential alternative to chemicals in controlling bacteria associated with bovine mastitis by means of bactericidal and antibiofilm effects.

M38 - Evaluation of *Aspergillus oryzae* enzymatic characteristics for koji and innovative miso production

Presenting Author – *Catarina Prista, Linking Landscape, Environment, Agriculture And Food (leaf), Associated Laboratory Terra, Isa, Ulisboa, Portugal*

Author/s – *Rafaela Santos, Ana Catarina Costa, Mariana Mota, Anabela Raymundo, Catarina Prista*

Abstract Content

Miso is a Japanese fermented semi-solid paste, with a strong umami flavour and ability to mask odours, highly nutritive, digestible and with physiological and functional benefits. It is usually made from soybeans, koji and salt, together with yeast and lactic acid bacteria. Koji results from inoculating *Aspergillus oryzae* in steamed rice and plays a crucial role in miso production, providing enzymes capable of hydrolysing proteins and polysaccharides.

With the focus on producing an innovative miso using traditional Portuguese protein-rich pulses (lupin, chickpea and grass pea) instead soybeans, the first step to koji production was to select the *A. oryzae* strain, considering fungus' growth and hydrolytic potential.

A. oryzae strains, obtained from culture collections (AL and AJ) and from a commercial koji (AB and AS) were tested for cell viability under salt stress, and for proteolytic, amylolitic and lypolitic activity in the absence and in the presence of 3% and 12% NaCl. Amylolytic, proteolytic and lipolytic activity assays were performed according to the procedures described in [2], [3], and [4], respectively.

Results obtained led to select AS and AJ, as the best strains with the highest values for amylolytic (0.620 and 0.615 U/mL) and proteolytic activities (12.125 and 7.340 U/mL) without salt. Their enzymatic performance was also high in the presence of salt. AS presented the highest value for lipolytic activity (1.122 U/mL), as well as a high growth rate.

Misos from lupin, chickpea and grasspea, using AS strain koji, were produced. Their fermentation is currently under analysis.

M39 - Isolation and characterization of wine yeasts of indigenous Georgian grape variety Shavkapito

Presenting Author – *Tamar Sachaneli, Georgian Technical University, Faculty of Agricultural and Biosystems Engineering Science, Georgia*

Author/s – *Tamar Sachaneli, Ketevan Kochiashvili, Sophi Meladze, Tamar Zedginidze, Giorgi Kvartskhava*

Abstract Content

Background: The grapevine ecosystem is diverse and unique, however, one of the key microorganisms living in this microbiome is yeasts. They are found in specific habitats and formed by various species. Mukhrani and Vaziani villages, in the Kartli region, are one of those places in Georgia where all important and interesting aspects gather and create unique environments (terroir) for vine growing and winemaking but are not properly observed.

Consequently, the species isolated and identified in further research, represent the native microbiota found in these two areas of the Shavkapito grape variety.

Objectives: The research aimed to establish and characterize autochthonous yeast strains of the grape, grape juice, and wine of the Shavkapito grape variety and based on the study of main technological characteristics selection of prospective strains as potential wine starters.

Methods and Results: Characteristic *Saccharomyces* and Non-Saccharomyces Yeast strains of the grape Shavkapito have been isolated. The morphological and physiological characteristics and the main technological properties of isolated yeast have been studied.

According to different morphological (shape of cells and colonies, staining), physiological (temperature), and biochemical (catalytic activity) properties, different species were identified: *Hanseaspora*/*Kloeckera*, *Saccharomyces cerevisiae*.

Chemical composition has been examined of the grape sample, juice before fermentation, and from wine Vaziani and Mukhrani areas. Examination of the main technological properties of yeast revealed strains with high potential for use in the wine industry.

M40 - Metabolic Characteristics and Aroma Compounds of P*enicillium roqueforti* Isolates from Turkish Blue Cheeses

Presenting Author – *Hatice Ebrar Kirtil, Istanbul Sabahattin Zaim University, Turkey*

Author/s – *Hatice Ebrar Kirtil, Banu Metin*

Abstract Content

Penicillium roqueforti is the principal mold that gives the blue-green color to Turkish blue cheeses such as Konya Kuflu Tulum and Erzurum Kuflu Civil in the ripening period. In this study, we aimed to determine the technological and metabolic characteristics, and aroma profile of *P. roqueforti* isolates from Turkish blue cheeses. We identified 120 *P. roqueforti* isolates from 61 traditional Turkish blue cheeses. Twenty isolates representing the population were selected based on the sequence types defined in previous studies. Mycelial growth at 10°C and 25°C, salt resistance (1%, 3%, 6% NaCl), proteolytic activity, and lipolytic activity of the isolates were determined. A deletion in the mycophenolic acid biosynthesis gene, mpaC, described in some *P. roqueforti* strains in European cheeses, was not detected in Turkey-originated isolates. Future studies will concentrate on the production of secondary metabolites including mycophenolic acid, isofumigaclavine A, roquefortine C, and andrastin A using LC-MS/MS. The aroma profile of the isolates will also be determined using GC-MS. The detection of different technological, and metabolic characteristics, and aroma profiles of *P. roqueforti* isolates from Turkish blue cheeses will indicate their potential as secondary starters for mold-ripened cheese making.

M41 - Metabolic characteristics of *Leuconostoc mesenteroides*, a major kimchi lactic acid bacterium, for mixed sugars

Presenting Author – *Ju Hye Baek, Chung-Ang University, Korea, Republic of*

Author/s – *Min Woo Lee, Dong Min Han, Che Ok Jeon*

Abstract Content

Microbes have the ability to metabolize a variety of carbon sources and show different preferences for utilizing carbon sources, which may be a major factor to determine their environmental niches. Vegetables, major raw materials of kimchi (a representative Korean traditional fermented food), contains various carbon sources such as glucose, fructose, and sucrose and the preference of microbes for carbon sources affect microbial succession during kimchi fermentation. Therefore, to understand microbial succession and metabolic features during kimchi fermentation, it is necessary to investigate the metabolic characteristics of kimchi microbes for major carbon sources. In this study, we investigated the metabolic characteristics of *Leuconostoc mesenteroides*, a major kimchi lactic acid bacterium, for mixed sugars. We cultivated *Leu. mesenteroides* J18 in culture media supplemented with two each of glucose, fructose, xylose, lactose, and sucrose, and analyzed carbon compounds, along with the growth of *Leu. mesenteroides* J18, pH, and fermentation products, over time. In addition, we analyzed the transcriptome of *Leu. mesenteroides* J18 to investigate the carbon metabolic features of *Leu. mesenteroides* J18 and metabolic regulations by carbon compounds. This study will provide a better understanding of kimchi fermentation and we will discuss more about them in the poster section.

M42 - Plasma Functionalized Liquid for controlling pathogenic biofilms in the poultry processing chain

Presenting Author – *Soukaina Barroug, University College Dublin, Ireland*

Author/s – *Soukaina Barroug, Paula Bourke*

Abstract Content

Background: Biofilm formation enables bacteria adhesion to abiotic surfaces in the poultry processing chain and ensures a high risk of cross-contamination, spoilage, and product quality deterioration. Residues like chicken juice promote microbial growth and biofilm formation. Non-thermal plasma is a novel-technology applied under development to preserve the microbial safety of an increasing range of foods.

Objectives: This study considers the impacts of poultry processing environmental conditions on biofilms generated by *Campylobacter jejuni* and *Salmonella* Typhimurium while identifying the bactericidal potential of plasma functionalized liquid (PFL).

Methods: Biofilms were generated on six abiotic food-contact materials using a bioreactor and a poultry juice model with variations in time and temperature. PFL generated by a Midi-Plex Microwave-discharge plasma system was applied to biofilms by washing (0-10min). Microbial recovery, metabolic activity, and fluorescence analyses were used to identify the antimicrobial effect. Biofilm structure, topography, and three-dimension were examined by scanning electron microscopy, atomic force microscopy and confocal laser scanning microscopy.

Results: Results illustrate that temperature, incubation time, media, and surface materials were all factors affecting the adhesion and resistance of biofilms. PFL induced 4-7.log10CFU/mL reduction with the disorder of the total cell metabolic activity following 60-sec washing. Damages to structure and topography were identified in addition to the compromise of cell membrane and release of intracellular fluids. PFL is a promising and scalable approach to ensure rapid sanitary conditions in the fresh poultry processing environment by eradicating pathogens in suspension, biofilms attached to abiotic surfaces, or on the surface of the meat.

M44 - Antibacterial activity of a modular, engineered phage lysin against foodborne pathogen *Salmonella* sp.

Presenting Author – *Aleksandra Kocot, University of Gdansk, Poland*

Author/s – *Yves Briers, Magdalena Plotka, Dennis Grimon*

Abstract Content

Background: According to the World Health Organization the most common foodborne diseases are caused by Gram-negative bacteria. One of the alternatives for antibiotics are phage-encoded lysins. Most of them are investigated for their use in the clinical sector, whereas their potential application in the food industry remains much less explored.

Objectives: The aim of our study was to obtain a modular lytic enzyme (MLE) with increased antibacterial activity against Gram-negative bacteria and to evaluate its anti-*Salmonella* activity for use in the food sector.

Methods: The VersaTile DNA assembly method was applied to obtain an engineered lysin. Plate counting and epifluorescence microscopy coupled with LIVE/DEAD staining was then used to evaluate the antibacterial activity against *Salmonella* sp.

Results: MLE-13 used at the concentration of 250 and 500 µg/mL completely inhibited the growth of Salmonella sp. in the plankton and biofilm form, respectively. Carrot inoculated with *Salmonella* (~106) was treated with 500 µg/mL of MLE-13 and stored at temperatures 4, 20 and 37℃ for 3 h. MLE-13 caused significant reduction of bacterial counts at 37℃ (1.05 log units, p<0.05) in the comparison to the untreated control. When carrot was incubated for 3 h with MLE-13 (500 µg/mL) prior to *Salmonella* contamination, bacterial counts were significantly reduced at all storage temperatures relative to controls, indicating the possibility of using MLE-13 as a food protector against contamination by Salmonella sp.

M45 - The impact of indole on intestinal and hepatic cells exposed to recombinant *Clostridium perfringens* enterotoxin (CPE)

Presenting Author – *Chao Wang, Research group of Food Microbiology and Food Preservation, Belgium*

Author/s – *Tom Defoirdt, Evy Goossens, Andreja Rajkovic*

Abstract Content

Background: *C. perfringens* enterotoxin (CPE) toxico-infections are the most frequent foodborne diseases in several countries. Low-dose of CPE can disrupt cellular signaling in hosts. Indole has been reported to protect hosts against toxins by improving tight junctions (TJs). However, the mechanisms of the potential impact of indole on intestinal and hepatic cells when acutely exposed to CPE are unclear.

Objectives: To investigate the impact of indole on mitochondrial functioning of Caco-2 and HepG2 cells when exposed to CPE, and to determine whether indole can protect the host against CPE in a short acute exposure.

Methods: The Seahorse XF96 was used to determine the real-time kinetic response of Caco-2 and HepG2 cells pre-treated with indole for 24h, followed by exposure to recombinant CPE (rCPE) for 2h. Meanwhile, the impact of indole on TJs of both cell lines was determined before and after exposure to rCPE by Western blot.

Results: The oxygen consumption rate of both cells was measured after exposure to rCPE, and the maximal mitochondrial respiration increased in the indole pre-treated group. This indicated that indole could protect cells from rCPE. The level of TJ occludin was higher in indole-treated Caco-2 cells before exposure to rCPE. However, no significant difference was observed after exposure to rCPE in both cells. This suggests that indole could improve tight junctions of Caco-2 cells to maintain their barrier properties, but it has no impact on HepG2 cells. Finally, TJs were quickly disrupted in both cell types by exposure to a low dose of rCPE for a short time.

M46 - Inhibition of quorum sensing related spoilage activities of the meat spoiler *Pseudomonas fragi* by using thyme essential oil

Presenting Author – *Yasemin Şefika Küçükata, Istanbul Sabahattin Zaim University, Turkey*

Author/s – *Yasemin Küçükata, Hasan Yetim, Banu Metin*

Abstract Content

Quorum sensing (QS) mechanism utilizes cell-to-cell communication to regulate the cell density and expression of virulence factors via the signal molecules such as autoinducer-1 (acylated homoserine lactones, AHL) and autoinducer-2 (AI-2). *Pseudomonas fragi* is a major meat spoiler in refrigerated raw meat and causes deterioration resulting in economic losses. It was reported that *P. fragi* could not produce AHL but recognized and responded to AI-2. Inhibition of the QS system, called quorum quenching, is a promising strategy to disrupt the QS mechanism and spoilage-related activities such as biofilm, proteolytic activity, and motility. The QS system of *P. fragi* has yet to be studied in detail. The present study aimed to determine the anti-quorum sensing potential of thyme oil against *P. fragi*. For this purpose, 325 *Pseudomonas* species were isolated from beef and minced meat samples collected from 12 local butchers. Using species-specific PCR, 100 isolates were determined to be putative *P. fragi.* Phylogenetic analyses using rpoD sequences indicated that 56% of the isolates were *P. bubulae*, a recently defined species close to *P. fragi.* The AI-2 production, biofilm formation, proteolytic activity, and motility of the isolates will be analyzed with and without thyme oil to determine the quorum quenching effect. The results will give information on the inhibition effect of thyme essential oil on the QS system and spoilage-related activities of the meat spoiler *P. fragi*.

M48 - Sub-lethal exposure of *Lacticaseibacillus paracasei* to atmospheric non-thermal plasma alters its membrane and response to low pH

Presenting Author – *Dragana Mladenović, University of Belgrade, Serbia*

Author/s – *Jovana Grbić, Predrag Petrović, Aleksandra Djukić-Vuković, Saša Lazović, Ljiljana Mojović*

Abstract Content

Lactic acid bacteria have an important role in food production, as probiotics and producers of lactic acid. Due to the generation of oxygen and nitrogen-based reactive species and UV radiation, non-thermal plasma (NTP) was found to be effective in microbial inactivation and food processing. In this study, we investigated the effect of atmospheric NTP on *Lacticaseibacillus paracasei* NRRL B-4564 survival, membrane alternations, and the response of treated cells to acidic stress.

Cell suspensions in water were subjected to different treatment time intervals using a custom-made plasma needle. Argon was used as a feed gas, with a flow of 0.5 slm, while the distance between the needle tip and suspension surface was 1.5 cm. Immediately after the treatment, the viable cell number was estimated by the pour plate method, while cell membrane alternations were studied by analyzing zeta potential and membrane permeability (Crystal Violet assay). To ascertain if sub-lethal NTP stress could influence *L. paracasei* survival in an acidic environment, NTP-treated cells were challenged by pH 2.5 for 3h.

The results demonstrate that the negative surface potential of the bacterial membrane (-29.83±1.49 mV for untreated) was gradually shifted towards neutrality (-7.59±0.54 mV after 180 s) with prolonged treatment time. Increasing cell exposure to NTP resulted in higher membrane permeability, which was correlated with viable cell reduction. The cells exposed to shorter treatment time (30 and 60 s) kept viability and showed better survival in low pH compared to untreated cells, suggesting the application of NTP in probiotic food processing.

M49 - Development of next generation yeast for baking industrial improvement

Presenting Author – *Sangmin Shim, SPC, Korea, Republic of*

Author/s – *Sangmin Shim, Sungho Lee, Moonyoung Jung*

Abstract Content

In the baking industry, baker's yeast is one of the most important factor affecting the quality of bread, since yeast produces carbon dioxide and various metabolites that have an influence on dough rheology, bread texture, volume and flavors. Currently, bread types and distribution channels are diversifying in the baking industry. Commercial bread suppliers produce lean and sweet dough and distribute them in frozen and refrigerated form, therefore it is crucial for the producers to develop distinctive yeasts that can exhibit optimal fermentation performance in the dough where various environmental conditions. SPC Research Institute of Food & Biotechnology (RIFB) developed the *Saccharomyces cerevisiae* Y76LT in 2022 by improving SPC native yeast (*Sac. cerevisiae* SPC 70-1) developed in 2015 and applying Mating Technology (non-GMO method). As a consequence, the Y76LT has various advantages in baking compared with the parent strain. Since the Y76LT has an excellent maltose utilization and stress tolerance (sugar, freeze), it has been confirmed that it is not only available for bread dough at various sugar levels (0~25%) but also suitable for making frozen dough. In particular, the Y76LT produces much less carbon dioxide in dough than that of the parent strain at 0 to 10℃, whereas the normal fermentation activity is restored when the temperature is raised to 25℃ or above. In this study, we introduce a novel yeast that can be applied to all types of dough and helps to control fermentation in positive cold conditions thus maintaining dough quality during the freeze-thaw process.

M50 - An investigation into changes in immunogenic proteins associated with cold temperature adaptation in *L. monocytogenes*

Presenting Author – *Federica D'Onofrio, University Of Teramo, Italy*

Author/s – *Federica D'Onofrio, Luigi Iannetti, Maria Schirone, Ivanka Krasteva, Francesco Pomilio, Manuela Tittarelli, Francis Butler*

Abstract Content

Listeria monocytogenes can survive under different stress conditions and its virulence appears to increase when exposed to stress factors used to control its growth.

The objective of this study was to identify immunogenic proteins (IP) of *L. monocytogenes* associated with adaptation to cold temperature.

L. monocytogenes cultures were grown at 12 and 37°C (control). Bacterial cells were collected during late exponential growth phase. The proteins were extracted, purified, quantified by BCA method, in-gel trypsin digested and analyzed by nLC-MS/MS. Proteins were identified with at least 2 peptides per protein against the *L. monocytogenes* Uniprot database. The IP were predicted by an in silico analysis approach. The proteins were sublocalized (Cello v2.4, SignalP 3.9) considering for the immunogenic prediction (VirulentPred) only the non-cytosolic proteins.

The IP were clusterized by STRING v11.05, applying a minimum required interaction score equal to 0.7.

As result, two main different networks were determined at 12 and 37°C. In response to low temperature, proteins associated with cell motility (FliM), chemotaxis (lmo0723) and oxidative stress (sod, trxB) were identified. Moreover the above mentioned proteins are absent at 37°C. At optimal growth condition, L. monocytogenes codified for several virulence factors associated to adherence of epithelial cells (Iap) and their invasion to colonize the host gastrointestinal tract (lmo1422 and lmo0129). These results were similar to previous literature reports. the datasets obtained can be useful for further studies on listeriosis pathogenesis and role of environmental stress in determining the virulence factors expression of different strains.

M51 - Autochthonous lactic acid bacteria as potential starter culture in plantbased fermentation

Presenting Author – *Charlotte Bauer Munch-Andersen, Norwegian University Of Life Sciences, Norway*

Author/s – *Charlotte Bauer Munch-Andersen, Martine Hilstad, Davide Porcellato, Hilde Marit Østlie*

Abstract Content

Plant-based protein (PBP) sources have an incomplete protein-profile on their own. However, their amino acid composition can complement each other to yield high-quality protein. Therefore, they represent good alternatives to animal protein. Yet, many PBP sources contain various antinutritional factors (ANF), that can cause irritation and discomfort for the consumer, making them problematic as alternative protein sources.

Bioprocessing methods like fermentation are known to reduce ANFs, and it is widely recognized that microorganisms autochthonous to the raw material are suitable starter cultures in fermentations.

The objective of this study was to establish a strain collection with well-defined fermentation properties. The technological properties of selected strains were characterised, and their potential use as starter culture for the fermentation of legumes and (pseudo-)cereals was evaluated.

Lactic acid bacteria (LAB) from spontaneous sourdough fermentation of legumes and (pseudo-)cereals was isolated and identified by culture-dependent methods, including genome sequencing. Selected isolates were screened for their acidification potential, proteolytic activity, exopolysaccharide (EPS) production, phytase activity and fermentation pattern. The assessment of technological properties was collected in a strain library, and isolates showing promising properties were tested as single-strain sourdough starters. The selected single strain cultures included an EPS-producing *Lactiplantibacillus plantarum*/*pentosus*, a *Leuconostoc mesenteroides*/*pseudomesenteroides* showing good acidification properties, and a phytase-active *Lacticaseibacillus rhamnosus*. Acidification, breakdown of flour compounds, metabolite changes, and production of volatile aromatic compounds were determined in fermentations using these starters. The result of this study adds to the exploration of autochthonous microbes to become new starter-cultures in fermentations of plant-based, protein-rich food.

M52 - Exploring combined antimicrobial activity of curcumin and nisin against *Listeria monocytogenes*

Presenting Author – *Loredana d' Ovidio, University Of Sao Paulo, Brazil*

Author/s – *Bernadette Dora Gombossy de Melo Franco, Svetoslav Dimitrov Todorov, Débora Preciliano de Oliveira*

Abstract Content

Background: Listeria monocytogenes is a foodborne pathogen with adaptations ability to different environmental biotopes, allowing its persistence in food production. Ready-to-eat meat products can be excellent carrier for *L. monocytogenes* and are considered as elevated risk for consumers, thus novel antimicrobials are important for ensuring food safety.

Objective: To explore combined antimicrobial activity of curcumin and nisin versus *L. monocytogenes*.

Methods: Different *L. monocytogenes* strains (3 from meat products, 2 serotype 1/2a -Δprfa ΔSigB and isogenic- and ATCC 7644) were investigated. The Minimum Inhibitory Concentration (MIC) of nisin for all strains were performed at neutral pH (NN) and 6.0 (N6), and curcumin heated at 60°C/30 min (CH) and non-heated (CNH). The Checkerboard Assay (CBA) was performed for all combinations of treatments and fractional inhibitory index (FICI) was obtained. Wilcoxon treatment (p<0.05) was used for statistical analysis.

Results: The MIC results showed that N6 and CH were more effective (p<0.05) when compared to NN and CNH. The CBA results showed that the only synergic (FICI≤0.5) combination against all strains was N6+CNH, followed by N6+CH. This last combination showed synergistic effect for only 4 tested strains. The other treatments (NN+CNH and NN+CH) were indifferent for all strains. These results could be explained by the acid pH which enhances nisin antimicrobial activity and stability of curcumin. The comparison between L. monocytogenes 1/2a wild strain and its mutant showed no influence of prfa and sigB genes (p>0.05) in response to the antimicrobials.

M53 - Depicting the suitability of FTIR to improve the differentiation of persistent and sporadic *Listeria monocytogenes*

Presenting Author – *Rui Meneses, Centro de Biotecnologia e Química Fina - Universidade Católica Portuguesa, Portugal*

Author/s – *Clara Sousa, Paula Teixeira*

Abstract Content

Listeria monocytogenes is a ubiquitous Gram-positive pathogen that is particularly harmful to immunocompromised individuals. In food processing environments (FPEs), certain strains of *L. monocytogenes* are routinely isolated, while others are only encountered sporadically. One possible explanation for this recurrent isolation correlates with the presence of heterogeneous subpopulations, some of which can withstand adverse conditions encompassing high salinity, low temperature, and low pH, along with other FPEs-related stressors. We set out to evaluate the suitability of Fourier Transformed Infrared Attenuated Total Reflectance (FTIR-ATR) spectroscopy to discriminate between the fittest persistent specimens from the remaining sporadic subpopulations. 72 *L. monocytogenes* isolates, encompassing both persistent and sporadic *L. monocytogenes*, were grown in microtiter plates in the presence of different food-associated stressors (pH, T ºC, % NaCl), with growth curves obtained by measuring the optical density (OD600). After reaching the late exponential phase, the cell pellets were collected and analysed by FTIR-ATR spectroscopy. Additionally, infrared (IR) spectra of isolates grown in agar plates at 37ºC were collected. Partial Least-Squares Discriminant Analysis classification models based on collected IR spectra were developed to determine the viability of FTIR spectroscopy to discriminate persistence in different conditions. The gathered data showed no significant differences in growth rates and lag phases of persistent and sporadic *L. monocytogenes* in a planktonic state when grown at 37ºC or under the food-associated stressors tested. FTIR analysis has shown promising results in differentiating persistent and sporadic isolates belonging to the same serogroup, achieving correct assignment rates of over 70%.

M54 - Cytopathogenicity of *Bacillus thuringiensis* Isolates from an emetic illness on human hepatoma HepG2 Cells

Presenting Author – *Jintana Pheepakpraw, Chiang Mai University, Thailand*

Author/s – *Jintana Pheepakpraw, Thida Kaewkod, Aussara Panya, Yingmanee Tragoolpua, Thararat Chitov*

Abstract Content

Bacillus cereus is a foodborne pathogen that can cause emetic foodborne illnesses, which are caused by emetic toxins. In this study, we investigated the cytopathogenicity of some strains of *Bacillus thuringiensis* (BT) associated with emetic food poisoning, which were obtained from food involved in the food poisoning incident (FC2) and vomit samples from one patient (FC7 and FC8). Based on the ISSR-PCR result, these strains were genetically different from each other. The cytotoxic effects of the heat-treated culture supernatants of the BT strains and a reference emetic *B. cereus* strain (F4810/72) on human hepatoma (HepG2) cells were determined. At a concentration of 50% (v/v), the BT culture supernatants caused vacuolation and exhibited toxicity to HepG2 cells, with CC50 values of 60.37, 68.24, and 72.21 µg/mL for FC2, FC7, and FC8, respectively, by the PrestroBlue assay for 48 h. The degrees of toxicity were less than that of *B. cereus* F4810/72, which had a CC50 value of 39.00 µg/mL. The flow cytometry with the Annexin V/PI assay revealed HepG2 cell destruction through both apoptosis and necrosis mechanisms, which were also observed in *B. cereus* F4810/72. However, for the BT strains, late apoptosis was the main pathway of the apoptotic mechanism, whereas both early and late apoptosis were observed in *B. cereus* F4810/72.

M55 - Biomimetic magnetic nanoparticles for magnetic concentration and qPCR detection of bacteria in liquids

Presenting Author – *Monica Jimenez-Carretero, University Of Granada, Spain*

Author/s – *Javier Rodríguez-López, Cristina Ropero-Moreno, Juan Granada, Josemaría Delgado-Martín, Manuel Martinez-Bueno, Antonia Fernandez-Vivas, Concepcion Jimenez-Lopez*

Abstract Content

Background: The development of biosensors to detect pathogens in food is of paramount importance. Magnetic nanoparticles (MNPs) have gained popularity in this context due to their large surface area, which maximizes the interaction with target microorganisms; and their magnetic susceptibility, that allows to concentrate the nanoparticles and the attached microorganisms using external magnetic fields.

Objectives: In this study, biomimetic magnetic nanoparticles (BMNPs) were used to concentrate microorganisms from saline solution and milk. The specific detection of *Staphylococcus aureus* (model bacterium) was achieved by qPCR.

Methods: BMNPs were synthesized by precipitation with the mediation of the magnetosome protein MamC from Magnetococcus marinus MC-1. These nanoparticles were added to saline solution and milk inoculated with bacteria. After magnetic concentration, DNA extraction was performed, and *S. aureus* was detected by qPCR.

Results: Compared to existing MNP-based biosensors, the surface properties of BMNPs, provided by the presence of MamC in the external layers of the magnetite crystals, allow a direct electrostatic interaction between microorganisms and nanoparticles without the need of post-production coatings. Our results show that the binding of Gram-positive and Gram-negative bacteria on BMNPs is efficient, and the application of external magnetic fields enables to concentrate them easily. Although the binding is unspecific, the specificity for detection is given by qPCR, which permits the detection of bacterial loads as low as 10 CFU/mL. Our system is simpler than current MNP-based biosensors and maintains (or even improves) the detection limit for *S. aureus*, becoming a cost- and time-effective alternative for bacterial detection in liquids.

M56 - Isolation of ethyl hexanoate-producing *Saccharomyces cerevisiae*

Presenting Author – *Jeong-Ah Yoon, Kangwon National University, Chuncheon, Republic of Korea*

Author/s – *Yu-Jeong Lee, Seong-Wook Cho, Myoung-Dong Kim*

Abstract Content

Ethyl hexanoate (ethyl carbonate) is a volatile ethyl ester with a floral/fruity aroma. It is produced by the reaction of ethanol and fatty acid by yeast fermentation and is an essential flavor component in alcoholic beverages. Based on the fact that cerulenin-resistant yeast exhibits excellent ethyl hexanoate production, the cerulenin resistance of *Saccharomyces cerevisiae* strains isolated from traditional Korean foods were compared. Among the three cerulenin-resistant strains, MBY1334 isolated from nuruk showed the superior production of ethyl hexanoate in YEPD medium. Furthermore, it produced 187.13±9.69 ppb of ethyl hexanoate in rice hydrolyzates, corresponding to 3.6 folds enhancement in ethyl hexanoate concentration compared to the control.

Keywords: *Saccharomyces cerevisiae*, Ethyl hexanoate, cerulenin-resistance, rice hydrolyzates, Nuruk

M57 - Evaluation of mycocins antimicrobial conjugated effect produced by *S. cerevisia*e and *D. hansenii* against foodborne pathogens

Presenting Author – *Patricia Branco, University of Lisbon, Portugal*

Author/s – *Patricia Branco, Inês Mendes, Catarina Prista*

Abstract Content

Nowadays, to avoid microbial spoilage, food industry applies mainly chemical preservatives. However, these preservatives can provoke several health problems [1,2]. Thus, with emergent foodborne diseases and aligned with the increased interest in more natural products, the search for effective biopreservatives, non-toxic to human health, can be a sustainable and safer alternative to the commonly used preservatives.

Over the last years, some yeast, such as Saccharomyces cerevisiae and *Debaryomyces hansenii* have been reported as mycocins producers [3,4]. In the present study, we evaluated the antimicrobial effect of those mycocins, alone and combined, against five foodborne pathogens: *Candida albicans*; *C. krusei*; *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella* sp.

Mycocins were obtained as described in [3,4]. To evaluate their antimicrobial effect, we performed bioactivity-assays in 96-well plates, and O.D600 nm and colony-forming unit (CFU) of the above mentioned pathogens were determined for 48h in the presence and the absence of the mycocins. Results for bioactivity-assays revealed that the mycocins produced by *S. cerevisiae* presented a fungicidal and bactericidal effect on all the tested microorganisms except on *C. krusei*. However, the *D. hansenii* mycocins only showed a bactericidal effect on *E. coli* and *Salmonella* sp. Nonetheless, a synergistic antimicrobial effect was observed when the mycocins from both yeasts were combined, leading to a significant CFU reduction in all microorganisms tested, i.e., approx. 4-orders of magnitude.

Hence, this work is an important step forward for *S. cerevisiae* and *D. hansenii* mycocins evaluation as potential biopreservatives in food products.

M58 - Effect of water activity and lactate ion on the modelling of lactic acid inhibition in food

Presenting Author – *Olav Sliekers, Corbion, Netherlands*

Author/s – *Frank Segers, Anh Linh Nguyen, Catalin Iancu, Juliana Lane Paixiao dos Santos*

Abstract Content

Background: It is generally accepted that the undissociated form of the lactic acid molecule is responsible for the antimicrobial action in food and fementation. In predictive models, usually the effect of undissociated lactic acid is fitted and the concentration is calculated using the pH.

Objective: We show, that this approach can lead to models with less quality when predicting the inhibiting effect of lactic acid on more resistant spoilage bacteria like lactic acid bacteria.

Methods: We used *Lactococcus lactis* as an example and two datasets were obtained in broth. On one dataset we fitted three different models, including one correcting for the water activity effect and one including the dissociated form as well. The other dataset was used for validation with the accuracy and bias factors.

Results: By taking the effect on the water activity in account first and then fitting both the effect of undissociated and of the dissociated lactic acid molecule, the standard error of fit is diminishing drastically, especially by including the water activity effect. This can be easily observed visually in the parity plots, but also the accuracy and bias factor improve drastically.

Although the inhibition by dissociated lactate is much less, it seems to play a role in the total inhibition. The theoretical explanation of the effect of the lactate ion cannot be established with modelling. However, a larger potential across the membrane may drive also diffusion of lactic acid across the membrane or counteract export of lactate out of the cell.

M59 - Novel non-antibiotic antimicrobials for the control of bacterial contamination in food production chain

Presenting Author – *Michał Zaród, Mossakowski Medical Research Institute, Polish Academy Of Sciences, Poland*

Author/s – *Justyna Czarnecka, Ana Astorga, Gabriela Ryk, Małgorzata Korzeniowska, Maciej Wolak, Izabela Sabała, Elżbieta Jagielska*

Abstract Content

Sustainable and safe food production, due to growing demand of fresh food in the diet, requires development of novel solutions to control microbiological safety at each stage of the food chain: from production and harvest to processing, storage, preparation and consumption. It should be immediately addressed, including reduction of development and spread of antibiotic resistance among bacteria in the food chain, and discovery of novel, targeted strategies towards emerging pathogens which have a high impact on human health, as well as on the food industry. We are working on alternative infection prevention and food preservation, which specifically target pathogenic bacteria leaving natural microbiome untouched. The aim of the project is to develop novel antimicrobials, enzybiotics, recruited among peptidoglycan hydrolases, which cleave specific bonds in peptidoglycan structure of bacterial cell walls, causing their instant lysis and death. We have discovered enzybiotics targeting three pathogens identified in salmon production chain, particularly in salmon aquaculture (*Yersinia ruckeri*), processing and storage of raw or smoked salmon (*Staphylococcus aureus*, *Listeria monocytogenes*). Novel anti-Listeria, anti-Yersinia and anti-staphylococcal enzymes were tested against targeted pathogens in vitro by monitoring cell lysis in suspension or calculating the number of surviving cells. All of them displayed significant antimicrobial potential, killing at least 99% of bacteria present in the samples. Also, it was confirmed that they are safe for eukaryotic cells (fibroblasts, keratinocytes) and not toxic to model organisms, *Danio rerio* and *Galleria mellonella*. Overall, our results show high antibacterial potential of newly designed enzybiotics to be used in food industry.

M60 – Characterization and safety evaluation of *Kocuria Salsicia* isolates from traditional Montenfrin food

Presenting Author – *Beatriz Daza, Faculdade Ages, Austria*

Author/s – *Nadja Raicevic, Ana Jovanovic, Adriana Cabal, Robert L. Mach, Werner Ruppitsch, Aleksandra Martinovic, Anna Stoeger, Johann Ladstaetter*

Abstract Content

Background: The quality and sensory properties of artisanal food products are a reflection of their microbiome composition. The genus *Kocuria* may contribute to the typicity of artisanal food products but has been described also as an opportunistic pathogen. Thus, detailed characterisation of bacterial species obtained from artisanal food is a basic imperative for consumer safety and food producer.

Objectives: Whole genome based characterization of *Kocuria salsicia* isolates obtained from artisianal Montenegrin food products.

Methods: Isolates were obtained from three cheese and five sausage samples using standardized procedures. Illumina and Nanopore genomic libraries were prepared using NexteraXT or Rapid Barcoding kit and sequenced on a NextSeq2000 or on a R9.4.1 flow cell on a MinION Mk1C. An ad hoc core genome multilocus sequence typing scheme was created using SeqSphere+. CARD, Nucleotide DB, AntiSMASH and BAGEL4 were used to detect ARGs, VGs, secondary metabolites and bacteriocin genes. Antimicrobial susceptibility was determined by E-test.

Results: Isolates showed a high diversity in their core genome and had a minimum of 873 allelic differences to GenBank strain G1. Detection of secondary metabolites- and bacteriocins genes indicate a contribution to the typicity of artisanal food products. All isolates carried the vanY accessory gene of the vanM ARG group but were sensitive to vancomycin, which in combination with the absense of VGs indicate that these isolates can be regarded as safe for consumers.

M61 - General features of M23 endopeptidases and their potential use as enzybiotics.

Presenting Author – *Imdik Pan, Laboratory of Protein Engineering, Poland*

Author/s – *Magdalena Kaus-Drobek, Alicja Razew, Paweł Mitkowski, Izabela Sabała*

Abstract Content

One of the major problems of the 21st century is the rapid development of multi-drug-resistant bacterial strains. To address this problem, new solutions have to be developed. A promising alternative to antibiotics is enzyme-based antimicrobials (enzybiotics), like the M23 peptidase family. The M23 enzymes are zinc-dependent peptidoglycan hydrolases (PGHs), with the characteristic H(x)3D and HxH motifs. PGHs comprise glycosidases, muropeptidases, amidases, and other endopeptidases that are able to cleave specific peptide bonds in bacterial peptidoglycan (PG). In nature, PGHs have an important role during cell division, remodeling of the cell wall PG, and even for virulence, but also serve as microbiological weapons to eliminate competing bacteria in the same ecological niche.

The aim of our work is the biochemical and structural characterization of the M23 peptidases to create potent antimicrobial drugs against multi-resistant bacteria. We have performed an in silico analysis of over 90 000 protein sequences, predicted to contain the conserved motifs of the M23 peptidase family. We have analysed in detail their modular architecture and their surface net charge, characteristics that were shown to play a crucial role in enzyme activity and specificity. Based on these results, we have gained a better understanding of activity and specificity determination of the whole family of M23-containing proteins that can be used as a platform for creating and improving novel enzybiotics.

M62 - Prevalence and molecular characteristics of AmpC β-Lactamase producing Enterobacteriaceae and carbapenem resistant Enterobacteri

Presenting Author – *Tak Fai Wong, The Hong Kong Polytechnic University, Hong Kong*

Author/s – *Iain Chi-Fung Ng, Franklin Wang-Ngai Chow, Emily Wan Ting Tam, Shun-Wan Chan, Polly Hang-Mei Leung, Gilman Kit-Hang Siu, Lam-Kwong Lee, Ka-Yee Fung, Jiaying Zhang, Choi-Ying Wong*

Abstract Content

Background: Ready-to-eat (RTE) food is a popular choice of food in Hong Kong. However, RTE food may also be a vehicle of antimicrobial resistance bacteria (AMRB) as it is consumed directly without further heat-treatment to eliminate the bacteria. Although antimicrobial resistance (AMR) issue has been official addressed by local government recently, research about AMRB in Hong Kong RTE food has been scarce. Therefore, we aim to characterize the AMRB landscape in Hong Kong with this comprehensive study.

Objectives: This study aims to reports recent AMR data on AMRB prevalence, antibiogram and molecular resistant profile of the AMRB.

Methods: A total of 299 sashimi and 304 vegetable RTE samples were collected by random sampling from local retail spots. Antimicrobial resistant bacterial isolated from selective agar were identified by MALDI-ToF MS. Phenotypic resistance was confirmed by disk diffusion method. Genetic information was extracted from whole genome sequencing with Oxford Nanopore technology. Molecular resistant profile was composed with bioinformatics pipeline.

Results: Multi-drug resistant organisms (MDRO) were found in 6% of sashimi and 2.6% of vegetables samples. AmpC β-Lactamase producing Enterobacteriaceae contributed for 90% and 61.5% of the MDRO in sashimi and vegetable samples respectively. Unique AmpC profile of Citrobacter (blaCMY, blaCMY-2, blaCMY-79, blaCMY-176), Enterobacter (blaACT, blaACT-12, blaACT-38), Hafnia (blaACC-1a, blaACC-1d) and Serratia (blaSFDC) could be commonly found. Besides, carbapenemase gene NDM-5 and blaIMI-2 were identified from *E.coli* and *Enterobacter* bacteria. Transferrable resistance genes found in plasmid or composite transposons further extended the antimicrobial resistance to non-beta-lactam antibiotics.

M63 - Impact of antibiotic resistance genes on physiological properties of *Salmonella enterica*

Presenting Author – *Václav Peroutka, University Of Chemistry And Technology Prague, Czech Republic*

Author/s – *Milada Šolcová, Veronika Bočková, Sabina Purkrtová*

Abstract Content

Considering the clinical, food, and economic implications, one of the most discussed topics in current microbiological research is the alarming increase in bacterial resistance to antibiotics. The source of this resistance is the genesis, transfer, and accumulation of specific antibiotic resistance genes (ARGs) and antibiotic resistance bacteria (ARBs). This occurs, among other things, in the environment of the food chain. In addition to resistance, the presence of ARG can have both positive and negative effects on the physiology of its bacterial carrier. The goal of this study is to clarify this impact; better comprehension of these mechanisms is crucial for understanding, monitoring, and controlling the spread of ARBs as well as the spread of ARGs themselves in the food bacteriome. Massive parallel sequencing techniques allow relatively easy whole-genome sequencing, further genome assembly, and mapping is optimal for determining complex genotypic ARG profiles of bacteria. Using the nanopore sequencing method (Oxford Nanopore Technologies), we assembled a whole-genome sequence of several selected Salmonella enterica isolates of the resistant phenotype. Obtained sequences were compared with different ARGs sequences libraries to determine overall resistance profiles. The specific growth rate and ability to form biofilm were chosen as the physiological traits to be correlated with antibiotic resistance profiles to evaluate ARGs impact. Optimizing conditions and protocols for measuring these phenotypical traits was part of this project and resulted in the designing of a methodology for assessing these phenotypic properties and their correlation with genotypic ARG profiles.

M64 - Characterization of microbiota associated with fruit and vegetable storage places in households

Presenting Author – *Damien Ballan, LUBEM, Univ. Brest, France*

Author/s – *Jérôme Mounier, Louis Coroller, Clément Bovo, Sylvie Tréguer, Emmanuel Coton, Adeline Picot, Stella Debaets, Univ Brest, Audrey Pawtowski*

Abstract Content

Background: Fresh fruits and vegetables (FFV) are among the most wasted food products (35-50% of food losses). An important part of this waste, mainly due to spoilage microorganisms, takes place at consumer's level.

Objectives: This study aimed at describing the microbiota associated with FFV storage places in households and its potential relation with waste.

Methods: Nearly 400 swabbings were performed on fridges and fruit baskets in about 50 French households (Brittany region), during the summer and fall periods, while the weight of FFV waste was continuously monitored. Swab samples were plated on various media to enumerate different microbial groups (mesophilic and psychrophilic bacteria, lactic acid bacteria, sporulating bacteria, *Pseudomonas*, enterobacteria and fungi) and also processed for ITS and 16S metagenetic analyses.

Results: FFV storage compartments represented an ecological niche for spoilage-related microorganisms, especially in refrigerators (notably *Pseudomonas* spp. with 1.2x10^6 CFU/cm² and enterobacteria with 2.9x10^4 CFU/cm² in average), while fungi were predominant in fruit baskets (1.5x10^5 TFU/cm² in average). FFV waste was significantly higher during the summer (36.3 ± 30.0 grams/day/person vs.8.9 ± 7.9 in the fall). However, no significant correlation was found between microbial abundances and waste amount. The ongoing metagenetic analyses, combined with identification of microorganisms isolated from spoiled FFV, will provide more data about the relation between microorganisms and waste. Moreover, the integration of behavioral (related to hygiene and purchase practices) and socio-demographic data of households will provide a better understanding of factors contributing to household microbiota and FFV waste.

M65 - Development of Real-Time PCR (RT-PCR) Method for Rapid Detection and Quantification of 19 Probiotics Strains

Presenting Author – *Joon-Gi Kwon, Seoul National University, Korea, Republic of*

Author/s – *Ju-Hee Park, Sujeong Lee, Jihye Yang, Sung-Woo Choi, Li-Ha Kim, Ju-Hoon Lee*

Abstract Content

In some recently marketed probiotics products, a contamination accident occurs in which the displayed strain and the actual strain are different. To solve this problem, the unique gene of each species was designated based on pan-genome analysis to accurately detect the probiotics species. And a RT-PCR method was developed to rapidly identify and quantify each probiotic species. To select the target gene of each bacteria, pan-genome analysis was performed with a total of 4,345 complete genome sequences. Then, the specificity of the selected genes was verified using BLASTN. After designing primer and probe sets with species-specific genes, these sets were validated by conventional PCR and RT-PCR method (triplicates). According to conventional PCR and RT-PCR, primer and probe sets successfully distinguished each species. Also, we constructed the standard curve in RT-PCR to quantify each species. To verify the accuracy of the standard curve through experiments for food application, a model was created in which 1*108 CFU/ml strains were inoculated in sterile milk and the RT-PCR and direct counting method were compared. Standard curves test results, slopes for the specific primers ranged from -3.00 to -3.50 and the lowest R^2 value of the standard curve was >0.991. With these standard curves, we performed RT-PCR and viable cell count and compared them using Mann-Whitney U test. There were no significant differences between RT-PCR and the viable cell count method. These results suggest that the novel detection method based on RT-PCR can replace the existing method.

M66 - Antimicrobial and antibiofilm assay of bioactive compounds and probiotic strain for the development of functional food packaging

Presenting Author – *Valeria Poscente, University Of Tuscia, Italy*

Author/s – *Luciana Di Gregorio, Costanzo Manuela, Chiara Nobili, Roberta Bernini, Luigi Garavaglia, Annamaria Bevivino, Bindo Arianna*

Abstract Content

Background: The increasing focus on packaged and ready-to-eat products has enhanced the risks associated with foodborne illness, demanding the development of advanced microbiological monitoring systems. Natural bioactive compounds incorporated in innovative packaging represent an extremely promising alternative for preventing food contamination and spoilage and for extending products' shelf-life.

Objectives: The aim of this study was to assess the antimicrobial and antibiofilm activity of selected bioactive compounds against foodborne pathogens and spoilage microorganisms. Both the traditional culture-based methods and Flow Cytometry (FCM) were applied to evaluate the potentiated action of Lactobacillus plantarum probiotic strain together with natural antimicrobials on the culturability and viability of pathogenic and spoilage strains.

Methods: Traditional microbiological methods and FCM [1] were applied to evaluate the effect of antimicrobial treatment of natural compounds (Thyme essential oil (EO), Origanum EO, Basil EO, Citrus Limon EO, Carvacrol, Limonene, Gallium(III) nitrate hydrate, Nisin) at different concentration (50-100-250-500 ppm) against the planktonic and sessile cells of four selected strains (*Escherichia coli* ATCC25922, *Pseudomonas fluorescens* ATCC 13525, *Listeria monocytogenes* 54ly, *Lactobacillus Plantarum* DSM 20174).

Results: The results obtained showed a higher efficacy of Carvacrol and Thyme EO, highlighting an overestimation of the dead population using the culture-based method; in fact, when the FCM method was applied, the prevalence of injured bacterial cells in a viable but non-culturable (VBNC) state was observed. When bioactive molecules were applied to a preformed biofilm of *L. plantarum*, an enhanced effect was observed. The latter could represent a promising alternative to functionalize antimicrobial ready-to-eat product packaging.

M67 - Anti-diabetic effect of *Lactobacillus sakei* KCTC14037BP strain in db/db mouse model of type 2 diabetes mellitus

Presenting Author – *Se Young Kwun, Kangwon National University, Korea, Republic of*

Author/s – *Se-Young Kwun, Jeong-A Yoon, Eun-Hee Park, Myoung-Dong Kim*

Abstract Content

Background: Probiotics provide health benefits to human when administered in appropriate amounts. Recently, Probiotics have been studied to have positive effects on type 2 diabetes mellitus. The *Lactobacillus sakei* KCTC14037BP strain was selected potential probiotic candidate with anti-diabetic properties in vitro experiment.

Objectives: The objective of this study was to evaluate the antidiabetic effect of *Lactobacillus sakei* KCTC14037BP strain used diabetic C57BL6J-db/db mice.

Methods: To confirm the antidiabetic effect of the *L. sakei* KCTC14037BP strain, 5-week-old male diabetic C57BL6J-db/db mice was used. For the experiments, the diabetic animal model C57BL6J-db/db mice were divided into 3 groups: non-treatment control group, orally treated with 1x108 mg/kg/day dose of L. sakei KCTC14037BP and orally treated with 200 mg/kg dose of guava leaf. Also, The C57BL6J mice were used as the non-diabetic normal control (non-diabetic group).

Results: The result of *L. sakei* KCTC14037BP was orally administrated for 7 weeks was improved compared to the diabetic group in fasting blood glucose, plasma antioxidant capacity, glycated hemoglobin, and insulin concentration. The triglyceride content was about 30% or more and the plasma antioxidant activity was about 19% or more compared to the guava leaf extract administration group used as a control group. These results show that the intake of *L. sakei* KCTC14037BP may be effective in anti-hyperglycemia by the attenuation of glucose and lipid levels. The *L. sakei* KCTC14037BP containing diets or drugs may be beneficial for controlling diabetes mellitus type 2 in humans.

M68 - Isolation of potent *Saccharomyces cerevisiae* for maltose fermentation

Presenting Author – *Seong-Wook Cho, Kangwon National University, Korea, Republic of*

Author/s – *Jun-Hyeok Park, Yu-Jeong Lee, Myoung-Dong Kim*

Abstract Content

Maltose is a disaccharide produced by amylase during malt production. The metabolic capability of Saccharomyces cerevisiae for maltose utilization is directly related to alcohol production capacity. Therefore, it is an essential factor that could affect the beer's taste and flavor. Yeast was isolated from Nuruk, Makgeolli, and flowers and was identified by comparing nucleotide sequences of PCR-amplified D1/D2 region of 26S rDNA using BLAST. Thirty-three strains of *S. cerevisiae* with a higher maltose metabolic capacity than control strains were selected among four hundred strains by determining cell growth. The NIY576 strain isolated from Makgeolli significantly showed the highest cell growth using maltose.

M69 - Identification and characterization of antibiotic resistant, Gram-negative bacteria isolated from Korean fresh produce and agric

Presenting Author – *Gyu-Sung Cho, Max Rubner-Institut, Germany*

Author/s – *Sunyoung Jeong, Ile Kim, Bo-Eun Kim, Myeong-In Jeong, Kwang Kyo Oh, Gyu-Sung Cho, Prof. Dr. Charles M.A.P. Franz*

Abstract Content

Background: Fresh produce and fruits are well known to harbor naturally occurring microorganisms with high diversity and a great variety of microbial lineages. The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) reported that fresh produce and fruits are prone to microbiological contamination and researchers have found that fresh produce is a potential reservoir of food borne pathogens, antibiotic resistance bacteria, and/or resistance genes.

Objective: This study aimed to identify and characterize bacteria isolated from lettuce cultivated in Korea by using phenotypic and genotypic methods.

Methods and Results: Based on the 16S rRNA gene sequencing data from these samples, 184 strains could be assigned to the genera Pseudomonas (30.2%), Acinetobacter (14.4%), Pantoea (13.4%), Enterobacter (9.4%), Flavobacterium (6.9%), Serratia (4.5%), Lelliottia (5.0%), Erwinia (2.0%), Aeromonas (1.5%), Brucella (1.5%), Klebsiella (1.5%), Leclercia (0.5%) and Pluralibacter (0.5%) using the EzTaxon database. One hundred thirty-three (69.3%) and 105 (54.7%) strains showed a resistance phenotype to ampicillin and cefotaxime in antibiotic resistance test using the disc diffusion method respectively. The whole genomes of 15 selected strains were sequenced by Illumina Miseq and NextSeq platforms and Resfinder analysis detected acquired antibiotic resistance genes including a fosfomycin resistance protein fosA, aminoglycoside resistance (aac(6')-Ic), tetracycline resistance (tet(41)), antibiotic efflux pump qxA and QqxB, (fluoro)quinolone-resistance gene qnrE1 and β-lactamase genes blaMIR-6, blaACT-12, blaOXA-304, blaADC-25, blaSST1, blaSST-2, and blaOKP-A-11. This study supported to need to evaluate risks associated with foodborne pathogens and antibiotic resistant bacteria in fresh produce in South Korea.

M70 - Unrevealing the genetic background of antimicrobial resistance in bacterial species present in edible insect farming sector

Presenting Author – *Teresita Bello Gonzalez, Wageningen Bioveterinary Research, Netherlands*

Author/s – *Betty van Gelderen, Frank Harders, Alex Bossers, Olga Haenen*

Abstract Content

Background: The increasing interest in rearing insects for feed and food requires deep insight into the food safety, veterinary safety and One Health of insects rearing, considering that the insect farming sector is growing exponentially. The genetic background of antimicrobial resistance in bacterial species present in edible insect farms has been studied occasionally. However, little is known on the potential risk of food, veterinary and potential zoonotic bacteria present during the entire rearing process, from substrate to insect products.

Objectives: The aim of this study was to analyze the genetic background of antimicrobial resistance in 50 potential pathogenic bacteria isolated from morioworms (*Zophobas morio*), house crickets (*Acheta domesticus*), and band crickets (*Gryllodes sigillatus*) from culture facilities, to highlight their potential risk for animal and human health.

Methods: A selection of 50 bacteria isolated from morioworms (n=17), house crickets (n=15) and band crickets (n=18), including Gram-positive and Gram-negative aerobes and facultative anaerobes, were characterized further by whole genome sequencing to identify the presence of antimicrobial resistance genes and mobile elements.

Results: High levels of antimicrobial resistance genes were identified, mainly in the group of Gram-negative isolates (61.2% isolates from morioworms, 55.6% from band crickets, and 46.7% from house crickets). Plasmid mediated resistance was identified in *Enterobacter cloacae*, *Klebsiella pneumoniae* and *Bacillus cereus* isolates. The beta-lactams, macrolides, aminoglycosides, fosfomycin, quinolone, phenicol, and tetracycline resistance genes were identified. The mecA gene was identified in all the *Staphylococcus sciuri* isolates. Our results support the need for monitoring the entire rearing process at insect farms.

M71 - Lactic acid as a targeted antimicrobial against food and gut related clostridia

Presenting Author – *Lucia Huertas Díaz, Department of Biological and Chemical Engineering, Denmark*

Author/s – *Clarissa Schwab*

Abstract Content

Clostridia are strictly anaerobic spore-forming bacteria that can be found in almost any environment on Earth. Some of them, have been classified as pathogens, either carried by food or by feces. One example of pathogenic clostridia is *Clostridioides difficile* that causes intestinal infection that can cause death in elderly during the ingestion of oral antibiotics. This, like many others from the taxa, can be ingested in food by its bacterial form or by its spores, constituting a big threat to society. Short chain carboxylic acids have been shown to act as antimicrobial against food pathogens, however their mode of action or their specificity towards different taxa has not been completely revealed.

In this study, the antimicrobial activity of short carboxylic acids such as lactate or acetate was evaluated against different strains of *C. difficile* at pH: 4.5, 5.5 and 6.5 by minimal inhibitory concentration (MIC50) assay. Additionally, we monitored how short chain carboxylic acid accumulation affected growth profiles.

Our results showed strains of *C. difficile* and *C. perfringens* were more inhibited at pH 4.5 and 5.5 compared to 6.5. Strains were more sensitive to lactate than to acetate in a pH dependent manner with *C. difficile* strains being the most sensitive. Interestingly, lactate did not follow the weak acid theory with our strains, for which acetate should have had higher antimicrobial activity, giving the possibility of a specific inhibition structure-dependent towards Clostridia.

Taken all together, lactate might be a good candidate food additive for the prevention of the clostridia contamination.

M72 - Survival of *Listeria monocytogenes* during natural and starter culture sauerkraut fermentations

Presenting Author – *Leon Maughan, Teagasc Food Research Centre, Ireland*

Author/s – *Leon Maughan, Declan Bolton, Paul Whyte*

Abstract Content

Fermented foods are becoming increasingly popular, especially due to their publicised health benefits. However, there is limited information on the fate of pathogens in sauerkraut and the implications on food safety. The objective of this study was to investigate the survival of *Listeria monocytogenes* during Irish sauerkraut fermentation and storage.

Raw cabbage (*Brassica oleracea*) was inoculated with a cocktail of *L. monocytogenes* strains (5 log10 cfu/g) and fermented using a commercial starter culture and using the indigenous microorganisms (both with 2% salt at 25°C). Samples were extracted periodically over a 2 week period and L. monocytogenes counts obtained using Brilliance Listeria Agar and an overlay technique to enumerate stressed cells. Fraser enrichment broth was utilised when the cells were below the level of detection. The fermentation was characterised by enumerating lactic acid bacteria (LAB) with De Man, Rogosa and Sharpe agar and also by monitoring pH.

When the starter culture was used, *L. monocytogenes* decreased from 5 log10 cfu/g to 3.4 log10 cfu/g during the first 2 days, followed by a further decline and was not detectable after 5 days, even using enrichment. During the same period the LAB count increased from 4.5 log10 cfu/g (day 0) to 8.4 log10 cfu/g (day 4) and the pH decreased from 6.1 to 4.7. A similar pattern was observed when the fermentation was driven by the natural microflora on the cabbage.

It was concluded that *L. monocytogenes* does not survive the sauerkraut fermentation process.

M73 - Rapid metagenomic long-reads sequencing based on PCR applied to milk samples with low microbial biomass

Presenting Author – *Vinícius Duarte, Norwegian University Of Life Sciences, Norway*

Author/s – *Davide Porcellato*

Abstract Content

Bovine mastitis has been the main infectious disease leading to a loss in milk production and lower animal welfare worldwide. Notwithstanding this fact, there are limited protocols available to study the interaction between the udder microbiome and mastitis-causing pathogens in milk samples with a low microbial load. In this study, milk samples from seven cows with a low SCC (< 100,000/mL) and a high SCC (> 200,000/mL) were collected for method implementation. After the microbial pellet was obtained, host DNA depletion and metagenomic DNA extraction/enrichment were conducted with MolYsis complete5 kit. For the PCR reaction, LongAmp Taq 2X Master Mix and the SQK-RPB004 rapid barcoding kit were used according to the protocol developed by Alcolea-Medina et al., 2022. The amplified mgDNA was sequenced on FloMIN 106 R9 version flowcell mk1 with MinKNOW version 18.12.4. Metagenome-assembled genomes were obtained by using the combination of Flye and Racon. The taxonomical assignment was carried out with Kraken2. Taken together, our results indicate that this protocol is suitable for milk samples with high SCC, but still needs some improvements for samples with low SCC. The genomes of major pathogens such as *Staphylococcus haemolyticus*, *Streptococcus uberis*, and *Enterococcus faecium* were successfully reassembled and contigs belonging to less abundant taxa such as Aerococcus were also noticed and can serve as a basis to study the interplay between the microorganisms inhabiting the udder of dairy cattle. This is a new and adapted protocol for milk samples and will serve to investigate the udder microbiome of dairy cows in health/disease.

M74 - Potential prebiotic activity of coffee silverskin

Presenting Author – *Marlene Machado, Faculty of Pharmacy of the University of Porto, Portugal*

Author/s – *Josman Palmeira, Hélder Puga, Maria Oliveira, Helena Ferreira, Rita Alves*

Abstract Content

Demand for prebiotic ingredients, those that promote gut health by stimulating beneficial bacteria while inhibiting pathogenic, has increased in recent years. Recent studies show that by-products of the agro-food industry can have prebiotic properties due to their richness in soluble and insoluble dietary fiber (1). Thus, the aim of this work was to evaluate the potential prebiotic activity of silverskin, a by-product of the coffee industry. For this, an aqueous silverskin extract was produced using the multi-frequency multimode modulated ultrasonic vibration technique (2). The extract was freeze-dried and then incorporated into glucose-free MRS broth at different concentrations (0.5 to 4%) along with 10% *Lactobacillus paracasei* inoculum. Glucose, glucose-free MRS broth, and inulin were used as positive control, negative control, and reference prebiotic, respectively. After 48 h of incubation, counting was performed on MRS agar plates after successive decimal dilutions, and pH was measured (in triplicate). The freeze-dried silverskin extract promotes *L. paracasei* growth in a dose-dependent manner (Δ CFU/ml = 2.30 to 3.00 x 10 8). The beneficial bacterium is able to metabolize the freeze-dried silverskin extract and produce acidic metabolites that lower the pH of the medium (in the case of the 4% concentration, Δ pH = 0.6). In conclusion, silverskin contains compounds that stimulate the growth of the beneficial bacterium *L. paracasei* and may be of interest for the development of functional foods with a prebiotic effect, but further studies are needed.

M75 - Clostridia and other microbiota in raw milk and milk products: importance for product quality and food safety

Presenting Author – *Misti Finton, Norwegian University Of Life Sciences, Norway*

Author/s – *Misti Finton, Davide Porcellato, Siv Borghild Skeie, Marina Elisabeth Aspholm*

Abstract Content

Spore-forming bacteria are the most complex group to eliminate from the dairy production line due to their ability to withstand heat treatment used in dairy processing. These ubiquitous microorganisms have ample opportunity for multiple points of entry into the milk chain, creating issues for food quality and safety. Certain spore-formers, namely Bacilli and Clostridia, are more problematic to the dairy industry due to their possible pathogenicity, growth, and production of metabolites and spoilage enzymes. This research investigated the presence and persistence of spore-formers along the dairy processing line, starting when the raw milk arrives at the dairy plant through the cheesemaking stages until ripening. Samples were collected by three Norwegian dairy plants over a two-year period and examined in a culture-independent and dependent manner, followed by high-throughput 16S rRNA amplicon sequencing and metagenomic analysis. A total of 953 isolates were identified at the species level. The majority of the spore-forming isolates belonged to two genera, Bacillus and Clostridium, with the latter dominant in enriched gas-positive tubes, raw milk, and bactofugate samples. Results showed a great seasonal variation of Clostridia detected in milk and during processing, however, three species were found over the entire sampling period (*Cl. tyrobutyricum*, *Cl. sporogenes* and *Cl. beijerinckii*). Additionally, the bactofugation step was shown to impact the level and quantity of Clostridia, and to a lesser extent, Bacillus. In conclusion, our findings highlight the significance of spore-formers on dairy quality and may facilitate future strategies that reduce loss throughout the dairy production line caused by spore-formers.

M77 - Development of targeted Nanopore sequencing for the detection of *Listeria monocytogenes* in microgreens and sprouts

Presenting Author – *Hongsheng Huang, Canadian Food Inspection Agency, Canada*

Author/s – *Sohail Naushad, Marc-Olivier Duceppe, Amit Mathews, Mingsong Kang, Dele Ogunremi, Isaac Firth, Hongsheng Huang*

Abstract Content

Background: Listeria monocytogenes is a major causes of foodborne illnesses in humans. The detection of this organism has been mainly based on traditional bacterial isolation, and molecular- and immunological-based assays. Whole genome sequencing technologies have evolved rapidly and have improved the detection and characterization of foodborne bacterial pathogens including *L. monocytogenes*.

Objective: This study aimed to develop a targeted or adaptive metagenomics sequencing approach using Oxford Nanopore sequencing platform for rapidly detecting *L. monocytogenes* in food.

Methods: This study evaluated an adaptive sampling algorithm which employs a rapid mapping of raw electrical signal to specified reference genome and eject the non-targeted reads during the sequencing process. Both the stand-alone computer and portable Mk1C device, on MinION and Flongle flow cells have been used to determine suitability for various laboratory and in field settings. So far, DNA samples tested included those extracted from a) two pure isolates as positive control, and b) from microgreens and sprouts experimentally spiked with *L. monocytogenes* (n=9) and non-spiked (n=4) and incubated in a selective enrichment broth overnight.

Results: Targeted sequencing showed 100% specificity in all cases. Further evaluation to determine the limit of detection and establish specificity on large number of samples is in progress. This study showed that adaptive sequencing process is specific, rapid, low cost, and portable that can potentially be applied to different food matrices and in various laboratory settings.

M78 - Bacterial composition of goat milk and its relations with somatic cell count during lactation

Presenting Author – *Francesca Desidera, Norwegian University Of Life Sciences, Norway*

Author/s – *Siv Skeie, Davide Porcellato*

Abstract Content

An increase in somatic cell count (SCC) usually indicates poor udder health and possible mastitis, although, in goats, SCC is not always related to an infection. The Norwegian dairy goat industry has observed an increase in SCC during the summer mountain pasture. Other causes than bacterial infection might be at the origin of this problem and, to get a better overview, this study aimed to investigate the milk composition, the goat milk microbiota, and changes in bacterial counts in one herd over on lactation period and to correlate these observations to the level of somatic cells. Forty goats were sampled monthly during lactation from March to October, including the pasture period (June to August). Amplicon sequencing of the 16S gene was used to study microbiota changes. The presence of mastitis-related species in the microbiota data was then correlated to the SCC. A positive correlation was detected between the bacterial count and SCC, and each sequence variant was correlated to a specific genus of bacteria. Among the bacteria present in the samples, some of the sequence variant (SV) showed a correlation between each other. Interestingly, when the correlation was studied within each month, some months showed a positive correlation between somatic cell count and bacteria count (or some SV), while others did not show any correlation. This study highlights some of the relationships existing between bacterial count and somatic cell count in goat milk and their influence on the milk microbiota composition.

M80 - Novel genes essential for biofilm formation of *Vibrio vulnificus* are identified by large-scale transcriptomic analysis

Presenting Author – *Sang Ho Choi, Seoul National University, Korea, Republic of*

Author/s – *Hojun Lee, Hanhyeok Im, Seung-Ho Hwang, Duhyun Ko*

Abstract Content

Vibrio vulnificus, a foodborne pathogen, forms biofilms to survive under host immune defenses and environmental stresses. However, conventional differential expression (DE) analysis of the genes in biofilm and planktonic cells under a single condition has limitations to identify the genes essential for biofilm formation. In this study, a machine learning algorithm named independent component analysis (ICA) was adopted to comprehensively identify the biofilm genes of *V. vulnificus.* ICA analyzed the large-scale transcriptome data of *V. vulnificus* cells under various biofilm and planktonic conditions and then identified 72 sets of independently co-regulated genes, iModulons. Among the iModulons specifically activated in biofilm cells, BrpT-iModulon mainly consisted of known genes of the regulon of BrpT, a transcriptional regulator controlling biofilm formation of *V. vulnificus.* Interestingly, the BrpT-iModulon additionally contained two novel genes, cabH and brpN. cabH and brpN were shared in other *Vibrio* species and not yet identified by DE analyses. Genetic and biochemical analyses revealed that BrpT positively regulates the expression of cabH and brpN by binding directly and specifically to their upstream regions. The deletion of cabH and brpN impaired the robust biofilm and rugose colony formation. cabH, predicted to encode a protein carrying calcium-binding repeats, was essential for attachment of *V. vulnificus* to the surface. brpN, predicted to encode a protein containing an acyltransferase-3 domain, was crucial for exopolysaccharide production. In conclusion, ICA identified two novel genes, cabH and brpN, which are regulated by BrpT and essential for the development of biofilms and rugose colonies of *V. vulnificus.*

M81 - Pulsed electric fields – influence on goat's cheese microbiome

Presenting Author – *Paulo Fernandes, CISAS, Escola Superior De Tecnologia E Gestão, Instituto Politécnico De Viana Do Castelo, Portugal*

Author/s – *Carla Barbosa, Alberta Araújo, Alexandre Romão, M. Rui Alves*

Abstract Content

The efficacy of pulsed electric fields (PEF) on microbial inactivation depends on several factors such as the composition and physical-chemical characteristics of the medium (e.g. conductivity), and also the type and size of the cells to be inactivated. As goat's milk has a lower thermal stability than cow's milk, we used an alternative processing to reduce the microbial load by about 5 log. Cheeses were made with heat-treated goat's milk at 75ºC for 3.4 s and also with milk treated with PEF (10 Kv/cm, 50 µs pulses width, 3 Hz) followed by heat treatment at 63ºC for 6.0 s. The cheeses produced with the milk obtained with the two different treatment conditions were ripened for 25 days and samples were collected after 5, 15 and 25 days. After DNA extraction and purification, 16S amplicon high throughput sequencing was performed on MiSeq Illumina sequencing platform. Comparing the alpha-diversity indices of the bacterial populations of both types of cheese, namely Simpson, ACE, Chao1, and Shannon, indicated identical species richness and abundance, although with a tendency for the cheese produced with milk treated with PEF to show a lower diversity of species with a more uneven distribution of relative abundance. However, the composition of the microbial communities of the two types of cheese, namely the presence/absence of each species and their relative abundance, are effectively different as revealed by a Weighted UniFrac analysis (p=0.026) and by an Analysis of Similarities (ANOSIM, p=0.040), with impact in the development of the starter culture.

M82 - Triacylglycerols hydrolysis and hydroxy- and epoxy-fatty acids release during fermentation of walnuts as model system: inter- an

Presenting Author – *Pasquale Filannino, University of Bari Aldo Moro, Italy*

Author/s – *Giuseppina Fiorino, Ali Zein Alabiden Tlais, Ilario Losito, Pasquale Filannino, Marco Gobbetti, Raffaella Di Cagno*

Abstract Content

Background: Although lactic acid bacteria (LAB) encompass a wide and heterogenous group of microbial genera, only few lactobacilli were previously associated to a specific lipolytic activity or to the conversion of fatty acids into hydroxy- or oxo-derivatives. The investigation of fatty acids metabolism by LAB was mainly limited to the fermentation of animal matrices (dairy and meat-based products) or to in vitro studies by using pure fatty acids as substrate.

Objectives: This study aimed to fill the current knowledge gaps on lipid metabolism during lactic fermentation of plant matrices, due to the involvement of a plethora of microbial players and enzymes.

Methods: Liquid Chromatography-High-Resolution Mass Spectrometry (LC-HRMS) was employed for the detection and characterization of lipids, including native and oxidized fatty acids and mono-/di-acylglycerols, in lipid extracts of Persian walnut subjected to fermentation by a diversified pool of LAB and in related control samples.

Results: Most of the fermented walnut samples exhibited an increase of free fatty acids (linoleic, α-linolenic, palmitic, and oleic acids). The increase of diacylglycerols and, especially, monoacylglycerols levels in fermented walnuts confirmed that strain-specific bacterial lipolytic activities hydrolyzed triacylglycerols during walnuts fermentation. Twelve hydroxylated or epoxidized derivatives arising from oleic, linoleic, and linolenic fatty acids, divided into five groups of isomeric compounds, were also identified. A prominent role of *Weissella cibaria*, *Enterococcus faecalis*, and *Leuconostoc mesenteroides* emerged for the lipolytic activities and the capability to release hydroxy- and epoxy-fatty acids, in addition to the well-known role of some lactobacilli.

M83 - Selection of exopolysaccharides-producing lactic acid bacteria strains isolated from plant based fermented foods

Presenting Author – *Angel Angelov, University Of Food Technologies, Bulgaria*

Author/s – *Angel Angelov, Mariana Petkova, Aneliya Georgieva, Manol Ognqnov, Emanoila Todorova, Velitchka Gotcheva*

Abstract Content

The microflora of plant-based fermented foods is dominated by lactic acid bacteria (LAB) which produce various substances with health-promoting benefits and diverse applications in the food industry. Production of exopolysaccharides (EPS) by LAB attracts particular interest due to their rheological properties.

The current study aimed to isolate LAB from plant-based fermented foods, to evaluate their potential for EPS synthesis, and to characterize the obtained EPS.

LAB strains were isolated using the reference method ISO 15214:1998. 16S rRNA gene sequencing was followed by sequence similarity search in the GenBank data library using the BLAST program. Nucleotide substitution rates were calculated and phylogenetic tree was constructed by the neighbour-joining method using MEGA 11 software. After isolation, EPS were characterized by monosaccharide composition analysis, molecular weight distribution and Fourier transform infrared spectroscopy.

We isolated 43 strains of LAB from five plant-based fermented foods – "Boza", sauerkraut, and „Dèguè". The isolates were attributed to *Lactiplantibacillus plantarum*, *Pediococcus pentosaceus*, *Pediococcus acidilactici*, *Limosilactobacillus ferment* and *Enterococcus faecium*. Screening for EPS production was performed, and six strains were positive. Since culture media composition and especially the carbon source are a critical factor influencing the yield of bacterial EPS, the impact of glucose and fructose on EPS synthesis by the selected strains was explored. Further, the monosaccharide composition, molecular weight and IR spectra of the obtained EPS were determined.

M84 - Should we need to change and introduce new referral values for aflatoxins in dairy products in "official gazette" of Bosnia an Herzegovina

Presenting Author – *Amir Ibrahimagić, Institute For Health And Food Safety, Bosnia and Herzegovina*

Abstract Content

Background: The European Commission established a maximum limit of 0.05 µg/kg of AFM1 in milk and limit for AFM1 of 0,025 µg/kg in infant formula, while other regulatory authorities and the Codex Alimentarius Commission have set a higher limit of 0,5 µg/kg AFM1 in raw milk. Some countries on National level have limit values such as Austria and France (cheese – 0,25; butter – 0,02), Egypt (dairy products – 0 µg/kg).

Objectives: Bosnia and Herzegovina have only limit regulation for AFM1 in milk -0,05 µg/kg (excluding other dairy products, such as cheese, jogurt, butter), respectively.

Methods: During 2021-2022, a total of 167 samples (105 milk and 62 feed) were analysed on aphlatoxins AFM1 and AFB1 using enzyme-linked immunosorbent assay (ELISA) specific kits (Eurofins Technologies, Budapest, Hungary). Quality control material with the standard concentration (FAPAS QC) were used.

Results: Among 105 milk samples, AFM1 was present in 104 samples (mean 0.039; range 0.000 – 0.270 µg/kg). Twenty-eight milk samples (out of 104; 26.9%) had higher concentration (mean 0.097; range 0.049-0.270 µg/kg) than maximum level set by National legislation (0.05 µg/kg). In the same time, aflatoxin B1 was present in 61 samples (mean 3.241; range 0.000-13.300 µg/kg). Fourteen samples were contaminated with AFB1 over the EU regulatory limits (mean 7.750; range 5.100-13.300; reference value <5 µg/kg), respectively.

M85 - The bacterium *Latilactobacillus curvatus* FAM25164 possesses a decarboxylase that produces tryptamine

Presenting Author – *Stefan Irmler, Agroscope, Switzerland*

Author/s – *Stefan Irmler, Tharmatha Bavan, Eliane Binz, Reto Portmann*

Abstract Content

Background: Biogenic amines are undesired in cheese. They are formed during cheese ripening by the activity of bacterial amino acid decarboxylases. Tryptamine is one of the biogenic amines that occurs less than others, and there is little data on its formation. We identified and isolated a tryptamine-producing *Latilactobacillus curvatus* strain, named FAM25164, from cheese.

Objectives: Tryptamine is probably produced by the enzymatic decarboxylation of tryptophan. Since bacterial tryptophan decarboxylation has rarely been reported, we wanted to know whether FAM25164 actually possesses a tryptophan decarboxylase.

Methods: The genome of FAM25164 was sequenced and assembled. The contigs were then searched for genes encoding amino acid decarboxylases. Genes of interest were cloned in frame with a poly-histidine tag and heterologously expressed in *Escherichia coli*. The medium incubated with recombinant *E. coli* was analyzed for the presence of tryptamine using HPLC. Furthermore, the recombinant proteins were purified and assayed for tryptophan decarboxylase activity.

Results: The study reports the identification and characterization of a *L. curvatus* gene encoding a novel decarboxylase involved in tryptamine biosynthesis. Neither sequence comparisons with known tryptophan decarboxylases nor database searches (e.g. InterProScan) could predict a unique function for it. When *E. coli* was transformed with the gene, tryptamine was formed. The purified protein decarboxylated tryptophan to tryptamine *in vitro*. Genomic comparisons showed that the tryptophan decarboxylase gene is rare in *L. curvatus*. Furthermore, it is part of a cluster that includes three additional genes encoding a DUF544 domain-containing protein, a TetR/AcrR family transcriptional regulator, and a tryptophan--tRNA ligase.

M86 - Antimicrobial-resistant pathogens in fruits and vegetables from retail and home-gardens in southwestern Nigeria

Presenting Author – *Afolake Olanbiwoninu, Ajayi Crowther University, Nigeria*

Author/s – *Afolake Olanbiwoninu, Theresa Awotundun, John Olayiwola, Yinka Somorin*

Abstract Content

Fruits and vegetables are sources of dietary nutrients, yet they have been identified as vehicles for the transmission of pathogenic antimicrobial-resistant (AMR) microorganisms. This is of food safety concern, thus requiring continuous surveillance. This study aimed to profile antimicrobial resistance bacteria present in selected fruits and vegetables retailed in markets and from home gardens (HG) in southwestern Nigeria. Watermelon, cucumber, tomato, and garden-egg samples were collected and analyzed using standard microbiological procedures and the susceptibility of the isolates to eight commonly administered antibiotics was determined. Multi-drug resistant isolates were screened for the presence of AMR genes by PCR. A total of 53 bacteria were isolated and identified, belonging to the genera *Bacillus, Corynebacterium, Listeria, Aeromonas, Enterobacter, Erwinia, Salmonella, Serratia, Shigella* and *Vibrio.* Thirty-six (67.93 %) isolates demonstrated phenotypic resistance to five out of the eight antibiotics tested being the most prevalent pattern observed. BlaTEM and blaCTX-M were detected in *Salmonella enterica* isolated from retailed tomato; blaTEM, blaSHV, blaCTX-M and erm(B) were detected in *Listeria monocytogenes* from retailed watermelon; blaSHV and blaCTX-M were detected in *Bacillus cereus* from retailed tomato; while blaTEM, blaSHV, blaCTX-M and erm(F) were detected in *Staphylococcus* aureus isolated in garden-egg from home garden. The presence of multidrug-resistant pathogens in fruits and vegetables, often consumed raw, could pose a considerable food safety and public health risk, hence requiring continuous surveillance and appropriate interventions to mitigate them.

M87 - Whole genome-based characterization of *Enterococcus faecium* recovered from retailed meats in Akungba Akoko, Nigeria

Presenting Author – *Ayodeji Charles Osunla, University of Saskatchewan, Canada*

Author/s – *Ayodeji Charles OSUNLA, Olayemi Stephen Bakare, Aderonke Mary Fayanju, Dorcas Oladayo Fatoba, Daniel Gyamfi Amoako, John P Giesy, A. Smith*

Abstract Content

Background: Enterococci are commensal bacteria that live in both human and animal gastrointestinal systems and are developing as opportunistic pathogens, although they have received little attention as food concerns.

Objectives: To better understand the role of enterococci in the mobility of antibiotic resistance genes, virulence genes, and mobile genetic elements from farm to table.

Method: Ten Enterococcus faecalis were isolated from raw retailed chicken, beef, and turkey meats purchased at Ibaka market and were sequenced on the Illumina Nextseq platform.

Results: The multilocus sequence typing (MLST) analysis showed two sequence types (STs), ST16 and ST477. Our findings showed that the recovered isolates harboured different multiple antibiotics resistance genes, including those conferring resistance to inter alia Lincosamide-Macrolide-Streptogramin a,b (erm (B), lsa (A)), Aminoglycoside (aac(6'), aph(2''), ant(6)-Ia), Chloramphenicol (cat), and Tetracycline (tet(L), tet(M)). The ARGs were associated with some MGEs such as insertion sequences (lS6 and IS256), Plasmids (repUS43 [CDS12738 (DOp1)], and rep9c [repA(pTW9)]) and transposon (Tn6000). Also, detected in the isolates were thirty-three virulence genes, including those encoding Biofilm formations (efaAfs, gelE, fsrB), Collagen adhesin precursor (ebpC, ebpA, ace), and Sex pheromone cAM373 precursor (camE). The whole-genome sequencing of the isolates revealed the presence of *Enterococcus faecalis* with diverse ARGs, virulence genes, and MGEs in the retailed meats (Beef, chicken, and turkey). Their ability to survive in the food chain could pose a risk to human health as they could serve as vehicles for the mobility of resistance and virulence genes from the farm to human-proximal ecologies.

M88 - The microbial ecosystem of the Greek PDO cheese Anevato determined by metagenomic analysis

Presenting Author – *Konstantinos Papadimitriou, Agricultural University of Athens, Greece*

Author/s – *Maria Govari, Dimitra Tsoliakou, Maria Gkerekou, Panagiotis Skandamis, John Kapolos, Marina Papadelli*

Abstract Content

Background: Anevato, a customary soft cheese produced in the Grevena area in Western Macedonia in Greece has been classified as a PDO product. This cheese is made using sheep, goat, or a blend of both types of milk.

Objectives: To assess the microbial populations of the Anevato cheese.

Methods: Twelve samples were obtained from three different Anevato cheese producers, four samples from each producer. Culture-based microbiological analysis as well as shotgun metagenomics were used for the identification of the cheese microbiome.

Results: The abundant microbial populations were Lactic Acid Bacteria (LAB) and yeasts. In one of the samples coliforms, Enterobacteriaceae, *Staphylococcus* spp., *Escherichia col*i and *Pseudomonas* spp. were detected. The main identified microbial species were LAB, such as *Lactococcus lactis*, *Lactococcus raffinolactis*, *Streptococcus thermophilus Lactobacillus helveticus*, *Streptococcus parauberis*, *Lactiplantibacillus plantarum,* etc. At lower population levels, yeast species were also identified such as *Kluyveromyces lactis* and *Saccharomyces cerevisiae*. Furthermore, metagenome-assembled genomes (MAGs) were identified.

Conclusions: Shotgun metagenomics proved to be an important toolbox for the identification of the microbial ecosystem of Anevato cheese. Microbial analysis of Anevato cheese resulted in significant information for the production of this cheese. Identified LAB species could be used as starter cultures, improve the quality characteristics and lead to an extension of Anevato cheese shelf life.

M89 - A Novel A novel peptide nucleic acid against DNA gyrase of *Sphingomonas paucimobilis* extends fresh produce shelf life

Presenting Author – *Su Jin Yum, Chungnam National University, Korea, Republic of*

Author/s – *Hee-Gon Jeong, SeonYeong Yu*

Abstract Content

Sphingomonas paucimobilis found in the natural environment is a putrefying bacterium as well as an opportunistic pathogen. We used gene-specific oligonucleotides such as peptide nucleic acid (PNA) oligomers to control S. paucimabilis without antibiotic problems. Based on the genome sequence of *S. paucimabilis* AP023323.1, a set of bacterial penetration peptides ((KFF)3K) were synthesized to target two essential genes (gyrA and rpoA) and their antibacterial properties were evaluated. Two peptide-conjugated PNA (gyrA and rpoA target P-PNAs) have inhibitory effects on the growth of *S. paucimabilis* in a dose-dependent manner (> 0.2 mM and 2 mM, respectively). The antimicrobial activity of gyrA target P-PNA was higher than that of rpoA target P-PNA. Two peptide-conjugated PNA treatments of infected lettuce samples have been shown to significantly inhibit the decay of fresh produce through the reduction of bacterial loads. These results suggest that P-PNAs can efficiently inhibit bacterial growth and have the potential as a novel treatment for multidrug-resistant *S. paucimobilis* in the environment.

M90 - Isolation and immunosynbiotic characterization of lactic acid bacteria from swine milk to generate a *Lactobacillus* library

Presenting Author – *Binghui Zhou, Tohoku university, Japan*

Author/s – *Ryusuke Ohgi, Taiga Sakuma, Sudeb Saha, Mitsuki Sakurai, Yuka Nakano, Fu Namai, AKM Humayun Kober, Wakako Ikeda-Ohtsubo, Keita Nishiyama*

Abstract Content

Background: The emergence and spread of antibiotic resistance threats forced us to explore alternative strategies for improving the resistance to pathogens in livestock production. Probiotic lactic acid bacteria (LAB) can be an interesting alternative for this aim.

Objective: In this work, LAB strains from porcine colostrum and milk were isolated, identified and characterized in terms of their abilities to modulate immunity in porcine intestinal epithelial (PIE) cells.

Methods and results: Isolates were identified by 16S rRNA. Among isolated Lactobacilli, *Lactiplantibacillus plantarum* species were dominant. PIE cells (10^4 cells/ml) were stimulated with the strains (10^8 cells/ml) and then challenged with enterotoxigenic *Escherichia coli* (10^7 cells/ml) for 48 h to evaluate their immunomodulatory potential. Pgrp, Nod and pBD expressions were evaluated by qRT-PCR. It was found that the three genes were increased by the *L. plantarum* strains compared to the control. The most pronounced effect was observed for $4M_4326$ and $4M_4417$ strains. Furthermore, PIE cells were stimulated with *L. plantarum* strains and then challenged with poly(I:C) to trigger TLR3-mediated inflammation. The determination of IFN-α and IFN-β expression by qRT-PCR demonstrated that *L. planatrum* $4M_4326$ and $4M_4331$ had a remarkable ability to increase IFN-α, hile $4M_4326$, $4M_4338$, $4M_4347$, and $4M_4417$ strains significantly increased IFN-β in poly(I:C) challenged PIE cells. Our results suggest that the supplementation *L. planatrum* $4M_4326$ or $4M_4417$ could reduce the severity of intestinal infections and improve immune health status in piglets, although in vivo studies are necessary to find out an efficient way for immunosynbiotics development.

M91 - Assessment of effect of different packaging materials on sensory evaluation and microbial quality of locust beans during storage

Presenting Author – *Ayodele Ogunlade, The Federal Polytechnic Ado, Nigeria*

Author/s – *Oluwatoyin Aladejana, Omolara Afolabi, Dayo Fakomiti, Ugochi Azunnaya*

Abstract Content

Food packaging is just one among many ways of food preservation and packaging purposely to protect the products from environmental contamination throughout period of shelf life. Therefore, the packaging becomes an integral part of the food product. Locust beans (Dawadawa) is a popular condiment used as taste and flavor enhancer in soup and dishes in Africa. It is traditionally produced from locust beans seed (*Parkia biglobosa*) and preserved using different packaging materials before use to prolong its shelf life. The objective of this study is to assess the effect of different packaging materials on the Microbial quality and Sensory attributes of Locust beans during storage. The locust beans were separately wrapped with plastic container, nylon and dry banana leaves, allowed to ferment for 5days and thereafter its sensory and Microbial quality were assessed using 9-point Hedonic scale and standard methods respectively. Locust beans wrapped with plastic container was mostly preferred based on sensory scores. Samples wrapped with dry banana leaves had highest total bacterial count (8.20x103cfu/g) and total colony count (3.40x103 cfu/g) while Samples wrapped with plastic container had the lowest total bacterial count (4.83x103 cfu/g) and total colony count (2.60 x103 cfu/g). Bacterial species that persistently populated the samples include *Salmonella* spp, *Staphylococcus aureus*, *Streptococcus Lactis*, *Pseudomonas aeruginosa*, *Lactobacillus plantarum*, *Bacillus cereus*, *Bacillus subtilis*, *Leuconostoc* sp, *Escherichia coli* and *Micrococcus* sp. Findings from this study showed that locust beans wrapped with plastic containers should be encouraged in order to reduce contamination by pathogenic organisms.

M92 - Absolute quantification of viable bacteria coupled with 16S rRNA next-generation sequencing

Presenting Author – *Jekaterina Kazantseva, Center of Food and Fermentation Technologies, Estonia*

Author/s – *Aili Kallastu, Esther Malv, Anne Meikas*

Abstract Content

The discrimination between life and death is crucial when it is associated with the microbes around us. Coupled with the quantitative data, this knowledge is what we need to know about the safety, nutritional value of food or predict an ecological outcome. Cell plating is an affordable and simple method that allows getting an overview of the total amount of alive microorganisms in the product. However, not all bacteria are cultivated, taxonomic characterisation is often missing, and it might take up to a week to get the results. Thus, currently used microbiology methodologies don't give the full answer to how many definite alive bacteria species are present in a sample.

In our work, we combined the 16S rRNA next-generation sequencing (NGS) approach with spike-in control and viability qPCR to get absolute quantification of alive bacteria in a sample. For method development, 20 isolated bacterial species were chosen, and their viability was evaluated using flow cytometry, microscopy, and qPCR with specific primers. These data were compared to the elaborated quantitative NGS methodology. Furthermore, actual food samples were taken and analysed using a created pipeline.

As a result, we created and validated a methodology that defines a taxonomic composition of alive bacteria and estimates their number.

M93 - Importance of pre-milking udder hygiene to reduce transfer of clostridial spores from teat skin to raw milk

Presenting Author – *Johanna Burtscher, University of Natural Resources and Life Sciences, Vienna, Austria*

Author/s – *Johanna Burtscher, Tamara Rudavsky, Ulrike Zitz, Viktoria Neubauer, Konrad Domig*

Abstract Content

Butyric acid producing clostridia (BAPC) are the cause of the so-called late blowing defect, a serious quality problem in semi-hard and hard cheeses. Their endospores are ubiquitous and contaminate raw milk during milking. Teat cleaning prior to milking is a key factor in preventing bacterial contamination of the milk. However, different cleaning methods are used and little information is available on the effectiveness of routine teat cleaning in reducing clostridial endospores. The main objectives of this study were to assess the extent of udder contamination with BAPC spores and to investigate the effect of routine teat cleaning on BAPC spore counts in milk. Eight dairy farms were visited during five sampling events. Clostridial spore counts were quantified from teat skin before and after routine teat cleaning, in pooled quarter milk samples from individual cows, and in bulk tank milk samples using a most probable number method. In addition, farm management data were collected periodically through a survey and average cow cleanliness was assessed by a veterinarian. On average, teat cleaning resulted in a 0.6 log reduction in BAPC spores on teat skin and a strong positive correlation was found between BAPC spore concentrations on teat skin after cleaning and in pooled quarter milk samples. Seasonal variations and the potential influence of differences in farm management were also noted. Interestingly, average cow cleanliness correlated strongly with BAPC spore levels, suggesting the potential for a quick and rough estimation method of clostridial contamination that could be implemented by farmers.

M94 - Horizontal transfer of the pCER270 megaplasmid within the *B. cereus* group

Presenting Author – *Markus Kranzler, University of Veterinary Medicine Vienna, Austria*

Author/s – *Monika Ehling-Schulz*

Abstract Content

The emetic lineage of *B. cereus*, which is the causative agent of sever foodborne intoxications, harbours the pXO1-like mega-plasmid pCER270 that encodes the structural genes ces for the non-ribosomal biosynthesis of the emetic toxin cereulide. Next to the ces operon, 222 coding sequences are predicted on pCER270, including genes for sporulation and germination as well as transcriptional regulators. Since horizontal gene transfer by plasmids is a driving force in bacterial adaptation, we aim to elucidate the role of pCER270 transfer in pathogen emergence and adaptation to the environment.

Thus, we tagged pCER270 in the emetic reference strain F4810/72 with an antibiotic-resistance cassette using CRISPR/Cas9 technology and transferred it to other *B. cereus* group members by conjugation. Since pCER270 is not conjugative by itself, we used a pXO16-derivative to mobilize its transfer. The conjugation was carried out in a liquid medium to measure transfer kinetics. Tested recipients received both plasmids with saturating ratio of 10-3-10-2 of pCER270 to pXO16 transfer frequency. Interestingly, very early after donor-recipient mixing, the frequency of pCER270 transfer was comparable or even up to ten-fold higher than that of pXO16.

Transfer of pCER270 was confirmed by PCR targeting the origin of replication (repX) and ces genes. Phenotypic analysis of transconjugants and their parentals indicated specific metabolic traits linked to the mega-plasmid, and co-cultivation assay in laboratory media and an insect model host revealed a significant impact of the mega-plasmid on bacterial fitness.

M95 - Investigation of lettuce susceptibility to *B. cinerea* after treatment with LED light

Presenting Author – *Lina Dėnė, Lithuanian Research Centre for Agriculture and Forestry, Institute of Horticulture, Lithuania*

Author/s – *Alma Valiuškaitė, Neringa Rasiukevičiūtė, Simona Chrapačienė, Giedrė Samuolienė, Akvilė Viršilė, Viktorija Vaštakaitė-Kairienė, Kristina Laužikė, Rūta Sutulienė, Aušra Brazaitytė*

Abstract Content

Background: Lettuce (*Lactuca sativa* L.) growing plays a significant part in controlled environment agriculture systems (CEA). This lettuce contains valuable nutritional elements and is consumed worldwide. However, due to the morphology of lettuce plants, they are susceptible to various pathogens, like *B. cinerea*, and have a short shelf-life. In CEA, supplemental light plays an important role, as its necessary for plant development and accumulation of nutritional elements. Moreover, such technologies could provide a safe, non-toxic possibility to increase the shelf life of lettuce and lower the production loss during storage.

Objectives: This research aimed to evaluate lettuce susceptibility to *B. cinerea* after preharvest treatment with different wavelength LED light at 1) daytime and 2) night-time.

Methods: Plants were grown under daylight and standard high-pressure sodium lamps (HPS) (SON-T Agro) with supplementary LED lightning 50 µmol m-2 s-1 (4h photoperiod, different wavelenghts) in day-time and night-time. After the growth period, detached lettuce leaves were examined for the susceptibility of B. cinerea after performing inoculation with mycelium discs. Disease percentage was calculated: disease percentage (%) = (wound width/leaf width) × 100.

Results: The disease percentage was reduced in several LED light treatments, however, no significant differences were observed. Results of the study indicate the promising effect of 400 and 455 nm LEDs light supplementation HPS during night-time to lower the susceptibility to postharvest *B. cinerea*, as these treatments reduced disease percentage compared to non-treated lettuce.

M96 - Development of specific primer-probe sets and reference materials using nineteen probiotics

Presenting Author – *Changwoo Park, Korea Research Institute Of Standards And Science, Korea, Republic of*

Author/s – *Seil Kim, Jinyoung Park*

Abstract Content

Background: The use of 16S rRNA gene-based microbial community analysis has been widely used for the investigation of beneficial and harmful microorganisms in different fields and environments using NGS (next-generation sequencing) technology. However, the results of microbiome analysis can be influenced by various factors, such as sample handling, storage conditions, PCR, library preparation, sequencing, and bioinformatics tools.

Objectives: Development of reference materials for probiotics using ddPCR quantification with primer-probe sets for specific strains.

Methods: Nineteen probiotics were obtained from the Korean Collection for Type Cultures (KCTC) and their gDNA was extracted and quantified using ddPCR. Then the extracted gDNA was mixed into mock communities based on various ratios of copy numbers. The mock communities were sequenced by NGS platforms. Biases that may occur in the microbiome analysis were analyzed through quantification as copy number and sequence reads through ddPCR and NGS platforms in the following steps: quantification of gDNA, quantification of specific gDNA within a mock community, and metagenome sequencing.

Results: The specific primer-probe sets were designed based on the 16S rRNA gene sequence alignment of nineteen probiotics. Our results showed that specific primer-probe sets were able to specifically quantify target gDNA within a mock community using quantitative polymerase chain reaction (qPCR), and bias from a microbial community based-on NGS can be investigated. In conclusion, a mock community with ddPCR quantification can be used as an external standard in probiotic microbiome analysis.

M97 - Characterization of aerobic spore-forming bacteria associated with ropy bread spoilage

Presenting Author – *Nicola Pacher, University of Natural Resources and Life Sciences, Austria*

Author/s – *Nicola Pacher, Johanna Burtscher, Denisse Bender, Lars Fieseler, Konrad Domig*

Abstract Content

Sticky and stringy degradation of the bread crumb, discoloration, and an odor reminiscent of rotting fruit are typical attributes for ropy bread spoilage (RBS). Several aerobic spore-forming bacteria (ASF) have been reported to cause ropiness, but the underlying mechanisms behind RBS remain unclear as only few isolates have been fully characterized with regard to their spoilage potential.

The aim of this study was to characterize and establish the rope-forming potential (RFP) of different ASF isolated from bakery-associated environments based on a comprehensive screening protocol. Hence, 67 isolates were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and species identity was confirmed by 16S rDNA, gyrA and gyrB gene sequencing. Genetic fingerprints were determined using rep-PCR, RAPD-PCR and MALDI-TOF-MS. Phenotypic characteristics were evaluated by cultivation on six different growth media. Rope formation and its physiochemical spoilage characteristics were assessed by inoculating autoclaved bread slices with bacterial spores. High species diversity comprising *Bacillus amyloliquefaciens*, *B. velezensis*, *B. subtilis*, *B. inaquosorum*, *B. licheniformis*, *B. cereus* sensu lato, *Priestia megaterium*, *Peribacillus simplex* and *Paenibacillus* spp. was observed. Most isolates expressed high motility on swarming plates and amylase production on staiiirch-agar. The intraspecies variety of rep-PCR, RAPD-PCR and MALDI-TOF-MS fingerprints was reflected in strain-specific effects on bread slice properties, as rope formation, discoloration and odor strongly depended on the bacterial strain. Our findings allowed a better understanding of the ASF involved in RBS and their RFP.

M98 - Evaluation of the microbial composition and safety evaluation of greek PDO cheese Sfela and its artisanal variants

Presenting Author – *John Kapolos, Greece*

Author/s – *Natalia Tsouggou, Olibia Tsipidou, Aleksandra Slavko, Maria-Chrysanthi Kafentzi, Athanasia Koliadima, Marina Papadelli, Konstantinos Papadimitriou*

Abstract Content

Background: Sfela is a white cheese, made from ovine or caprine milk and is ripened in brine. It is a Greek PDO product that is produced in the Messinia and Laconia regional units, and despite its popularity, little is known about its microbial composition and safety, which is crucial for its quality control and the health of the consumer. In this study, we investigated Sfela cheese as well as two closely related variations, Sfela touloumotiri and Xerosfeli.

Objectives: We aimed to investigate the microbial composition and safety of Sfela and its two artisanal variants.

Methods: Two methods were used: 16S rDNA amplicon sequencing and shotgun metagenomic analysis. The first allowed us to identify the various populations present in the cheese at the genus level while the second at the species level. We also investigated the presence of metagenome-assembled genomes (MAGs) in our sequencing data.

Results: Lactic acid bacteria (LAB) were dominant in all samples, with *Streptococcus thermophilus*, *Lactococcus lactis*, *Levilactobacillus brevis*, *Latilactobacillus curvatus*, and *Lactobacillus delbrueckii* being the most prevalent species in Sfela. *Debaryomyces hansenii*, *Tetragenococcus halophilus*, and *Lactococcus lactis* were the most widespread species in *Sfela touloumotiri*, while *Streptococcus thermophilus* and *Lactobacillus delbrueckii* were the two predominant bacterial species in Xerosfeli. Additionally, we identified partial MAGs of *Bacillus cereus*, *Acinetobacter baumannii*, *Klebsiella oxytoca*, and *Pseudomonas putida* in specific samples. The presence of MAGs of spoilage species and potential pathogens highlights the need for quality control measures during the production and storage of these cheeses.

M99 - Volatile compounds and sensory analysis of natural and pulped natural coffees from different Brazilian regions inoculated with yeasts

Presenting Author – *Rosane Schwan, Universidade Federal de Lavras, Brazil*

Author/s – *Silvia Martinez, Nadia Batista, Ana Paula Bressani, Disney Ribeiro Dias*

Abstract Content

Different regions, environments, coffee varieties, and fermentation methods allow the production of coffees with distinctive sensory profiles. This research evaluated the volatile and sensorial profile of un/blended coffees from 7 regions, a mixture inoculated with yeasts and fermented using the self-induced anaerobic fermentation (SIAF) method. SIAF fermentations were performed with starter yeasts: CCMA0543 *Saccharomyces cerevisiae*, CCMA0535 *Saccharomyces cerevisiae*, and CCMA0684 *Torulaspora delbrueckii*. Volatile profile evaluation was performed with a gas chromatographer-mass spectrometer (GC-MS) and the sensorial through-the-cupping test. More exclusive compounds were found for CCMA0535 than the other yeasts and high-quality specialty coffees were obtained (86-90.25). The highest scores belonged to coffees O (90.25- Mantiqueira de Minas with yeast 0543) and H (90- Cerrado Mineiro with yeast 0684). The SIAF fermentation allowed the production of high-quality specialty coffees, and the inoculated yeasts contributed positively by improving its sweetness, uniformity, clean cup, acidity, and body.

M100 - Investigating the antilisterial effects of carrot juice against an outbreak strain, *Listeria monocytogenes* LL195

Presenting Author – *Irmak Şah, Ludwig Maximillians University, Germany*

Author/s – *Jana Walter, Claudia Guldimann, Thomas Nothnagel, Frank Dunemann, Irmak Şah*

Abstract Content

Listeria monocytogenes poses a constant challenge in food safety due to its high resilience under challenging environmental conditions. Taking measures to avoid *L. monocytogenes* in food is of high importance for the food industry. In this context, identifying new, naturally occurring substances, which can either be used as a disinfectant or food additive, is a promising strategy given the consumer's high demand for natural products.

According to the literature and our preliminary studies, some carrot strains show antimicrobial properties. However, neither the active substance nor the mechanism behind this effect has ever been identified. This step of our project aimed to screen different carrot strains for their antilisterial effects.

We received 52 accessions of carrots including commercial cultivars, ancient landraces, and wild forms. These accessions have already been characterized in detail with respect to their genetic and chemical composition and were chosen based on their differing concentrations of several candidate antimicrobials. The antilisterial effects of these cultivars were tested by inoculating *L. monocytogenes* LL195 cultures into carrot juices, with subsequent evaluation of the survival of bacteria in a time-series ranging from three minutes to two hours. So far, out of these 52 species, 19 have demonstrated a strong antilisterial effect within three minutes after inoculation.

To reveal the molecular mechanisms of *L. monocytogenes* in response to this stress, we will investigate the transcriptional response of *L. monocytogenes* exposed to the carrot accessions with the strongest antilisterial effect.

M101 - Genomic insights on Salt susceptibility of *Weizmannia coagulans*

Presenting Author – *Do-Won Jeong, Dongduk Women's University, Korea, Republic of*

Author/s – *Tao Kim, Sojeong Heo*

Abstract Content

Weizmannia coagulans is spore forming, and lactic acid-producing bacterium that was recently reclassified from *Bacillus coagulans*. In our previous study, 31 *W. coagulans* strains were evaluated safety and functional properties to select starter candidates. Through this experiment, it was confirmed that *W. coagulans* were susceptible to salt compared with other *Bacillus* genus. Therefore, in this study, the genomes of *W. coagulans* and four *Bacillus* genus were compared to identify the basis for the salt sensitivity of *W. coagulans*. We selected a total 23 strains including *W. coagulans* species and four species-*B. licheniformis*, *B. siamesis*, *B. subtilis*, *B. velezensis*- of genus and analyzed for comparative genomics. As a result, *B. siamesis*, *B. subtilis* and *B. velezensis* possesses five Opu transporters: OpuA, OpuB, OpuC, OpuD, OpuE and *B. licheniformis* has four Opu transporters except OpuE, but *W. coagulans* has only OpuC transporter. These results assumed that the lack of the Opu system contributes to the salt-susceptibility of *W. coagulans*.

M102 - Characterization and *in-vitro* antibacterial potential of exopolysaccharides (EPS) from lactic acid bacteria isolated from fermented foods

Presenting Author – *Racheal Fashogbon, Ajayi Crowther University, Nigeria*

Author/s – *Itor Moses, Bukola Adebayo-Tayo, Racheal Fashogbon*

Abstract Content

Characterization and in-vitro antimicrobial potential of Lactic Acid Bacteria (LAB) isolated from fermented foods was investigated. LAB EPS was produced, characterized using HPLC, FTIR, SEM, antimicrobial and cytotoxicity potential was evaluated. From the twenty LAB isolated from the food samples, four were high EPSs producers, eight were moderate while eight were non-EPS producer. The best two EPS producers had 98% and 97% relatedness to *Lactobacillus plantarum* and *Lactobacillus pentosus*. FTIR spectra of CW2EPS and SC5EPSs revealed the presence of eighteen and ten peaks which corresponds to hydroxyl (OH), methyl, alkyl halides groups as well as stretches of C-C, C-O-C and C-O of alcohol. The HPLC analysis of all the EPS revealed the presence of galactose, glucose, mannose, glucosamine, and fructose, Glucose had the highest concentration (25.20% and 25.40%) in CW2EPS and SC5EPSs. SEM revealed a porous structure of different sizes of oval matrix with irregular lumps of varied appearances and soft surfaces. Antibacterial activity of the SC5EPS and CW2 EPS against the test strains ranged from 20.0 – 22.0 mm and 10.0 -15.0 mm. The highest activity was against E. coli and S. aureus. The EPS samples did not show any observable antifungal and cytotoxic activity against the test fungi. In conclusion, the *Lactobacillus plantarum* and *L. pentosus* produced hetero-polymeric substances with antibacterial and non-cytotoxic effects and could be used as non-toxic bio-degradable polymer in edible film production, nanomedicine formulation and in food industries as bio-thickener and viscosifying agent

M106 - Gut dysbiosis induces the colonization of foodborne pathogenic *Salmonella* in edible crickets

Presenting Author – *Shuma Tsuji, Okayama University, Japan*

Author/s – *Kazuyoshi Gotoh, Osamu Matsushita*

Abstract Content

Background: In recent years, edible insects have been reevaluated as a sustainable food source with low environmental impact and excellent nutritional value. However, little is known about the potential risk of pathogen contamination in edible insects.

Objectives: The objective of this study is to evaluate the colonization of human foodborne bacteria in edible crickets when their feed is contaminated. The goal is to provide knowledge on the safety of edible insects.

Methods: The edible cricket, *Gryllus bimaculatus*, and the bacteria *Salmonella Enteritidis* ATCC 31194 were used as a colonization model. Agar blocks containing the bacteria were fed to the crickets ad libitum for 24 hours. After feeding, the digestive tract of crickets was removed and homogenized. Suspensions were spread on mannitol lysine crystal violet brilliant green (MLCB) agar, and the number of *S. Enteritidis* colonies were counted. Agar blocks containing an antibiotic mixture were used to destroy the intestinal bacteria. Bacterial abundance in the gut was estimated by quantitative polymerase chain reaction (qPCR) using 16S rRNA gene primers.

Results: Twelve days of antimicrobial treatment reduced the gut bacteria by 1/1000. Although untreated crickets cleared Salmonella within 2 days of bacterial inoculation, in the antimicrobial-treated group, Salmonella were still detected at 105 cfu/g or higher after 7 days. This suggests that gut commensal bacteria may inhibit Salmonella colonization. However, further study is required to confirm this hypothesis. As some antimicrobial pesticides are commercially available, it is possible cricket feed is contaminated with these substances, raising concerns about feeding edible crickets.

M108 - Characterization and antibacterial effect of bacteriophage cocktails and endolysin to biocontrol pathogenic *Enterococcus faecali*

Presenting Author – *Chen Wang, Kyushu University, Japan*

Abstract Content

Background: *Enterococcus* spp., is a leading opportunistic pathogen. Several virulence factors of *E. faecalis* are involved in the infections. Many *E. faecalis* strains have evolved antibiotic resistance. Thus, an alternative agent for treating pathogenic *E. faecalis* strains has become increasingly required.

Objectives: *E. faecalis* strains were isolated from food commodities and the antibiotic susceptibility, and virulence genes were evaluated in the isolates. The novel bacteriophages specific to the pathogenic *E. faecalis* were isolated. The characteristics of the phages were determined for further application to in food.

Methods: The strains of *Enterococcus* spp. were isolated and identified by determining the nucleotide sequence of 16SrDNA. Seven virulence factors, phenotype and antimicrobial susceptibility of the isolates were investigated. For bacteriophage isolations, double layer agar method was used and the whole genomic DNA sequencing was performed for further analysis and endolysin expression.

Results: All the 29 strains of *E. faecalis* isolated from fecal and chicken meat samples contained three or more virulence genes, and five strains were resistant to eight or more antimicrobials. Among 16 lytic phages isolated, three phages showed the broadest lytic spectra, strong specificity, and unique different host range. They showed strong bactericidal activities against *E. faecalis* JCM7783. The cocktail of three phage showed wider host range and significantly inhibited the regrowth of the bacterium. Four novel endolysins found in three phages will be used for expression and antimicrobial assessment.

M109 - Beneficial Bacteria Isolated from the Korean traditional fermented food

Presenting Author – *Sumin Ryu, Dankook University, Korea, Republic of*

Author/s – *Hyunok Doo, Gi Beom Keum, Eun Sol Kim, Jinok Kwak, Sriniwas Pandey, Hyeun Bum Kim,*

Abstract Content

Background: Kimchi is a popular fermented food in Korea. It is traditionally prepared by natural fermentation without using starter culture at low temperature. Even though Kimchi is known to have several beneficial bacteria, there is limited information on microorganisms in Kimchi and their biotechnological potentials.

Objectives: The aim of this study was to isolate beneficial bacteria from Kimchi and evaluate their functional potentials.

Methods: Kimchi samples were collected from the local markets in South Korea, then they were cultured on a variety of selective agars (MRS, modified RCM, M17 and Enterococcosel agar). The isolated colonies were identified by the full-length 16S rRNA gene sequencing. MEGA software was used for the sequence analysis. The isolates were tested for acid and bile tolerance at pH 3.0, 5.0, 7.0 and bile concentrations of 0%, 0.3% and 0.5%, respectively. Lactate dehydrogenase assay was performed with 1x108 CFU/ml bacteria using RAW264.7 cells to confirm the cytotoxicity of bacteria. In addition, the hydrogen peroxide production of the isolates capable of inhibiting the pathogen, *Gardnerella vaginalis*, was confirmed.

Results: A total of 9 beneficial bacterial species were isolated from Kimchi. The genus Bacillus was most abundant, and it included *B. subtilis*, *B. velezensis* and *B. amyloquefaciencs*. Other beneficial bacteria identified included *Latilactobacillus sakei*, *Leuconostoc mesenteroides*, *Ligilactobacillus animalis*, *Lactiplantibacillus plantarum*, *Limosilactobacillus fermentum* and *Staphylococcus hominis*.

Among them only *L. plantarum* and *L. fermentum* had the bile and acid tolerance. *L. fermentum* produced hydrogen peroxide and inhibited *Gardnerella vaginalis*. Also, it did not show cytotoxicity on RAW264.7 cells.

M110 - Rapid and accurate identification method for beer-spoilage microbes using nanopore-based sequencer and genome database

Presenting Author – *Satoshi Shimotsu, Asahi Breweries, Ltd., Japan*

Author/s – *Hitomi Fujimoto, Akina Okada, Masayuki Omote, Tadashi Imanishi, Atsuo Uyama Uyama*

Abstract Content

MALDI-TOF MS and DNA sequencers are the instruments commonly used for identification of microorganisms; MALDI-TOF MS is fast and easy to operate, while DNA sequencers have excellent identification capability. However, DNA sequencing analysis requires PCR for the amplification of target genes, which takes time to obtain identification results. Recently, the nanopore DNA sequencer, MinION, has emerged and attracted much attention. In addition, the combination of MinION with a genome search toolkit (GSTK) and a genome database (GenomeSync) has made it possible to perform molecular detection and identification of microbial DNA in a short time. We applied this system to develop and evaluate a rapid identification method for beer-spoilage microbes by direct analysis of genomic DNA. DNA sequence data in FAST5 format was obtained from approximately 1000 reads, using the Rapid Sequencing kit. Then, genome analysis was performed using GenomeSync and GSTK. As a result, species-level identification was accomplished for all of the tested microorganisms, including lactic acid bacteria, strictly anaerobic gram-negative bacteria and wild yeasts. Furthermore, it was possible to clearly identify and distinguish genetically closely related species, such as bacteria belonging to the *Lacticaseibacillus casei* group. The developed method does not require PCR amplification of genes, such as 16S rDNA, and is applicable to any microbial species, belonging to bacteria, yeast and fungi. Furthermore, identification of microbes was possible within 30minutes after obtaining genomic DNAs from beer-spoilage microbes. These results suggest that this method is one of the effective means of quality control in the brewing industry.

M111 - Polymerase chain reaction-based evaluation of bacterial spore heat resistance to optimize heat sterilisation conditions

Presenting Author – *Yosuke Ito, Kao corporation, Japan*

Author/s – *Jun Sato, Naofumi Shigemune*

Abstract Content

Background and objective: In designing heat sterilisation for foods and beverages, the most important microbial phenotype to consider is heat resistance. It is especially diverse among strains of spore-forming bacteria, such as *Bacillus subtilis*, *Weizmannia coagulans*, and *B. cereus*, which grow at approximately neutral pH. A rapid and simple evaluation method of spore heat resistance is required to establish appropriate heat sterilisation conditions. However, the conventional method—the thermal-death-time-tube method—has some disadvantages in terms of rapidity, simplicity, and comprehensiveness.

Method: We developed a novel multiplex polymerase chain reaction (PCR) method to detect the orthologous marker genes of the above three spore-forming bacteria by designing specific primers focused on domains of unknown function DUF421 and DUF1657 containing gene that enhances the heat tolerance of *B. subtilis* spore.

Results: The spore heat resistance was estimated by detecting the presence or absence of the marker genes using multiplex PCR. In total, 237 isolated strains were evaluated from tea-leaf materials, and no marker genes were detected. The sensitivity and quantitativeness were improved using most-probable-number PCR. Furthermore, trial production with the absence of high heat resistant spores revealed that the green tea flavour was improved by decreasing sterilisation conditions with no microbiological deterioration. These results suggest that optimisation of heat sterilisation can be achieved through comprehensive evaluation of spores in raw materials and the environment, using our novel method.

Conclusion: This novel method will contribute to the development of improved and efficient manufacturing controls for the production of foods and beverages, especially low-acid beverages.

M112 - Immunomodulatory effects of exopolysaccharides produced by *Streptococcus thermophilus* SBC8781 from soy or cow milk fermentation

Presenting Author – *Hajime Nakata, Tohoku university, Japan*

Author/s – *Sudeb Saha, Masanori Hiramitsu, Takashi Inoue, Julio Villena, Haruki Kitazawa*

Abstract Content

Exopolysaccharides (EPS) from lactobacilli gained attention not only because of their technological applications but in addition because their ability to modulate immune responses. However, the immunomodulatory capacities of EPS produced by *Streptococcus thermophilus* were less explored. In this work, EPS from *S. thermophilus* SBC8781 were isolated after soy or cow milk fermentation, identified, and characterized in terms of their abilities to modulate immunity in porcine intestinal epithelial (PIE) cells. Supernatant of soy or cow milks were inoculated with *S. thermophilus* SBC8781 (7 log CFU/ml) and incubated at 37oC for 24h. Extraction of EPS was performed by ethanol precipitation method. EPS was characterized by chromatography and NMR. For immunomodulatory assays, PIE cells (3×104 cells/well) were stimulated with 100 µg/ml EPS from soymilk (EPS-s) or cowmilk (EPS-m) for 48h to evaluate their immunomodulartory activities after the stimulation with poly(I:C). EPS-s had higher number of acidic polysaccharides, however, its molecular weight was comparable to EPS-m. At atomic level, four NMR signals (2.5-2.8 ppm) were found for EPS-m, while multiple signals (3.5-4.3 ppm) were detected in EPS-s. EPS-m and EPS-s yield produced by *S. thermophilus* SBC8781 was 200-240 mg/l and 50-70 mg/l, respectively. Studies in PIE cells showed that the EPS-s significantly reduce the expression of IL-6, IFN-β, A20 and Bcl-3 compared to EPS-m in response to TLR3 stimulation. Results indicate that EPS structure and immunomodulatory capacities produced by *S. thermophilus* SBC8781 vary according to the fermentation substrate. Soymilk fermented with the SBC8781 strain could be a new immunologically functional food.

M113 - Monitoring *Listeria* in a dynamic frozen vegetable processing environment

Presenting Author – *Nadja Pracser, Austrian Competence Centre For Feed And Food Quality, Safety And Innovation (ffoqsi), Austria*

Author/s – *Nadja Pracser, Andreas Zaiser, Ariane Pietzka, Hui Min Katharina Ying, Kathrin Kober-Rychli, Martin Wagner*

Abstract Content

Listeria (L.) *monocytogenes* is a foodborne pathogen, causing the rare but severe disease listeriosis linked to consumption of various types of contaminated food. Contaminated frozen vegetables were recently described as a public health risk due to outbreaks in the US (2016) and the EU (2015-2018). In the current study, Listeria were monitored in a frozen vegetable processing facility over the course of 2019-2020. We aimed to identify Listeria hotspots, transmission routes and in-house Listeria strains, and phenotypically and genetically characterize *L. monocytogenes* strains. The processing facility maintains a sophisticated sampling system including environmental swab samples and product samples to monitor pathogens. Therefore, a large dataset was analysed to investigate the temporal and spatial distribution of Listeria. Whole genome sequencing data of *L. monocytogenes* isolates were used to identify in-house clones based on MLST, cgMLST and whole genome SNP analysis. The presence of plasmids, stress resistance and virulence genes in the assembled genomes was assessed and the ability of *L. monocytogenes* strains to grow under low temperatures and survive multiple freeze-thaw cycles was examined. Identification of three Listeria hotspots disclosed conveyor belts as a major contamination source. Highly interconnected transmission underlined the dynamic process structures in the processing facility. Overall, five different in-house clones were identified. Although differences in growth at low temperatures were detected between sequence types, growth potential and resilience to freeze-thaw cycles, as well as the stress resistance gene profile could not explain the colonization of the processing environment by in-house clones.

M114 - A study on the control of phage-resistant *Escherichia coli* by inhibition the priA gene product

Presenting Author – *Neo Chen-Yu Lin, Kyushu University, Japan*

Author/s – *Tomoka Murayama, Koshiro Futada, Shota Tanaka, Yoshimitsu Masuda, Ken-ichi Honjoh, Takahisa Miyamoto*

Abstract Content

The phage resistance is the biggest problem in phage therapy. Therefore, the understanding of the functions of genes that participated in the mechanism of phage resistance and susceptibility is necessary for killing foodborne pathogens effectively by using phages. In this study, 3,909 single gene-deletion mutants of *Escherichia coli* BW25113 from the Keio collection were individually tested for screening the genes involved in the phage resistance. The phage S127 BCL3 (Myoviridae family) isolated from the chicken liver in our laboratory was used in the screening. The diameter of the head of the phage was 78.03 ± 8.23 nm and the length of the tail was 86.07 ± 4.14 nm. It also had a contractile tail. The phage has a genome size of 135,530 bp, an ORF of 215 and a GC content of 43.22%. The *E. coli* priA-deletion strain (□priA) showed increased susceptibility to the phage compared to the *E. coli* BW25113 parent strain (wild-type strain). The □priA strain also showed increased susceptibility to the T7 phage (Podoviridae family) compared to the wild-type strain. Furthermore, the substances, kanamycin (KM), chloramphenicol (CP), and kaempferol (KF), which have been reported to inhibit the function of PriA were used to confirm the increased phage susceptibility in the wild-type strain. The combined use of phage S127 BCL3 and CP at 2 or 5 μmol/L increased the phage susceptibility of *E. coli* BW25113 and retarded the re-growth of the phage-resistant cells. The combined effect of the phage and CP was confirmed on *E.coli* O157:H7.

M115 - Multispecies biofilms – A potential mechanism supporting persistence of food-associated *Listeria monocytogenes*

Presenting Author – *Lauren Alteio, University of Veterinary Medicine Vienna, Austria*

Author/s – *Lauren Alteio, Eva Voglauer, Nadja Pracser, Martin Wagner, Kathrin Kober-Rychli*

Abstract Content

Persistence of foodborne bacteria in food processing environments is a food safety and public health concern of global importance, as pathogens such as Listeria monocytogenes can be repeatedly detected in the same facilities over periods of months to years. Challenges remain in defining and studying persistence, and patterns of gene presence/absence have not yet been strongly linked to this phenomenon in *L. monocytogenes*. Biofilm formation is one mechanism hypothesized to support persistence of *L. monocytogenes*, as they provide protection against environmental fluctuations and disinfectants. To investigate the role of biofilms in persistence, we established a stable biofilm of co-occurring food spoilage organisms, *Pseudomonas fragi*, *Brochothrix thermosphacta* and *Carnobacterium maltaromanticum*, on stainless steel slides before introducing *L. monocytogenes* (CC121). All four strains were isolated from the same meat processing facility. After 4 hours, RNA was extracted from biofilms for analysis of gene expression. Additional slides underwent FISH to visualize patterns of colonization and multispecies interactions. Comparison of gene expression in *L. monocytogenes* inoculum versus the biofilm revealed upregulation of flagellar genes, likely involved in surface adherence. Additionally, iron acquisition genes were upregulated, indicating potential iron limitation by *L. monocytogenes* in the biofilm. Interestingly, gene expression patterns in the multispecies biofilm with and without *L. monocytogenes* revealed no significant enrichment for specific genes, indicating that Listeria may be able to successfully invade established multispecies biofilms relatively undetected. This work highlights the critical nature of incorporating gene expression and microbial ecology into understanding complex dynamics of persistence in food-associated *L. monocytogenes*.

M116 - Selection of surrogates for *Salmonella enterica* and EHEC strains based on thermal kinetics in fruit juices

Presenting Author – *Astrid Gedas, Universität Hamburg, Germany*

Author/s – *Herbert Schmidt, Agnes Weiss*

Abstract Content

Background: *Salmonella enterica* has caused outbreaks associated with the consumption of fruit juices. Due to the very low infectious dose foodborne enterohemorrhagic *Escherichia coli* (EHEC) also pose a serious concern for human health. Pasteurization reduces bacterial viable counts, but reduces vitamins, polyphenols and anthocyanins of the respective foods. To validate the effectiveness of classic and novel preservation processes, surrogates as non-pathogenic bacteria which mimic the behavior of pathogens to a particular treatment should be applied. These need to be appropriate for the specific treatments.

Objectives: The objective of the study was to investigate the heat resistance of five *Salmonella enterica* outbreak strains associated with fruit juices, namely *S. Senftenberg*, *S. Typhimurium*, *S. Saintpaul*, and *S. Enteritidis*, as well as of a *Salmonella* cocktail and two EHEC strains of the serovars O157:H7 and O113:H21. Furthermore, eight non-pathogenic bacteria were tested as potential surrogates based on thermal kinetics. Additionally, the matrix effect on heat resistance was investigated.

Methods: Thermal inactivation was performed at 60°C, 65°C, and 72°C in the two matrices phosphate buffered saline and strawberry nectar (12° Brix). The D- and z-values of microorganism-temperature-medium combinations were calculated.

Results: The results propose *E. coli* ATCC 11229 and *E. coli* ATCC 35218 as good surrogate candidates for *Salmonella enterica* and selected EHEC strains, respectively. The results show the differences in D-values in the tested matrices, which indicate a clear influence of the environment on heat resistance of bacteria, which may be caused, among others, by changes in heat transfer.

M117 - Foliar application of plant growth-promoting rhizobacteria to reduce *E. coli* contamination and its impact on microbial community

Presenting Author – *Boeun Kim, Rural Development Administration, Republic of Korea*

Author/s – *Husna Safi*

Abstract Content

The foodborne pathogen *E. coli* is responsible for widespread diseases and yield losses of important crops worldwide. The lack of effective control strategies and the increasing demand for organically grown food have stimulated research into biological control. The aim of this study was to evaluate the rhizosphere capacity of the commercially available inoculant *Bacillus velezensis* strain HN-Q-8, *B. velezensis* strain 19573-3 and *Acinetobacter* sp. AHP123 on lettuce growth and health and its effect on the phyllosphere bacterial community in the pot experiments. The use of plant growth promoting bacteria rhizobacteria as a bio stimulant is beneficial to increase crop yield and improve quality. The main purpose of this study was to investigate the role of PGPR to modulate microbial communities in lettuce phyllosphere under biotic stress (Analyzed the effects of PGPR application on the composition of active phyllosphere bacteria in lettuce). We studied the composition of the microbial community after 1 hour, 24 hours, 48 hours and 72 hours, using mRNA sequencing of the microbial communities in the phyllosphere of lettuce. We initiated the experiment with an inoculation of PGPR and foodborne pathogen *E.coli*, after 7 days of inoculation planted were further studied for experiment. Overall, our study shows that the composition of bacterial genera in the phyllosphere significantly differed between autoclaved cells and active bacteria, but that it was not strongly affected by foliar application of PGPR on lettuce leaves.

M118 - Alterations of the bacterial cell surface and pathogenicity by acquisition of mcr-1 in enterohemorrhagic *Escherichia coli*

Presenting Author – *Eunbyeol Ahn, Seoul National University, Republic of Korea*

Author/s – *Jinshil Kim, Byeonghwa Jeon, Sangryeol Ryu*

Abstract Content

Background: Dissemination of mcr-1 is a serious public health concern. Modification of the lipid A of lipopolysaccharides (LPS) by mcr-1 not only confers colistin resistance but also affect bacterial fitness and virulence.

Objectives: In this study, we investigated the effects of mcr-1 on bacterial fitness, cell surface properties, and virulence through the modification of LPS by mcr-1 using EHEC as a model bacterium.

Methods: The mcr-1-harboring plasmid (FORC82_3) was transferred to EHEC ATCC 43894 by conjugation, and an mcr-1 knockout mutant and a complemented strain were constructed. The LPS profiles and production were analyzed with a hot phenol-water method. Bacterial growth, biofilm formation, and swimming motility were measured to examine the bacterial fitness. Bacterial virulence was evaluated by measuring bacterial adhesion and invasion using HEp-2 cells and the *Galleria mellonella* infection model.

Results: The mcr-1-harboring plasmid was transferred to EHEC by conjugation and remained stable in the transformants. Although mcr-1 did not affect the bacterial growth, it decreased swimming motility and biofilm formation. Interestingly, horizontal transfer of mcr-1 resulted in the alteration of LPS profiles, particularly in the band patterns of core-lipid A and O-units. Also, the acquisition of mcr-1 increased bacterial adherence to HEp-2 cells but decreased invasion rates. Moreover, mcr-1 decreased virulence in the *G. mellonella* model, which is ascribed to the reduced production of endotoxins. Our results demonstrated that mcr-1 affects bacterial fitness and virulence in EHEC by altering the bacterial surface properties.

M119 - Cholesterol-lowering activities of *Lactococcus lactis* JJLC-140 and 167 and their influences on the gut microbiome in mice

Presenting Author – *Jihye Yang, Seoul National University, Republic of Korea*

Author/s – *Jihye Yang, Soo-Jeong Lee, Joon-Gi Kwon, Li-Ha Kim, Sung-Woo Choi, Ju-Hoon Lee*

Abstract Content

Cholesterol as a type of lipid is an important precursor of steroid hormones such as estrogen, and testosterone. However, a high concentration of cholesterol may increase the risk of heart attack, stroke, and even blood clot formation in the blood stream by hypercholesterolemia. Interestingly, some probiotics have been reported to have an cholesterol-lowering effect using several ways. In this study, cholesterol-lowering activity of *Lactococcus lactis* JJLC-140 and JJLC-167 isolated from Korean salted clam (Jeotgal) and their expected mechanism were evaluated *in vitro* and *in vivo*. These strains were selected from 1,589 bacteria through the screening system. *In vitro* cholesterol-lowering test revealed that *Lc. lactis* JJLC-140 and 167 could consume cholesterol in medium significantly. When analyzing the predicted mechanisms of cholesterol-lowering ability, both isolates lowered the cholesterol because they assimilated cholesterol significantly though they did not show BSH activity. Furthermore, there was the possibility of cholesterol degradation of *Lc. lactis* JJLC-140 and 167 considering the RNA-Seq analysis of this mechanism. To evaluate whether the lowering activity could reproduce *in vivo*, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol level was measured after the supplementation of *Lc. lactis* JJLC-140 or 167 in a high fat diet mice model. The supplementation of JJLC-140 could lower total cholesterol and triglyceride in serum and liver significantly, while JJLC-167 lowered the level in liver and increased the cholesterol level in feces, indicating JJLC-167 could induce the emission of cholesterol. Therefore, *Lc. lactis* JJLC-140 and 167 are promising functional probiotics which could modulate the cholesterol concentration.

M120 - Characterization of cold stress-tolerant *Campylobacter jejuni* and its impact on food safety

Presenting Author – *Jeong In Hur, Seoul National University, Republic of Korea*

Author/s – *Jeong In Hur, Jinshil Kim, Mi Seon Kang, Hyun Jung Kim, Sangryeol Ryu, Byeonghwa Jeon*

Abstract Content

Background: *Campylobacter jejuni* is transmitted to humans primarily by consuming contaminated poultry meat. Before causing human infection, *C. jejuni* must survive at low temperatures in the cold chain that normally distributes and stores poultry products in aerobic environments.

Objectives: We aimed to measure the survival of *C. jejuni* at low temperatures and assess its impact on food safety.

Methods and Results: We measured the survival of 90 C. jejuni isolates from retail raw chicken at 4°C under aerobic and microaerobic conditions. *C. jejuni* isolates exhibited various degrees of survival at 4°C. Notably, viability at refrigeration temperatures was higher under aerobic conditions than microaerobic conditions at all sampling times (7, 14, and 21 days). Based on the viability, cold-sensitive and cold-tolerant isolates were selected to measure survival on raw chicken skin at refrigeration temperatures. *C. jejuni* was not detected on refrigerated raw chicken after 4 days under microaerobic conditions but survived until 6 days under aerobic conditions. When survival models were fitted using the Weibull model, the inactivation time of cold-tolerant isolates was significantly longer than that of cold-sensitive strains under aerobic and microaerobic conditions. A high probability of illness estimated with the Monte-Carlo simulation indicated that cold-tolerant isolates are more at risk than cold-sensitive isolates at 4°C under both gas conditions. These results demonstrated that *C. jejuni* shows high strain-dependent variations in cold tolerance and that the enhanced survival of cold-tolerant *C. jejuni* at refrigeration temperatures can be a potential food safety concern.

M121 - Environmental pH and short chain carboxylic acid structure affect antimicrobial activity and biofilm formation of *Salmonella*

Presenting Author – *Ker Sin Ng, Department of Biological and Chemical Engineering, Denmark*

Author/s – *Maria Florencia Bambace, Clarissa Schwab*

Abstract Content

Short chain carboxylic acids are natural antimicrobials from plant metabolism and microbial fermentation with great potential as food biopreservatives. This study aimed to evaluate the effects of environmental pH and short chain carboxylic acid structure on the antimicrobial activity and inhibition of biofilm formation of the major food pathogen *Salmonella enterica* subsp. *enterica*. The minimum inhibitory concentration (MIC) of 21 structurally different carboxylic acids was tested using a high-throughput broth dilution method at pH of 4.5, 5.5 and 6.5. Optical density was determined after incubation, and biofilm formation was analysed with crystal violet stain.

In general, carboxylic acids inhibited *Salmonella enterica* in a pH-dependent with 57, 33 and 20% compounds reducing final density to 50% (MIC50) at concentration < 50 mM at pH 4.5, 5.5 and 6.5, respectively. Crotonic and caproic acid were the strongest antimicrobials. MIC values correlated positively with pH, topological polar surface area, and the presence of additional carboxyl and hydroxyl groups, and correlated negatively with pKa, log Kow and the occurrence of benzene groups. Caproic acid was the most effective in inhibiting biofilm formation at pH 4.5, while other carboxylic acids increased biofilm formation up to 2.5-fold at concentrations close to MIC50.

In conclusion, antimicrobial activity of carboxylic acids was higher at low pH environment and for more hydrophobic compounds. When stressed at concentrations close to the MIC50, carboxylic acids can enhance biofilm formation. In conclusion, environment pH and compound structure have to be considered when developing selected short chain carboxylic acids as food biopreservatives.

M122 - Growth characteristics of beer spoilage *Lactobacillus acetotolerans* and development of detection medium

Presenting Author – *Masaki Shimokawa, Asahi Breweries, Ltd., Japan*

Author/s – *Takaki Okamoto, Yuichi Nakamura*

Abstract Content

Microbial incidents in the food and beverage industry are often caused by lactic acid bacteria (LAB). LAB are also known as predominant beer spoilers. Beer-spoilage LAB are generally difficult to detect by culture media. In previous work, *Lactobacillus acetotolerans* has been characterized as being extremely difficult to cultivate due to VPNC (viable but putatively non-culturable) state. This study aimed to investigate appropriate culture conditions for *L. acetotolerans* and to develop its detection medium for quality control (QC) in breweries.

Our investigation revealed that beer-spoilage *L. acetotolerans* strains could not grow in media lacking beer, suggesting some component in beer was a growth factor. As a consequence of further study, mevalonic acid was identified as the growth-promoting factor in beer. It was also shown that the appropriate amount of mevalonic acid in a culture medium is that typically contained in pilsner-type beer. Beer-spoilage *L. acetotolerans* strains also exhibited hop tolerance, which is not a species-specific trait. This suggests beer-spoilage *L. acetotolerans* strain has been acclimatized to the brewing environment, and requires mevalonic acid as an essential growth factor. In addition, certain fatty acid sources were found to further enhance the growth of beer-spoilage *L. acetotolerans*, suggesting the lack of fatty acid synthesis pathway in beer-spoilage *L. acetotolerans* is responsible for the inability to grow in a conventional culture medium.

Taken together, the finding on the mevalonic acid requirement of beer-spoilage LAB, which is a novel insight in the brewing industry, is considered useful for the improvement of a laboratory QC medium.

M123 - The effect of probiotics on glycemic control in type 2 diabetes patients: a systematic review and meta-analysis

Presenting Author – *Kyong Park, Yeungnam University, Republic of Korea*

Author/s – *Kyong Park*

Abstract Content

Background: While there have been significant advancements in medical prevention and treatment of type 2 diabetes (T2DM) at the clinical level, diabetes still poses a major public health issue due to its potential for serious complications.

Objective: This study aimed to conduct a systematic review and meta-analysis of randomized controlled trials to determine the effect of various probiotics on glycemic control in T2DM patients.

Methods and results: A systematic review used three electronic databases to gather randomized clinical trials (RC) on the effect of probiotics on glucose level management in patients with T2DM. Considering the discrepancies, either fixed or random effect models were chosen to determine the effect size, and sensitivity analysis results as well as publication bias were also examined. A total of 33 trials were included, and the results showed that the use of probiotic supplementation was effective in lowering HbA1c, fasting blood glucose, fasting insulin, and HOMA-insulin resistance (HOMA-IR) compared to a placebo. The average difference between probiotics and placebo in pooled analyses were -0.18 (95% confidence interval (CI): -0.30, -0.06) for HbA1c, -0.98 (95% CI: -1.40, -0.56) for fasting blood glucose, and -0.96 (95% CI: -1.26, -0.65) for HOMA-IR in a positive dose-response manner. However, no significant association was found between probiotics and fasting insulin (MD: -4.93, 95% CI: -10.04, 0.17).

Based on the pooled results of the most recent studies, it appears that high-dose probiotics may be more effective in managing blood sugar levels for patients with T2DM compared to lower doses.

M124 - The composition and biocontrol features of fungal microbiota of rowanberries and sour cherries

Presenting Author – *Iglė Vepštaitė-Monstavičė, University, Lithuania*

Author/s – *Ramunė Stanevičienė, Juliana Lukša, Robertas Lisicinas, Eulalija Antanaitytė, Saulius Serva, Elena Servienė*

Abstract Content

Rowanberry (*Sorbus L.*) and sour cherry (*Prunus cerasus L.*) are attractive in the food industry and health care due to the high content of biologically active phytochemicals. However, berries are highly colonized by various microorganisms, demonstrating beneficial or harmful features to consumers. Therefore, it is important to characterize the berries-associated microbial communities and uncover beneficial constituents. During our study, the fungal microbiota of rowanberries and sour cherries along with biocontrol features of cultivable yeasts were investigated. Following the DNA isolation, DNA fragments of the ITS2 rRNA gene region of each sample were individually amplified and subjected to the high-throughput Next Generation Sequencing (Illumina). Yeast cultures were isolated by culture-dependent approaches and identified by molecular methods. Bioinformatics data indicate that the dominant fungal microorganisms on rowanberries are *Dothiora*, *Aureobasidium*, and *Cladosporium*, while on sour cherries prevailed *Aureobasidium*, *Metschnikowia*, *Taphrina*, and *Dothiora*. Cultivable yeast strains were isolated under fermenting conditions, and their biocidal activity was analyzed at different pH values. The biocontrol ability of yeasts was tested based on the antagonistic activity against control strains and a broad spectrum of microorganisms.

M125 - Change of microbial community and food quality of Gajami sik-hae fermentation with the addition of lactic acid bacterial starter

Presenting Author – *Ye-Bin Jang, Pukyong National University, Republic of Korea*

Author/s – *Ye-Bin Jang, So-Yeon Noh, Mi-Ru Song, Yeon-Ju Sim, Du-min Jo, Kyung-Jin Cho, Dokyung Oh, Seul-Ki Park*

Abstract Content

Gajami Sik-hae is a typical Korean fermented seafood made of flat fish Littlemouth flounder. Due to the low salt content of Sik-hae, over-fermentation is easily achieved during distribution and storage. Furthermore, because fermentation is heavily influenced by environmental factors, maintaining product homogeneity is difficult. The purpose of this study is to use a lactic acid bacterial starter to increase the storage stability and quality of Sik-hae. Among the five *Leuconostoc mesenteroides* strains isolated from diverse Sik-hae products, a strain (C6) exhibiting the highest salt and acid tolerances was chosen. Sik-hae inoculated with C6 was fermented at 20°C for 15 days, and microbiological, physicochemical, and sensory evaluations were performed. In addition, Next Generation Sequencing (NGS) was carried out to confirm alterations in the microbial flora during fermentation. In the treated group, *Leuconostoc* spp. predominated at an 80-90% rate, whereas diverse communities were observed in the untreated group. Furthermore, the growth of *Lactobacillus* spp., which causes a quality deterioration in Sik-hae products, was delayed in the treatment group. The treated group performed exceptionally well in the sensory evaluation. This is assumed to be owing to CO2 (texture enhancement) and mannitol (sweetness & cool flavor), which are metabolites of the early fermentation by *Leuconostoc* spp. This investigation demonstrated the growth inhibitory effect of *Lactobacillus* spp. during Sik-hae fermentation by the addition of lactic acid bacterial starter *L. mesenteroides* C6. In addition, it was also confirmed the food quality enhancement based on sensory examination.

M126 - Rapid pre-culture and DNA extraction method for detecting low population density of *Vibrio* spp. from seafood

Presenting Author – *Seulki Park, Korea Food Reserach Institute, Republic of Korea*

Author/s – *So-Yeon Noh, Yeon-Ju Sim, Ye-Bin Jang, Mi-Ru Song, Kyung-Jin Cho, DoKyung Oh, Du-Min Jo, Young-Mog Kim*

Abstract Content

A fundamental priority of the seafood industry is to maintain hygiene and product quality; therefore, it is critical to rapidly detect contamination of food-borne bacteria such as *Vibrio* spp. in seafood. The current study proposes a rapid pre-culture and DNA extraction method for detecting low-population density (100-102 CFU/g) of *Vibrio* spp. (*V. parahaemolyticus*, *V. vulnificus*, and *V. chorelae*) in seafood. Herein, we investigate the medium and salt concentrations that resulted in the most effective pre-culture of *Vibrio* spp. in 2 hours. Further, we present and verify an economical and rapid DNA extraction method. To validate the pre-treatment method, a shellfish (*Mytilus coruscus*) was artificially contaminated with low population density of *Vibrio* spp. at 100, 101, and 102 CFU/g. Thereafter, PCR was performed using a specific primer for each *Vibrio* spp. detection. Additionally, the purity of the DNA extracted by the new pre-treatment was compared to that extracted by a commercial kit to establish compatibility. In conclusion, PCR amplification products were confirmed as positive for all the artificially contaminated shellfish samples. The proposed method is exceptionally effective at rapid pre-culture and qualitative detection of *Vibrio* spp. contaminated in seafood when combined with the molecular biological detection method.

M127 - Biopreservation of fish fillets using lactic acid bacteria cell-free supernatant

Presenting Author – *Mi-Ru Song, Pukyong National University, Republic of Korea*

Author/s – *Mi-Ru Song, Yeon-Ju Sim, So-Yeon Noh, Ye-Bin Jang, Kyung-Jin Cho, Dokyung Oh,*

Du-Min Jo

Abstract Content

Seafood is highly perishable due to enzymatic processes and endogenous microbial growth during storage. Thus, keeping seafood freshness and increasing its shelf-life are major concerns in the food industry. Biopreservation using microbiota or antimicrobials is a method to preserve food and extend its shelf-life. Bacteriocin, lactic acid, and several other metabolites produced by lactic acid bacteria (LAB) possess antibacterial effects on pathogenic and spoilage bacteria as a potential source of biopreservation. This study aimed to confirm the effect of LAB cell-free supernatant on the quality and shelf-life extension of ribbonfish *Trichiurus lepturus* fillets. Two LAB strains, *Lactobacillus plantarum* SKD4 and *Pediococcus stilesii* SKD11, isolated from the Korean traditional fermented seafood Jeot-gal evaluated the possibility of preserving quality and extending the shelf-life of the fish fillets. The fish fillets were treated with LAB cell-free supernatant at 4°C and 25°C storage temperatures. Changes in its sensory, microbiological, and physiochemical properties [pH, color value, texture, and trimethylamine (TMA) content] were investigated by the treatment of LAB cell-free supernatant. The fish fillets treated with LAB exhibited a longer shelf-life without compromising their physicochemical and sensory properties. There was no significant increase in viable cell count and TMA content in the fish fillets treated with LAB was observed during the storage for 120 h at 4°C. This study produced a novel biopreservation technique for preserving and improving the quality of fish fillets. Furthermore, LAB for biopreservation technology is expected to have a wide range of industrial seafood applications.

M128 - Genomic features of *Listeria monocytogenes* species isolated from a butter plant

Presenting Author – *Zeinab Mousavi, Instituto Ramón Y Cajal De Investigación Sanitaria (irycis), Ireland*

Author/s – *Zeinabossadat Ebrahimzadh Mousavi, Francis Butler*

Abstract Content

Background: Food processing environments specially the moist ones are considered as one of the main route of contamination with *Listeria monocytogenes*. Various studies reported the incidence of listeriosis associated with butter consumption. The occurrence of *L. monocytogenes* in butter could be associated with poor hygiene standards in the processing environment.

Objective: In this study, the occurrence of *L. monocytogenes* in the plant was initially investigated. Furthermore, the ability of the isolates to cause pathogenicity and virulence, biofilm formation, and high resistance to antimicrobials and environmental conditions were characterized by Whole Genome Sequencing (WGS).

Methods: In this study, 3 positive *Listeria* isolates recovered from environmental samples were initially characterized using API® kit (Biomerieux) and subsequently by whole-genome sequencing (WGS). The prepared genomic libraries were sequenced using NextSeq platform (Illumina).The draft genomes were assembled and annotated using Spades and Prokka. The presence of antimicrobial resistance-encoding genes and virulence factors were also investigated.

Results: Two out of three positive listeria isolates were characterized as *L. monocytogenes* with identical sequence type (ST) of 174. One isolates was characterized as *L. innocua* with ST 602. All the three isolates carried clpl gene, encoding resistance to disinfectant and stress conditions and exhibited resistance to Fosmycin antibiotics. The most important virulence genes identified in the *L. monocytogenes* isolates were PrfA, PlcA, hly, mpl, ActA and PlcB, ActA, Imo0673, recA. These genes encodes biofilm and invasion factors. According to the results, stronger surveillance should be taken to prevent and control the clonal spread of *L. monocytogenes* isolates in butter processing plant in Ireland.

M129 - *Listeria monocytogenes* adapts its proteome during growth in UHT milk

Presenting Author – *Alba Espí Malillos, Universidad Ceu Cardenal Herrera, Spain*

Author/s – *Alba Espí-Malillos, Carla Palacios-Gorba, Inmaculada López-Almela, Pilar García-Ruiz, María Carmen López-Mendoza, Francisco García-Del Portillo, M Graciela Pucciarelli*

Abstract Content – *Listeria monocytogenes* (*Lm*) is an intracellular Gram-positive food-borne pathogen responsible for listeriosis. The latest European Food Safety Authority report considers listeriosis as one of the most severe zoonosis with the highest hospitalization and case-fatality rate (20-30%). Dairy products have been associated with approximately half of the reported listeriosis outbreaks in Europe and the United States. *Lm*, a common contaminant of raw milk, can be transmitted to Ultra High Temperature (UHT) milk as the result of post-pasteurization contamination due to poor sanitation practices. We hypothesize that *Lm* could remodel the cell wall and membrane proteome during growth in dairy products. The objective of this study was to identify *Lm* proteins differentially expressed during growth in UHT milk by using proteomic techniques. Liquid Chromatography and Mass Spectrometric analysis (LC-MS) was used to characterize the cell wall and membrane/cytosol proteins of *Lm* during growth in UHT milk and Brain Heart Infusion (BHI). Proteomic analysis identified upregulated and downregulated proteins in bacteria grown in milk compared to BHI. Upregulated proteins included bacterial factors involved in virulence as well as metabolism and stress response. Importantly, some of these proteins belonged to metabolic pathways that could be targeted with novel antibacterial agents to restrict *Lm* growth in dairy products.

M130 - *Lactococcus lactis* subsp. *lactis* LB 1022 attenuates fat accumulation and ameliorates insulin sensitivity in HFD-induced mice

Presenting Author – *Soyeon Ahn, Chung-Ang University, Republic of Korea*

Author/s – *Wonyong Kim*

Abstract Content

Background: Obesity, a global epidemic, has been closely related to a metabolic syndrome known as type 2 diabetes and is often clustered with health-risk factors, such as hyperglycemia, hypertension and dyslipidemia. *Lactococcus lactis*, which is famous species of lactic acid bacteria (LAB), is widely used in industry. Even though many probiotic LAB are reported to have anti-obesity effects, the beneficial effects of *L. lactis* subsp. *lactis* are unclear.

Objectives: The purpose of this study is to investigate the anti-obesity effects of *L. lactis* subsp. *lactis* LB 1022 in high-fat diet (HFD)-induced C57BL/6 mice.

Methods: The mice were fed HFD with oral administration of LB 1022 or were only fed HFD for 12 weeks. The fat mass of the mice was measured using dual-energy X-ray absorptiometry. Body weight increase was quantified via body mass index (BMI). Blood glucose was measured once a week, and homeostatic model assessment for insulin resistance (HOMA-IR) was calculated based on fasting glucose levels and insulin levels. The cholesterol levels and triglyceride levels in serum were evaluated with biochemical analysis.

Results: The treatment of LB 1022 group suppressed the increase of body fat mass and BMI compared with the HFD-treated group. Tissue weights containing liver, and white adipose tissue in LB 1022-treated group were significantly lower than in the HFD group. Furthermore, the treatment of LB 1022 induced a significant reduction of HOMA-IR levels. Serum triglyceride, total cholesterol and low-density lipoprotein cholesterol levels were lower in the LB 1022-treated group than in the HFD group.

M131 - Characterization of *Pectobacterium* bacteriophage POP21, POP22 and their Endolysins

Presenting Author – *Joonbeom Kim, Seoul National University, Republic of Korea*

Author/s – *Hyeongsoon Kim, Sumin Son, Hojun Shin, Minsik Kim, Sangryeol Ryu*

Abstract Content

Backgrounds: *Pectobacterium carotovorum* subsp. *carotovorum* is a plant pathogen that produces plant cell wall-degrading enzymes (PCWDEs) which lead to the necrosis of various crops. The cooper-based antibiotics were used to control this plant pathogen, but, alternatives to antibiotics need to be developed because of the emergence of multidrug-resistant bacteria. Bacteriophages and bacteriophage-derived cell wall degrading enzymes, called endolysins, have been drawn as promising biocontrol agents.

Objectives: We isolated the bacteriophage and endolysin to control *P. carotovorum* subsp. carotovorum as alternatives to pesticides.

Methods: *Pectobacterium* bacteriophages were isolated by a double-layer agar assay. The characteristics of the bacteriophages were analyzed by challenge assay, transmission electron microscopy and whole genome sequencing. Endolysins were isolated and analyzed using bioinformatics tools. They were purified with affinity chromatography, confirming the expression level by SDS-PAGE. The antibacterial activities of endolysins were examined against ethylenediaminetetraacetic acid-treated *P. carotovorum* subsp. carotovorum under various conditions.

Results: Bacteriophage POP21 and POP22 isolated from a sewage sample were classified as the Siphoviridae and the Myoviridae family, respectively. The two phages displayed a narrow host spectrum but showed strong inhibition effects against their host strain. Genome analysis revealed 165,377 bp and 151,800 bp chromosomes, respectively, containing endolysins LysPOP21 and LysPOP22 classified as peptidases. The endolysins exhibited strong lytic activities against membrane-permeabilized *P. carotovorum* subsp. *carotovorum*. Also, they not only exhibited the broad lytic spectrum in which bacteriophage could not infect but also were stable over broad pH (6.5-9.0), temperature (4℃-55℃), and salt (0-300 mM).

M132 - Adaptation to widely used biocide active susbtances selects for rifampicin resistance in *E. coli* biofilms

Presenting Author – *Raphael Charron, French Agency For Food Environmental And Occupational Health & Safety (ANSES), France*

Author/s – *Marine Boulanger, Pierre Lemée, Ornella Minlong, Paméla Houée, Julien Deschamps, Antoine Huguet, Christophe Soumet, Anses Fougères, Romain Briandet, Arnaud Bridier*

Abstract Content

Bacterial antibiotic resistance increased drastically in the last decades becoming one of the most important threat for human health. The understanding of phenomenon promoting resistance selection and dissemination from farm to fork is crucial to limit the emergence of resistant bacteria. Massive use of biocides on food chain may constitute an important selective factor, as bacteria can use similar defense mechanisms to resist against biocides and antibiotics, such as overexpression of multidrug efflux pumps. However, bacterial adaptation strategies to biocides and cross-resistance development remained unclear. In particular, very few studies have focused on biofilms, which nevertheless constitutes the main bacterial lifestyle in food processing environments and which can greatly influence bacterial tolerance and adaptive strategies against biocides.

In this study we investigate how the biofilm lifestyle influences the selection of antibiotic-resistant clones when exposed to biocides. Biofilms of 10 *E. coli* isolated along the food chain were exposed during one month to biocides and their resistances to three different antibiotics (rifampicin, gentamicin, and ciprofloxacin) were quantified each week. Exposure to triamine (N-(3-Aminopropyl)-N-dodecylpropane-1,3-diamine) and benzalkonium chloride increased significantly the quantity of rifampicin-resistant clones collected when comparing to controls exposed to water. WGS of 150 clones revealed recurrent genetic targets associated with each biocide adaptation. Triamine selects for mutations in the lipopolysaccharide formation genes (especially genes involved in O-antigen biosynthesis) while benzalkonium chloride led to mutations in the ribose metabolism pathway. Additional experiments are still under process to deeply understand collective biofilm adaptation to biocides and antimicrobial cross-resistance emergence in variants.

M133 - Investigation of probiotic formulas in the reduction of acrylamide

Presenting Author – *Siu Mei Choi, Technological and Higher Education Institute of Hong Kong (THEi), Hong Kong*

Abstract Content

In this study, the potential synergistic effects of probiotic formulas to reduce acrylamide (AA) was investigated. AA is a food common food processing contaminant that formed during high temperature processing of fried and baked food products. Five selected probiotic strains (*Lactobacillus paracasei*, *Lactobacillus plantarum*, *Lactobacillus bulgaricus*, *Bifidobacterium longum*, *Streptococcus thermophilus*) were examined for their potential ability to reduce acrylamide (AA) in selected food samples of potato chips and biscuits. The probiotic bacteria and formulas were incubated with (i) standard AA chemical solutions, (ii) food samples including biscuits and potato chips, and (iii) in-vitro digestion condition to evaluate the reduction of of AA. LC-MS was used to analyse levels of AA of samples. The results showed that all tested probiotic strains demonstrated ability to reduce AA in different extent. *Lactobacillus plantarum* showed the most significant AA reduction effect (51%) when exposed to 350 ng/mL AA standard chemical solution under 108 CFU/mL cell concentration. The combination of *Lactobacillus plantarum* and *Lactobacillus bulgaricus* showed higher AA reduction ability. The probiotic strains were also incubated in potato chips and biscuits. The findings showed that the combination of *Lactobacillus plantarum* and *Streptococcus thermophilus* caused higher AA reduction percentages than the combination of *Lactobacillus plantarum* and *Lactobacillus bulgaricus* in both food samples. In addition, the AA reduction percentage by probiotic formulas under *in vitro* digestion is much greater when compared with food samples only. The current results indicated the potential synergistic effect of probiotic formulas on AA reduction.

M134 - Shifts on microbiota of dotted gizzard shad associated with production and storage conditions

Presenting Author – *Seonyeong Yu, Chungnam National University, Republic of Korea*

Author/s – *Su Jin Yum, Hee-Gon Jeong, Seung Min Kim*

Abstract Content

The dotted gizzard shad is mainly consumed along the coast of Asia without heating process.

The dotted gizzard shad were collected 2 growth environments, and analyzed by 16S rRNA gene sequencing. The total bacterial loads and alpha-diversity indices of the microbiota on dotted gizzard shads were compared, and a significant difference between farm-raised(FR) and wild-caught(WC) samples were observed. The most abundant phyla were Proteobacteria (71.30% and 85.10%) and Bacteroidetes (27.80% and 7.10%) in both FR and WC. In the FR, *Psychrobacter* (54.41%), *Flavobacterium* (27.14%) and *Pseudomonas* (6.66%) were dominant genera. In WC, *Vibrio* (28.26%), *Pseudoalteromonas* (26.79%), *Shewanella* (9.21%), *Psychrobacter* (7.00%) and *Exiguobacterium* (5.79%) were dominant genera. Potential pathogenic genera *Staphylococcus*, *Pseudomonas*, *Escherichia*, and *Vibrio* were found on the microbiota of dotted gizzard shads. Within *Vibrio* genus, only *V. parahaemolyticus* was detected in the WC (2.75 log CFU/g, detection ratio; 64.71%) whereas, not in the FR. We compared and analyzed the effects on microbiota of FR and WC to simulate the cross-contamination processes over time by infecting the *V. parahaemolyticus*. In non-infection group, 3.29 ± 2.92 log CFU of *V. parahaemolyticus* were detected in WC which is higher than those of FR (2.26 ± 1.93 log CFU/g; p < 0.05) at 4 h of storage. In infection group, when storage for 8 hours, the total bacteria load of WC (8.85 ± 8.39 log CFU/g) was significantly higher than FR (7.81 ± 7.21 log CFU/g; p < 0.05). These suggest WC dotted gizzard shad can have more risk of food poisoning.

M135 - *Lactobacillus plantarum* and *Propionibacterium freudenreichii* can enhance folate and cobalamin content of a cereal-based food

Presenting Author – *Henok Ashagrie Deribew, Montpellier University, France*

Author/s – *Kaleab Baye, Benjamin Guibert, Isabelle Rochette, Christèle Humblot,*

Abstract Content

Background: Folate (vitamin B9) and cobalamin (vitamin B12) deficiencies are widespread with pronounced health impacts. Despite the strategies for preventing vitamin B deficiencies, it remains a global problem affecting millions. Some bacteria from the *Lactobacillus* and *Propionibacterium* genera possess folate- and cobalamin-producing capabilities. Therefore, their use in cereal fermentation could provide opportunities for a sustainable dietary vitamin B supply. Injera is a traditional, cereal-based, fermented staple widely consumed in Ethiopia, and its preparation follows backslopping fermentation.

Objective: The study aimed to evaluate the application of folate- and cobalamin-producing bacteria as an inoculum in injera fermentation to enhance folate and cobalamin levels.

Methods: Injera dough was prepared from teff flour using folate-producing *Lactobacillus plantarum* and cobalamin-producing *Propionibacterium freudenreichii* strains as inoculum, alone and in combination. Folate and cobalamin production of the strains were studied for 21 successive batches of injera fermentation through backslopping under non-sterile conditions to mimic traditional injera preparation. Folate and cobalamin contents were determined using microbiological assay techniques. The presence of the inoculated strains was monitored using real-time PCR.

Results: When applied as single inoculum, *L. plantarum* produced a fermented dough containing up to 57 µg/100g of fresh weight (FW) folate. The Cobalamin content of the *P. freudenreichii* inoculated dough reached 6 µg/100g FW. Co-inoculation of the two strains was not as effective since only 27 and 1.2 µg/100 g FW of folate and cobalamin were produced, respectively. Therefore, applying folate and cobalamin-producing bacteria to cereal fermentation could offer a sustainable alternative to combat vitamin B deficiencies.

M136 - Comparison of geno- and phenotypic characteristics of *E. faecalis* and *E. faecium* with whole genome sequencing results

Presenting Author – *Jolanta Rola, National Veterinary Research Institute, Poland*

Author/s – *Marlena Gołaś-Prądzyńska*

Abstract Content

Background: Enterococci are a group of bacteria of significant importance as they cause bacterial infections in humans. It is important to study this bacteria derived from dairy products, and enrich the presently insufficient data on the antimicrobial resistance and virulence factors.

Objectives: The aim of the study was genotypic characterization of archival strains obtained from milk and dairy products and comparison of the results with those obtained by phenotypic methods and classical PCR.

Methods and Results: A total of 36 strains from milk and dairy products were analyzed. The identification of *E. faecalis* and *E. faecium* species selected antibiotic resistance genes were detected using polymerase chain reaction. Panel of 18 antibiotics was performed using the broth dilution method. Deoxyribonucleic acid (DNA) isolation for sequencing was performed using the Maxwell Rapid Sample Concentrator kit. Quantitative and qualitative DNA assessments were performed and libraries created using the KAPA Hyper Plus kit. Sequencing was performed on the Nextseq instrument using a 2 × 150-bp paired-end protocol. Phenotypic resistance to tetracycline, kanamycin, streptomycin, erythromycin, chloramphenicol, tylosin and single strains resistant to vancomycin, gentamicin, teicoplanin and ciprofloxacin was determined. The tested virulence genes in most cases overlapped with the results of phenotypic resistance, with the exception of isolates sensitive to vancomycin (with VanA gene), erythromycin, tetracycline. Resistant straines lacked the vgaA and vatD gene. *E. faecalis* and *E. faecium* tetracycline-, erythromycin- and chloramphenicol-resistant genes found in the ResFinder 4.1 database were associated with resistance phenotypes.

M137 - Analyses of the blue pigment produced by *Pseudomonas carnis* provide important clues to its molecular identity

Presenting Author – *Tiziana Nicola, Max Rubner-Institut, Germany*

Author/s – *Christina Grimmler, Sabine Andrée, Illya Fedotenko, Dagmar Brüggemann, Sonja Lick*

Abstract Content

Pseudomonads are among the most important microbial spoilage organisms, due to their ubiquitous occurrence and the ability to modify food matrices through various mechanisms. As a result, their occurrence can cause great economic damage in food industry. In recent years, particular attention has been paid to some genetically closely related bacteria of the *Pseudomonas fluorescens* group, that can cause a blue discoloration of foods such as dairy and meat products through the production of extracellular pigments. Previous analyzes carried out by various research groups have not yet led to a final identification/characterization of the biosynthetic pathway or of the dye molecule itself and are sometimes very controversial. In this study, growth conditions under which the pigment was produced by *Pseudomonas carnis* type strain B4-1 were examined. The molecular structure and chemical properties of the dye were analyzed in order to draw conclusions about its biological function. Physical and chemical tests revealed that it is a polar molecule due to its behavior in various solvents. Coloration of the pigment depends on the growth and storage conditions, the oxygen exposure, pH value and temperature. These factors provide a range of color variants such as grey, blue, violet, red or brown. The best characterized blue-violet form shows characteristic absorption maxima around 400 nm and 590 nm which were used for detection of the pigment in reversed phase high-performance liquid chromatography (RP-HPLC) analysis and purification. HPLC mass spectrometry measurements revealed a molecular mass of 410 Da.

M138 - Influence of lactoferrin on growth and toxin production of enteropathogenic *Bacillus cereus*

Presenting Author – *Nadja Jessberger, University of Veterinary Medicine Hannover, Germany*

Author/s – *Clara-Sophie Jugert, Andrea Didier*

Abstract Content

Background: Lactoferrin is an iron-binding glycoprotein with antibacterial, antiviral, antifungal, antiparasitic, anti-inflammatory, anti-anaemic and anti-carcinogenic properties. It can have bacteriostatic or bactericidal effects on various Gram-negative and Gram-positive microorganisms. Due to its positive effects, lactoferrin, which is mainly derived from cow's milk, is used intensively in human medicine and as food supplement.

Objectives: The aim of this study was to determine the antibacterial effect of various lactoferrin-based food supplements on selected bacterial pathogens at strain level. Secondly, the effects on enterotoxin production of *Bacillus cereus* were investigated.

Methods: A total of 112 strains were used in this study. Antibacterial effects of lactoferrin were primarily tested in disc diffusion assays. Minimum inhibitory concentrations were determined in antimicrobial broth dilution assays. Enterotoxin production after lactoferrin exposure was tested via enzyme immuno assays as well as WST-1 bioassays on Vero cells. The transcriptional response of a *B. cereus* reference strain to lactoferrin exposure was determined by RNA sequencing.

Results: Growth inhibition and minimum inhibitory concentrations were subject to large strain-specific differences. Increased production of enterotoxins by B. cereus after incubation with lactoferrin was also detected in a strain-specific manner. RNA sequencing revealed changes in the total transcriptome of a selected reference strain after exposure to lactoferrin. The results suggest that lactoferrin-based supplements do not have a general antibacterial effect against specific pathogenic species. Rather, individual members of these species have developed strategies to escape the antibacterial effect. Strain-specifically, the protein can even promote growth and the production of toxins.

M139 - Phenotypic studies for targeted processes in a circular economy model

Presenting Author – *Laura Troiani, University of Parma, Italy*

Author/s – *Laura Troiani, Alessia Levante, Valentina Bernini, Erasmo Neviani, Camilla Lazzi*

Abstract Content – Microorganisms are the main representatives of biodiversity in every ecosystem. Among them, lactic acid bacteria (LAB) are involved in several applications in food industries. Over the time, LAB evolved the ability to metabolize different carbon sources and adapt to various environmental conditions, which provided them important benefits in colonizing many habitats. Food waste and by-products represent alternative substrates to produce industrially interesting compounds through biological methods. The use of these matrices as fermentation substrates provides significant economic benefits and encourages sustainable development promoting a circular economy.

This work aims to investigate the metabolic and physiological potentialities of a selected microbial core (SMC) from a wide collection of LAB, by exploring their biodiversity, in the perspective of carrying out targeted fermentation processes by recovering food waste and by-products.

The SMC consists of 150 strains of LAB of food origin belonging to the University of Parma Culture Collection (UPCC). Substrates utilization profiles of strains were determined on 71 different carbon sources by using BIOLOG GenIII MicroPlates. The ability of strains to grow in different extreme conditions of pH, temperature, and salt concentration was investigated by phenotyping microarrays.

Results highlight that UPCC's strains show the aptitude to metabolize several compounds proving the ability to colonize and ferment different matrices of both animal and plant origin. Physiological data show remarkable tolerance to conditions far from the optimal point, which represents an opportunity to avoid many pre-treatments on the biomass recovered, often required to make the substrates suitable for microorganisms.

M140 - Effect of transglutaminase and galactooligosaccharides addition on the viability of lactic acid bacteria and *bifidobacteria*

Presenting Author – *Ivana Hyrslova, Dairy Research Institute Ltd., Czech Republic*

Author/s – *Ivana Hyrslova, Jiri Stetina, Ladislav Curda, Dmytro Harkavchenko, Stepan Marhons, Anna Macurkova*

Abstract Content

Background: Last decades, the popularity of functional foods rapidly increases with emphasis on using of natural and healthy components for developing high sensory and quality of these foods. Galactooligosaccharides (GOS) prepared from whey are a food-grade prebiotics applicable into dairy products. GOS have proved a prebiotic potential and further benefits, such as selective promotion of beneficial microorganisms, increase in the absorption of minerals, improvement of the immune response and others. In addition, the microbial transglutaminase (MTG) represents another natural component, applicable to improve microstructural, textural, and sensory properties of non-fat fermented dairy products.

Objectives: The aim of the present study was to test the effect of addition of different concentrations of MTG or GOS on the viability of yogurt bacteria and bifidobacteria in yogurt during storage.

Methods: Viable counts of yogurt bacteria and bifidobacteria and sensory properties of the tested yogurts were investigated during 9 weeks of storage. Before fermentation (30 °C/16-18 h), GOS were added at 2, 10 and 20 % w/w into pasteurised milk. MTG (0.1, 0.5 and 1.0 U/g of protein) was applied to milk before pasteurization at 30 °C for 2 hours.

Results: Higher concentrations of GOS led to significantly increased viability of yogurt bacteria and bifidobacteria during 6 and 9 weeks of storage. Addition of MTG had no effect on bacterial viability, however 0.5 and 1.0 U/g MTG affected negatively the sensory quality of tested yogurts.

M141 - *Lactococcus lactis* LB 1022 alleviates alcohol-induced hepatic injury in Sprague-Dawley rat

Presenting Author – *YoHan Nam, Chung-Ang University, Korea, Republic of*

Author/s – *Jong-Hwa Kim, Jihye Baek, Wonyong Kim*

Abstract Content

Background: Alcoholic liver injury, characterized by impaired alcohol metabolism, is resulted from the excessive consumption of alcohol. Although many effectiveness studies of probiotics on alcoholic liver disease, the alcohol metabolisms by which probiotics function are still studied.

Objectives: The purpose of this study is to evaluate the functional effects of *Lactococcus lactis* LB 1022 on alcohol metabolism and alcohol-induced liver injury *in vitro* and *in vivo*.

Methods: Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) activities of *Lactococcus lactis* LB 1022 were evaluated using a modified Bergmeyer's method *in vitro* assay. *In vivo*, the sprague-dawley rats were fed with oral administration of *Lactococcus lactis* LB 1022 after 16 h of fasting. The rats were treated with 25% ethanol (10 mL/kg) after *Lactococcus lactis* LB 1022 administration. Biochemistry, blood alcohol, and acetaldehyde concentration were analyzed by serum. SIRT1/AMPK signaling-related mRNA expression and liver histology were analyzed by liver tissue.

Results: *Lc. lactis* LB 1022 increased alcohol metabolism by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), enhanced sirtuin-1 (SIRT1)/adenosine monophosphate-activated protein kinase (AMPK) signaling, and inhibited NF-κB signaling. Interestingly, probiotics induced peroxisome proliferator-activated receptor α (PPARα), a hepatic inflammatory injury-associated factor. In addition, probiotics reduced alcohol and acetaldehyde concentrations in the blood, aspartate transaminase (AST)/alanine transferase (ALT) levels, fat vacuole formation, inflammatory cell infiltration, and an irregular hepatocyte arrangement. Taken together, *Lc. lactis* LB 1022 could induce alcohol metabolism and alleviate alcohol-induced liver injury by regulating SIRT1 in fatty acid oxidation, AMPK in lipogenesis, and NF-κB in inflammation.

M142 - The occurrence and the whole-genome analysis of *Staphylococcus aureus* in dry-fermented salami

Presenting Author – *Miroslava Krzyzankova, Veterinary Research Institute, Brno, Czech Republic*

Author/s – *Martina Florianova, Kristyna Korena, Helena Juricova*

Abstract Content

Background: Dry-fermented salami (DFS) belong to popular and frequently consumed ready-to-eat food products. Their microbiological safety is ensured by the addition of well-defined starter cultures and subsequent fermentation processes resulting in high acidity, high salinity and low water activity of the product. However, some pathogens can survive and even grow in these conditions.

Objectives: Evaluation of the prevalence o*f Staphylococcus aureus* (*S. aureus*) in DFS from Czech retail, investigation of antimicrobial resistance and selected virulence genes in obtained isolates and estimation the possible source of *S. aureus* contamination in the final products.

Methods: 54 DFS samples from Czech retail were examined on the presence of *S. aureus*. Obtained isolates were confirmed with PCR, tested for antibiotic resistance, whole-genome-sequenced and analysed.

Results: The prevalence of *S. aureus* in DFS was 27.8 %. A total of five multidrug-resistant methicillin-resistant (MRSA) and sixteen methicillin-sensitive *S. aureus* (MSSA) isolates were characterized by whole genome sequencing. A total of eight different ST types and nine spa types were identified, with ST398-t011 predominating in MRSA and ST7-t091 in MSSA isolates. Methicillin-resistant ST398 isolates were classified as livestock-associated MRSA (LA-MRSA), and we hypothesized that the source of product contamination was raw pork. Different genes encoding staphylococcal enterotoxins, virulence factors, and factors mediating host invasion and immune evasion were identified exclusively in MSSA isolates, therefore the product contamination may occurred via human carriers. The obtained results suggest the importance of high standards of Good Hygienic Practices and Good Manufacturing Practices in food processing.

M143 - Dynamics of microbial succession in the cheese ripening process

Presenting Author – *Helena Juricova, Veterinary Research Institute, Brno, Czech Republic*

Author/s – *Kristyna Korena*

Abstract Content

Background: A large variety of fermented cheeses are produced using widely different manufacturing processes and with the addition of various starter or adjunct microbial cultures. Commercially available starter cultures are well-defined mixes of microorganisms that are deliberately added to milk in order to control and standardize the production process and to achieve better reproducibility of the final product.

Objectives: The aim of this study was to describe the succession of the microbial population during the production and ripening of smear-ripened cheese.

Methods: The composition of cheese microbiota and relative abundances of individual bacterial taxa were determined by amplicon sequencing of the V3/V4 variable region of 16S rRNA gene.

Results: In the first six days of production, cheese microbiota consisted exclusively of *Lactococcus* and *Leuconostoc* spp. originating from the starter culture. From day 7, bacteria from the production environment started to appear and from day 14 of the production, these bacteria completely dominated over the starter cultures. These included *Psychrobacter*, *Pseudoalteromonas* and *Vibrio* (phylum Proteobacteria), *Vagococcus* and *Marinilactibacillus* (phylum Firmicutes), *Malaciobacter marinus* (phylum Campylobacterota), *Psychrilyobacter* (phylum Fusobacteria) and *Psychroflexus* (phylum Bacteroidetes). The experimental ripening of the cheese under laboratory conditions excluded the contribution of the milk-derived microbiota since the microbiota of the final product after 55 days of ripening consisted only of the starter and adjunct microbiota.

M144 - Source and transmission analysis of *Listeria monocytogenes* using nanopore sequencing and Mash distances.

Presenting Author – *Astrid Heikema, University of Applied Sciences Leiden, Netherlands*

Author/s – *Koen Bossers, Marijke Mostert, Leratho Biekman, Mara Kröner, Walter Zuijderduin, Floyd Wittink, Angela Hoogenboom, Merry Torani, Kevin van den Berg*

Abstract Content

Background: Infection with foodborne *Listeria monocytogenes* is relatively rare but can result in severe disease with high mortality rates. The food industry must ensure its products are safe and undertake measures to prevent contamination.

Objectives: Determine the sources and transmission routes of *L. monocytogenes* in food processing companies using Nanopore whole-genome sequencing.

Methods: Nanopore whole-genome sequencing was performed on DNA isolated from more than 700 *L. monocytogenes* strains derived from various foods, ingredients and environmental locations of food processing companies. Libraries were prepared with the Rapid Barcoding Kit (Oxford Nanopore Technologies), and after quality control, genomes were assembled with the Flye assembler. The relationship between the genomes was determined with the SourMash tool, and a phylogenetic tree was constructed from Jaccard similarity distances. Five *L. monocytogenes* ATCC reference strains were used to validate Mash and set thresholds.

Results: Sequencing the DNA of five colonies of each *L. monocytogenes* ATCC reference strain in independent duplicates resulted in minimal similarity distances for each strain and enabled setting the Mash threshold for similarity. With this threshold, relatedness analysis was performed on the 700+ *L. monocytogenes* genome sequences. As a result, multiple genome clusters were identified, indicative of common sources of contamination. External environmental sources (mushroom culture soil, fish ingredient), as well as an internal persistent *L. monocytogenes* source (meat-processing plant), could be traced back and pinpointed.

Conclusion: Nanopore whole-genome sequencing, in combination with Mash distance analysis, are powerful tools to identify the source and transmission of *L. monocytogenes* in the food industry.

M148 - Effect of faecal fermentations and dairy fermentates on insulin secretion in an *in vitro* model of human EndoC-βH1 cell line

Presenting Author – *Enriqueta Garcia-Gutierrez, Teagasc Food Research Centre, Ireland*

Author/s – *A. Kate Falà, Helen Slattery, Laura Marroquí, Paul D. Cotter*

Abstract Content

Background: There is growing evidence of connections between gut microbiota dysbiosis and suboptimal health conditions, such as type I and type II diabetes, associated to some extent with changes in dietary habits in Western societies. Fermented foods and fermentates are attracting attention as healthier alternatives to Western diets as a strategy to help and/or alleviate conditions arising from an unbalanced gut microbiota.

Objectives: 1) To characterise the metataxonomic and functional changes of the bacterial communities induced by the different fermentates; 2) To characterise the short chain fatty acid (SCFA) profile produced in the faecal fermentations. 3) To evaluate the effect of the cell-free supernatants on the insulin secretion of EndoC-βH1 cells.

Methods: Faecal samples from six donors were collected and homogenised in a faecal slurry that was further used in an *ex vivo* faecal fermentation using a micro-Matrix bioreactor. Six dairy fermentates were digested following the INFOGEST guidelines and added to the faecal slurry at two concentrations (1 and 5 % v/v), where they were incubated in anaerobic conditions at 37 oC and 250 rpm for 24 h. Samples were sequenced using Illumina Seq Platform and SCFA composition was evaluated by HPLC. EndoC-βH1 were exposed for 2 h to the faecal fermentation cell-free supernatant and the secreted insulin was measured using ELISA.

Results: The concentrations of dairy fermentate were associated to different metataxonomic, functional and SCFA profiles in a faecal fermentation. Fermentates 1-4 exhibit a dose-dependent response on insulin release, whereas 5-6 did not.

M149 - Occurrence and prevalence of class 1 integrons in meat associated microbiota

Presenting Author – *Sebastian Knorr, Max Rubner-Institut, Germany*

Author/s – *Dagmar Adeline Brüggemann, Sonja Lick*

Abstract Content

Class 1 integrons are genetic elements in bacterial genomes responsible for the accumulation and expression of exogenous genes. Through the acquisition of antibiotic resistance genes (ARG) by an integrase (intI1) they are considered as potential markers for spreading ARG in microbial communities like meat associated microbiota.

Aim of the study was to analyse the burden of class 1 integron-integrase genes in retail meat samples and its contribution to antibiotic resistance among meat-associated microorganisms.

12 cold stored meat samples (4 chicken, 3 turkey, 3 pig, 2 cattle) were analysed for intI1 quantity via a newly developed real-time-qPCR-assay. A total of 1826 colonies were isolated from the samples and screened for intI1 presence via colony-PCR. Of all intI1 positive isolates we additionally determined the species (16S-rDNA-PCR), the integron-cassette structure (long-range-PCR) and minimal inhibitory concentration (MIC) to relevant antibiotics.

4 -12% of screened bacterial isolates on chicken meat, 4% on turkey and 0,003% on pig meat were positive on intI1. No integron-carrying isolates were found on cattle meat. Relative abundancies of intI1 gene copies (copy-number intI1 to rpoB) quantified by real-time qPCR were 10x higher in poultry meat samples compared to pig and cattle. It was possible to characterize gene cassettes from 12 of 36 isolates. Resistance genes related to aminoglycosides (aadA1, 2, 4) (*Aeromonas veronii*), trimethoprim (dfrA1) (*Escherichia coli*) and chloramphenicole (catB8) (*Aeromonas veronii*) were found. Cassettes carrying antibiotic resistance genes were exclusively found on chicken meat. In MIC assays tested isolates showed increased resistance to aminoglycoside antibiotics.

M151 - Influence of different yeast extract fractions on the growth behavior of selected lactic acid bacteria

Presenting Author – *Catharina Kleist, Hamburg University Of Technology, Germany*

Author/s – *David Sahar, Ana Malvis Romero, Dominik Spickermann, Raphaël Levesque, Andreas Liese*

Abstract Content

Yeast extracts are widely applied in food and industrial fermentation, due to their complex and nutritional composition. They are produced by releasing the intracellular contents of yeast cells and removing the cell wall debris. Some yeast extracts require additional purification steps to enrich target compounds, resulting in the formation of a by-product, hereinafter called TIDE. TIDE mainly contains free AAs and low molecular weight peptides (< 5 kDa).

Although being a promising source of nitrogen in fermentation, TIDE leads to worse growth performance compared to other yeast extract products, especially for lactic acid bacteria (LAB). LABs prefer diverse peptide sizes and free AA contents based on their proteolytic systems. This study investigates the influence of different size-fractions of TIDE on the growth behavior of *Streptococcus thermophilus*, *Lactobacillus helveticus*, and *Lactobacillus bulgaricus* to understand how TIDE's fermentation application can be improved.

TIDE was filtered at different cut-offs (2 kDa, 1 kDa, and 0.3 kDa). The resulting fractions were analyzed for free AAs and molecular size distribution and used as only nitrogen source in the fermentation of the selected organisms using a BioLector® II microbioreactor. It was investigated if there is a correlation between growth behavior and fraction composition.

The different LAB showed different preferences towards the fractions and untreated TIDE. However, only few significant correlations ($p < 0.05$) were found between free AA content, molecular size distribution, and growth performance, indicating that other factors also play an important role in TIDE's fermentation application.

M153 - Supplementation of cheeses with non-starter lactic acid bacteria by the addition of traditional animal rennets

Presenting Author – *Dimitrios Pavlidis, University Of Peloponnese, Greece*

Author/s – *Marina Papadelli, John Kapolos, Konstantinos Papadimitriou*

Abstract Content

Background: Several traditional cheeses are characterized by increased proteolysis, peptidolysis and production of aroma compounds during their ripening, physicochemical processes where contribute the non-starter lactic acid bacteria (NSLAB). Animal rennets could serve as a source of such microbial contamination.

Objectives: The aim of the study was to assess the microbial ecology of different traditionally prepared animal rennets used to produce traditional cheeses of Peloponnese region.

Methods: Two traditional animal (sheep and goat) rennets used in artisanal and/or industrial cheese coagulation were collected and added in UHT sheep's milk at 35 oC. Both liquid rennets and coagulated milk after 48 hrs were analyzed microbiologically. From each sample 15 random colonies were selected from MRS and M17 agar plates and identified through MALDI-TOF/TOF.

Results: The MRS counts ranged from ~5.50 to 6.50 logCFU/g in both rennets, while after 48 hrs their levels in coagulated milk were 8.50 logCFU/g. The numbers of Enterobacteriaceae, coliforms, and *Escherichia coli* varied between 2.5 to 4.0 logCFU/g, while no *Listeria monocytogenes* was detected. Maldi-TOF/TOF results highlighted *Limosilactobacillus reuteri*, *Levilactobacillus brevis*, *Enterococcus faecalis*, *Enterococcus faecium*, *Lactiplantibacillus plantarum*, and *Pediococcus pentosaceus* as bacterial species among samples. The findings are promising to assess the isolates for their potential technological and probiotic traits.

M154 - Physicochemical factors associated with the elimination of *Staphylococcus aureus* in aerobic composting of organic compost

Presenting Author – *Ping Lung Chan, Hong Kong Metropolitan University, Hong Kong*

Author/s – *Yuen Ting Chiu, Kit Ling Lam, Ki Ying Kwong, Kin Hang Ho, Po Yee Ling, Tsz Ho Lau, First Name: Ian, Wing Yin Mo*

Abstract Content

Background: Organic composts made of animal manure harbour pathogens, posing a food safety hazard. Aerobic composting is the primary measure for reducing the bacterial load in organic composts. Here we report the factors associated with the elimination of *Staphylococcus aureus* during aerobic composting.

Methods: Three piles of organic composts consisting of chicken manure, leaves, and coffee grounds with a C: N ratio of 14 and water content of 50% (w/v) were aerobically composted for 52 days. The composts were mixed every 3-5 days in the first two weeks and then once a week. Compost core temperatures were measured daily. Compost samples of each pile were collected from the top, middle, and bottom parts on Day 0, 3, 17, 30 and 52. The physicochemical properties of the composts were measured using standard methods. *S. aureus* counts of the composts were enumerated using Baird-Parker agar base with egg yolk telluride emulsion.

Results: *S. aureus* count decreased from Day 17 and was not detectable on Day 52 (Figure 1a). Principal component analysis and correlation analysis indicated that the *S. aureus* count was positively associated with the content of carbon and ammonium but negatively associated with the concentration of nitrate and nitrite in the composts, core temperature and pH (Figures 1b and c). These data suggested that a reduction in the organic carbon content and ammonium concentration and an increase in the nitrate and nitrite content, and the pH and temperature of the compost may favour the elimination of *S. aureus*.

M156 - Genomic analysis of novel bacteriophages to target cheese spoilage organism *Clostridium tyrobutyricum*

Presenting Author – *Melinda Mayer, Quadram Institute, United Kingdom*

Author/s – *Carmen Sánchez, Javier Calzada, Dave Baker, Rhiannon Evans, Marta Ávila, Sonia Garde, Arjan Narbad*

Abstract Content

Bacteriophages and their endolysins are potential biocontrol agents for the cheese spoilage organism *Clostridium tyrobutyricum*. In cheese trials, bacteriophage treatment was able to affect cheese spoilage symptoms, but only one phage of eight tested was successful.

We aimed to analyse the genomes of bacteriophages which lysed *C. tyrobutyricum* to identify novel species and new phage proteins and to investigate differences in biocontrol potential. Eight bacteriophages isolated from silage or soil from dairy farms in Spain were sequenced and their open reading frames identified by Prokka, Bakta and Blastp analysis. Phage taxonomy was explored with ViPTree using proteomic trees and VIRIDIC to calculate pairwise intergenomic similarities.

Phages vB_CtyS-FA3, vB_CtyS-FA21, vB_CtyS-FA29, vB_CtyS-FA52, vB_CtyS-FA59 and vB_CtyS-FA88 vB were highly similar at the nucleotide level (95%) to UK landfill isolate ΦCTP1(1), but all six contained one extra open reading frame encoding a predicted methylase. Phages vB_CtyS-FA67 and vB_CtyS-FA70 showed no similarity to ΦCTP1 but shared 93% nucleotide identity. None of the phages evidenced a lysogeny module. Taxonomic analysis of vB_CtyS-FA67 and vB_CtyS-FA88 indicated that both groups belong to the Siphoviridae family; vB_CtyS-FA67 had no close relatives and may represent a new genus, while the only close relative of vB_CtyS-FA88 was ΦCTP1. In previous cheese trials only vB_CtyS-FA67 showed potential to reduce cheese spoilage(2). Genomic comparison of vB_CtyS-FA67 and vB_CtyS-FA70 identified 5 open reading frames present in only one of each genome and differences in the amino acid sequences of predicted tail proteins. Further examination may reveal which characteristics are associated with improved biocontrol.

M157 - Genomic insights into antimicrobial resistance and virulence genes of *Campylobacter jejuni* strains

Presenting Author – *Jurgita Aksomaitiene, Lithuanian University Of Health Sciences, Lithuania*

Author/s – *Aleksandr Novoslavskij, Mindaugas Malakauskas*

Abstract Content

Background: WGS sequencing is a powerful method used for analysis of antimicrobial resistance with knowledge improvement on how bacteria become resistant and which bacteria genes are responsible for AMR. This method is an important step toward the reducing and spreading of antimicrobial resistance and its effect on human health.

Objectives: The aim of the study was to analyze the antimicrobial resistance of *C. jejuni* strains based on WGS sequencing data and perform the clustering of *C. jejuni* strains based on antimicrobial resistance genes (ARGs) and virulence factors.

Methods: In total fifty-three selected *C. jejuni* strains isolated from poultry products, dairy cattle, human stools, and wild birds were investigated using WGS Illumina MiSeq technology. *de novo* assembly of the reads was performed using SPAdes 3.15 genome assembler. cgMLST was performed using Ridom SeqSphere+ tool. AMR and virulence genes identification was performed using ABRicate, CARD, and RGI databases.

Results: The cmeD, cmeE, cmeF and cmeR genes, known to confer MDR, were found in all *C. jejuni* strains. The aminoglycoside resistance genes (aph(2)-d-prime, ant(6)-Ia, aph(3')-III) were found in strains assigned to ST-64111. Strains assigned to ST-64111 also harbored the sat4 gene coding the resistance to streptomycin. The genes involved in resistance to beta-lactams and tetracyclines were also found and detected as MGE. Strain assigned to ST-21 (CC21) harbored virulence markers associated with adhesion, invasion, biofilm formation, LOS, LPS. The genes involved in damage to the host immune system (kpsM, kpsS, kspT) and colonization (jlpA) were found in some of *C. jejuni* strains.

M159 - The activation of ICEKKS102Tn4677 conjugal tranfer by TraR, which is interacted with LysR motif in oriT region

Presenting Author – *Satoshi Matsumoto, Tohoku University, Japan*

Author/s – *Yuji Nagata, Yoshiyuki Ohtsubo*

Abstract Content

Background: *Acidovorax* sp. KKS102 carries ICEKKS102Tn4677, an integrative and conjugative element (ICE), which encodes PCB/biphenyl-degrading enzymes (Ohtsubo, et al., 2012). The transfer of the ICE from KKS102 to different bacterial strains has been demonstrated, but the mechanisms controlling the transfer remain to be fully elucidated. The traR gene, encoding a LysR-type transcriptional regulator, is located downstream of the bph operon and has been hypothesized to control the transfer.

Objectives: Our goal is to elucidate the function of the traR gene.

Methods: We overexpressed the traR gene and conducted ICE-mating experiments, as well as RNAseq analysis, reporter analysis, and an assay using a deletion series of a plasmid cloned from the oriT region.

Results: The overexpression of traR resulted in an increase in the ICE transfer frequency, demonstrating the function of TraR in coordinating bph operon induction and ICE transfer. RNAseq analysis revealed modest transcriptional induction of genes related to ICE transfer, but these results were not consistent with those obtained from the reporter analysis. The transfer frequency of the oriT plasmid was increased upon the overexpression of traR, and the traR-dependent increase required a LysR motif found in the oriT region. The direct interaction of TraR with this motif is currently under investigation.

M160 - Genetic characterization of an IncHI2 plasmid harboring a novel complex class 1 integron in a cephalosporin resistant *Salmonella*

Presenting Author – *Ainhoa Arrieta-Gisasola, University of the Basque Country UPV/EHU, Spain*

Author/s – *Victoria Garrido, Ilargi Martínez-Ballesteros, Lourdes Migura-García, Irati Martínez-Malaxetxebarria, María Jesús Grilló, Lorena Laorden*

Abstract Content

Background: *Salmonella* Typhimurium is a major foodborne zoonosis worldwide, whose increasing incidence in humans has been associated to pig products. The inappropriate use of antimicrobials has favoured the emergence of antimicrobial resistance (AMR). Is of particular concern the AMR to 3rd generation cephalosporins (3GC), as they are the treatment of choice for otitis, meningitis, and urinary and respiratory infections when other beta-lactams fail. Accordingly, the emergence of *S.* Typhimurium carrying mobile genetic elements (MGEs) containing 3GC-AMR genes should be monitored.

Objective: Characterize the MGEs involved in the dissemination of 3GC-AMR genes on a *S.* Typhimurium isolate collected from a fattening pig in Spain.

Methods: A cefotaxime-resistant strain phagetype-195; showing ASSuTNx3GC and a conjugative plasmid was selected. Whole-genome sequencing was performed by combining Illumina and Nanopore technologies. Plasmid sequence annotation and comparison was performed using NCBI Prokaryotic Genome Annotation Pipeline and BLASTN, respectively.

Results: The conjugative plasmid recovered was 306,822 bp, keeping the IncHI2 plasmid backbone and containing a novel AMR region of ~ 60 kb. The AMR region contained an unreported complex class 1 integron (IC1) with the extended-spectrum beta-lactamase gene blaCTX-M-9 integrated into a cassette with genes conferring multi-AMR to ASSu3GC and to heavy metals.

Conclusions: Overall, we describe a novel IC1 carrying multi-AMR genes located in a conjugative plasmid that should be monitored in both, animals and humans, since its clinical relevance. Furthermore, heavy metal resistance genes are being incorporated into new transferrable mega plasmids providing bacteria the ability to survive under selective pressure and favouring AMR spread.

M161 - Harnessing CRISPRi to unravel genetic mechanisms that underly persister formation and recovery in fluoroquinolone-treated *E. coli*

Presenting Author – *Silke Vercauteren, KU Leuven, Belgium*

Author/s – *Elen Louwagie, Lieze Agten, Sibylle Vonesch, Liselot Dewachter, Natalie Verstraeten, Jan Michiels, Daan Vermeiren*

Abstract Content

Antibiotics are indispensable for the treatment of bacterial infections. However, bacteria have developed a number of strategies to survive the lethal action of many antibiotics. One of these strategies relies on a subset of cells in an isogenic population that temporarily display an antibiotic-tolerant phenotype. After treatment, these so-called persister cells are able to recover and to establish a new bacterial population. Persistence thereby not only causes relapse of infections, but it can also lead to the emergence of antibiotic resistance. Despite its clinical importance, little is known about the mechanisms underlying bacterial persistence.

To close this knowledge gap, we performed a genome-wide CRISPR interference (CRISPRi) screen. This gene-silencing technique makes use of a catalytically dead Cas9 (dCas9) enzyme and single-guide RNAs (sgRNAs) that guide dCas9 to genes of interest, thereby blocking their transcription. By using a genome-wide sgRNA library, we have interrogated the role of each gene in both persister formation and recovery in *Escherichia coli*.

Our analysis indicates that widely different genes and gene categories are involved in persister formation or recovery phases. However, DNA repair and replication are important for both persister formation and recovery, pointing to similar mechanisms being active in both processes. Furthermore, active metabolism and biosynthesis pathways were identified as repressors of persister formation, and as activators of persister recovery, confirming that formation is related to dormancy while recovery of persisters relies on several active mechanisms. Combined, our data represent the most detailed picture generated to date of the genetic landscape underlying persistence.

M163 - An emerging MRSA clone, ST22-PT, positive for both Panton-Valentine leukocidin and toxic shock syndrome toxin-1 genes in Japan

Presenting Author – *Hiroshi Kaneko, Tokyo University of Pharmacy and Life Sciences, Japan*

Author/s – *Yuka Yanagi, Shogo Otake, Mayu Sato, Takumi Saito, Hidemasa Nakaminami*

Abstract Content

Background and Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) produces a wide range of virulence factors, including Panton-Valentine leucocidin (PVL) and toxic shock syndrome toxin-1 (TSST-1). Genes coding for PVL and TSST-1 have been generally used as epidemiological markers of *S. aureus* and are associated with severe infectious diseases. MRSA strains with PVL or TSST-1 genes are ubiquitous, but those with both genes are rare and sporadic. In this study, epidemiological and genomic analyses were conducted to characterize PVL- and TSST-1-positive MRSA strains isolated in Japan.

Methods: A total of 6,433 MRSA strains isolated from 54 healthcare facilities in Japan between 2015 and 2021 were used. PVL and TSST-1 genes were detected by polymerase chain reaction. Whole-genome sequences of PVL- and TSST-1-positive strains were determined and analysed.

Results: Among MRSA strains, 26 strains from 12 healthcare facilities were both PVL- and TSST-1-positive, and all were classified as clonal complex (CC) 22. The genomic features of these strains were sufficiently conserved can be considered as the same clone, which was named as ST22-PT according to a previous study. Some of ST22-PT strains were identified in patients with typical clinical symptoms caused by PVL-positive or TSST-1-positive *S. aureus*. Phylogenetic analyses revealed that PVL- and TSST-1-positive CC22 strains with high similarity to the ST22-PT strains had been isolated in several countries. ST22-PT possessed ΦSa2 with PVL genes and a unique *S. aureus* pathogenicity island with TSST-1 gene. Our report highlights the risk of international spread of a PVL- and TSST-1-positive MRSA clone, ST22-PT.

M164 - Comparative genomic analysis of the monocyclic aromatic hydrocarbons degradation potential in genus *Defluviimonas*

Presenting Author – *Jihye Baek, Chung-Ang University, Republic of Korea*

Author/s – *Jong-Hwa Kim, Wonyong Kim*

Abstract Content –

Background*:* Monocyclic aromatic hydrocarbons (MAHs) are major environmental pollutant including benzene, toluene, and xylene, it can cause skin irritation, dizziness, headaches, and blood disorders including anemia and leukemia in humans. Many marine bacteria have a variety of beneficial properties, such as degradation of organic compounds.

Objectives: The purpose of this study is to determine the beneficial function based on genomic analyses.

Methods: The whole genome of *Defluviimonas marina* CAU 1641T was determined using Hiseq Illumina platform. Genes were annotated using RAST and PATRIC server, and protein and their functions were predicted by eggNOG pipeline. The benzene degradation pathway was mapped based on KEGG.

Results: Whole genome size of *Defluviimonas marina* CAU 1641T was 5.02 Mb with 4,885 protein-coding genes and 92 core house-keeping genes. Compared with the strain CAU 1641T and 15 reference Rhodobacteraceae strains, 17,806 genes were classified as the accessory and unique genes. The genome of the strain CAU 1641T contains genes encoding the degradation pathway of benzene and phenol and *Defluviimonas marina* CAU 1641T was found to be able degrade MAHs based on the gene annotation and biosynthesis pathway analysis. These results suggest that *Defluviimonas marina* CAU 1641T, which is a strain that represents a novel species in genus *Defluviimonas*, is proposed that support for biodegradation of monoaromatic compounds.

M165 - Diversity of the functional machinery of native microorganisms for oil spills bioremediation

Presenting Author – *Maria Luis Boto, ICBAS-Instituto de Ciências Biomédicas Abel Salazar, University of Porto, Portugal*

Author/s – *Dhwani K. Desai, Julie LaRoche, Catarina Magalhães, Ana P. Mucha*

Abstract Content

To improve bioremediation techniques to mitigate oil spills, marine microorganisms with the capacity to degrade petroleum compounds have been broadly studied. The increased knowledge in microbial diversity combined with the recent advances in functional metagenomics, allows the exploration of hydrocarbon degradation potential in diverse marine environments by also encompassing uncultured microorganisms. The aim of this work is to unravel the distribution and diversity of marine prokaryotes capable of degrading petroleum hydrocarbons, in the NW coast of the Iberian Peninsula, and study its functional machinery involved in the degradation of those compounds. Seawater samples from 5 sites along the NW coast of the Iberian Peninsula were collected and incubated in microcosms spiked with crude-oil and nutrients, for 15 days. Both natural seawater and microcosms samples were filtered through SterivexTM, their DNA was extracted and sequenced by DNA nanoball. The metagenomic analysis is currently being processed using the ANVIO pipeline (https://anvio.org/). The analysis of the metagenomes from natural seawater and respective oil-enriched microbial communities, will allow to characterize both communities at a functional level and observe the potential of the natural communities to degrade petroleum compounds and compare it to the oil-enriched communities. Moreover, it is expected the recovery of genomes of promising taxa capable to degrade petroleum hydrocarbons and study its genes of interest. Based on the genetic potential gathered from this analysis, it will be possible to develop a workflow to recover the most promising bacterial strains capable of degrading petroleum hydrocarbons to help implement more efficient bioremediation approaches.

M166 - Multiple forms of a local regulator YihW from *Escherichia coli*

Presenting Author – *Anna Rybina, Skolkovo Institute of Science and Technology, Russian Federation*

Author/s – *Anna Rybina*

Abstract Content

Background: Few studies have focused on translation of several protein forms from one gene in bacteria. Preliminary experiments in our laboratory demonstrated production of a shortened form of regulator YihW in *Escherichia coli*. YihW is a local TF from the DeoR/GlpR family. It controls utilization of sulfo-sugar sulfoquinovose (SQ), and probably an alternative pathway of lactose degradation. Both SQ and lactose might act as effectors for YihW.

Objectives: Here we aim to analyze the occurrence pattern of the alternative translation start of YihW from *E. coli* among its homologs in different bacterial species. We intend to predict differences in binding of SQ and lactose between both protein forms of YihW.

Methods: Conservation of the alternative translation start for YihW across various bacterial species was studied using phylogenetic analysis. Blind molecular docking was applied to predict binding interactions of SQ and lactose with both YihW protein forms.

Results: Phylogenetic analysis of the protein YihW tree suggests that candidate start of the short YihW form originated in a common ancestor of Enterobacteriales. According to molecular docking, for both YihW protein forms, favorable poses of SQ corresponded to the positively-charged pocket near the linker between DNA-binding and effector-binding domains indicating possible role of SQ in decreasing affinity of YihW towards DNA. Lactose may similarly affect the shortened YihW form since its optimal binding mode was predicted in the same protein region. As for larger YihW form, lactose was predominantly docked to the C-domain thus might act differently as a effector disrupting protein oligomerization.

M167 - Reverse transcription-quantitative PCR (RT-qPCR) without the need for removal of template DNA

Presenting Author – *Damir Dermic, Ruđer Bošković Institute, Croatia*

Author/s – *Nunzia Santini, Alessandro Esposito, Maria Chiara Feliciello, Sven Ljubić, Isidoro Feliciello*

Abstract Content

Background: One of the major problems in transcriptome analysis is inability to completely eliminate template DNA, which is indistinguishable from cDNA, thus resulting in false positive signals.

Objectives: We developed a novel method for transcriptome analysis by RT-qPCR (Reverse-Transcription quantitative Polymerase Chain Reaction), which circumvents the need for elimination of potential DNA contamination, therefore being more precise, simpler and more reproducible than the commonly used methods.

Methods: The novel procedure involves the use of a modified specific primer during reverse transcription step, which contains mismatched bases, thus producing cDNA molecules not perfectly homologous to genomic DNA. By using the same modified primer in PCR amplification step, only cDNA template is amplified since genomic DNA template is not recognized by the primer.

Results: We determined the expression of *Escherichia coli* recA and sulA single-copy genes by RT-qPCR using either modified primers, or following the standard procedure. No recA and sulA sequence amplification was observed using our method unless cDNA was created by reverse transcription. The level of recA and sulA sequence amplification was unaffected by genomic DNA elimination from the sample. Conversely, the current method, which uses standard random/oligo-dT primers, showed a false positive signal even when reverse transcription step was skipped and the genomic DNA was (obviously incompletely) eliminated by DNase I treatment. Hence, our method of using a modified primer during cDNA synthesis produces a cDNA-specific PCR signal that is unaffected by genomic DNA and therefore quantifies gene expression much more accurately than the standard, commonly used method.

M168 - Identification of the locus of adhesion and autoaggregation (LAA)-like pathogenicity island of *E. coli* in *Klebsiella pneumoniae*

Presenting Author – *Ilaria Menozzi, Izsler, Risk Analysis And Genomic Epidemiology Unit, Italy*

Author/s – *Alessandra Dodi, Martina Tambassi, Melissa Berni, Marina Morganti, Erika Scaltriti, Stefano Pongolini*

Abstract Content

Background: *Klebsiella pneumoniae* (KP) is an opportunistic pathogen with an extraordinary ability in incorporating exogenous genes. This makes it an insidious health threat because of the onset of strains with many acquired virulence and antibiotic resistance (AR) genes causing severe infections. We previously identified two clinical isolates (KP34 and KP47) of ST 307, carrying the virulence gene flu, described in *E. coli* and associated with biofilm formation.

Objectives: Identification in KP of mobile genetic elements responsible for the acquisition of virulence and AR genes previously described in *E. coli*.

Methods: The genomes of KP34 and KP47 were sequenced producing short (Illumina Miseq) and long reads (Oxford Nanopore), assembled by hybrid-assembly and annotated. Moreover, 1,500 closed genomes of KP were downloaded from NCBI. All genomes were investigated for the presence of flu genes and their genomic context.

Results: Three different flu alleles and one copy of the colistin resistance mcr1.1 gene, nearby one of them, were detected in both the KP34 and KP47 genomes. We identified three large chromosomal insertions of roughly 30-40 Kbp, located inside phe and met tRNA chromosomal genes, each containing one flu allele. The annotation of these insertions revealed the presence of an integrase, the mcr1.1 gene and other virulence genes involved in cell adhesion, suggesting the possible acquisition by KP of the LAA-like pathogenicity island (PAI), previously identified in *E. coli*. Four-five % of the downloaded KP genomes were shown to harbour the LAA-like PAI.

M169 - Dancing the Nanopore limbo – Bacterial genome reconstruction from small DNA quantities using Nanopore long-read metagenomics

Presenting Author – *Sophie A. Simon, Environmental Metagenomics, Research Center One Health Ruhr of the University Alliance Ruhr, Faculty of Chemistry, Germany*

Author/s – *Katharina Schmidt, Lea Griesdorn, André Rodrigues Soares, Till L.V. Bornemann, Alexander J. Probst*

Abstract Content

Genome-resolved metagenomics has shaped our understanding of microbial and genetic diversity in environmental samples. While short-read assemblies seldom result in complete, closed genomes from metagenomes (MAGs), Nanopore long-reads greatly improve the assembly of complete MAGs and thus, facilitate in-depth genome analyses. However, the recommended DNA quantities for library preparation usually exceed the DNA amount of DNA recovered from samples of low-biomass environments. In this study, we assessed the quality of sequencing, community composition, assembly, and MAG recovery using a high-molecular weight (HMW) community standard. Decreasing the input DNA amount in nine levels from 1000 ng (the recommended DNA input amount) to 1 ng in triplicates, we generated 27 metagenomes. Evaluating the results at multiple levels of a genome-resolved metagenomics pipeline, we show consistent sequencing quality and read mapping accuracy across all input quantities. The relative abundance of species in the metagenomes was stable down to 50 ng of input material and high-quality MAGs (≥ 95% completeness, ≤ 5% contamination) were recovered down to 35 ng of input material. Our results show that Nanopore reads generated from 1 ng input material substantially improved the quality of hybrid assemblies compared to the assembly of Illumina short reads only. We conclude that the recommended DNA amount for Nanopore library preparation can be substantially reduced while preserving MAG recovery efficiency from metagenomes and recommend including Nanopore reads in every assembly-based metagenomic study. Future research endeavors in, e.g., low biomass environments will benefit from this work, enabling the recovery of high-quality microbial genomes from metagenomes.

M170 - Opposing forces govern plasmid evolution

Presenting Author – *Paula Ramiro Martinez, Instituto Ramón Y Cajal De Investigación Sanitaria (irycis), Spain*

Author/s – *Laura Jaraba Soto, Jeronimo Rodriguez Beltran*

Abstract Content

Plasmids are autonomously replicating DNA molecules that coexist with chromosomes in bacterial cells. These genetic elements are not only drivers of horizontal gene transfer, but also provide an important source of genetic diversity, playing a fundamental role in bacterial ecology and evolution. It has been suggested that plasmids evolve faster than chromosomes, as mutation rate per gene linearly increases with plasmid copy number. However, segregation of plasmid copies to daughter cells is random, creating an additional layer of genetic drift —known as segregational drift— that might delay plasmid evolution. The interplay between plasmid mutational supply and segregational drift determines the evolutionary rate of plasmid-encoded genes, yet the relative contribution of these opposite forces remains unclear. Mutation accumulation experiments impose extreme population bottlenecks of one cell, therefore minimizing selection and maximizing genetic drift. Here we developed an experimental system based on a tunable copy number plasmid, and performed a mutation accumulation experiment to quantify the relative contribution of increased mutational supply and segregational drift. Thirty-six lines of a hypermutator strain carrying low, medium, or high-copy number versions of the plasmid were allowed to accumulate mutations during ~700 generations of evolution. Whole-genome sequencing of the clones will be used to compare multicopy and low-copy plasmids and disentangle the effects of segregational drift and mutational supply on plasmid evolution. Together, our results shed light on the forces that shape plasmid-mediated evolution and contribute to explain their extreme prevalence across the bacterial phylogeny.

M171 - The genetic background and culture conditions only marginally affect *Pseudomonas aeruginosa* evolution toward colistin resistance

Presenting Author – *Matteo Cervoni, University Roma Tre, Italy*

Author/s – *Alessandra Lo Sciuto, Naida Babić Jordamović, Silvano Piazza, Olivier Jousson, Alfonso Esposito, Francesco Imperi*

Abstract Content

Background: Colistin represents a last-resort treatment option for *Pseudomonas aeruginosa* multidrug-resistant infections, but colistin resistance is emerging. In *P. aeruginosa*, colistin resistance is always associated with chromosomal mutations that induce the aminoarabinosylation of lipopolysaccharide (LPS). However, several studies have shown that the effect of LPS aminoarabinosylation on colistin resistance varies among *P. aeruginosa* strains and/or experimental settings, and that many other mutations, unrelated to LPS modifications, can influence the extent of colistin resistance.

Objectives: To verify whether the evolutionary trajectories leading to high-level colistin resistance in *P. aeruginosa* are conserved in different genetic backgrounds and/or under different culture conditions.

Methods and Results: *In vitro* evolution experiments in the presence of increasing colistin concentrations were performed for two phylogenetically-distant reference strains, that represent the two major *P. aeruginosa* lineages, in a standard laboratory medium and in media that mimic *P. aeruginosa* growth during infections (human serum and artificial sputum medium). A representative number of colistin resistant clones were subjected to whole genome sequencing to assess whether different strains follow similar evolutionary trajectories regardless of growth conditions or whether some mutations have a strain- and/or growth condition-specific impact on colistin resistance. This analysis revealed that most colistin-resistant mutants share mutations in genes that can be clustered in five functional groups: LPS modification regulators; LPS biosynthesis; polyamine biosynthesis; fatty acid metabolism; outer membrane protein assembly. The generation of deletion/conditional mutants and recombinant strains is in progress to characterize the contribution and mode of action of these pathways during the acquisition of colistin resistance.

M172 - Bacterial structural variations play an important role in host-microbe interactions

Presenting Author – *Lei Liu, University of Groningen, University Medical Center Groningen, Netherlands*

Author/s – *Lei Liu, Eleni Tsompanidou, Daria Zhernakova, Daoming Wang, Jingyuan Fu, Hermie Harmsen*

Abstract Content

The gut bacterial community plays a vital role in host health and disease. The composition of this gut microbiota is characterized by genetic and environmental factors. While the abundance of specific taxa can be associated with health or various diseases, little is known about how host and gut microbiota interact. Here we study the structural variations (SVs) in bacterial genomes that encode specific metabolic pathways for micro-nutrient degradation that mediate interactions between host and gut bacteria. A specific inositol degradation pathway at a SV region of *Anaerostipes hadrus* codes for a new propionate-producing pathway and the deletion of this region is associated with a higher body mass index. This finding was confirmed by the substrate utilization of specific *A. hadrus* strains. Intriguingly, inositol has been used clinically to treat patients who have Polycystic Ovary Syndrome accompanied with obesity and high insulin resistance, indicating that inositol metabolism has important implications for body weight and human reproduction. In addition, SV segments were found in *Faecalibacterium prausnitzii* which associated with specific human genetic trades determining the composition of gut mucin, a link that was confirmed by substrate utilization analysis of human gut-derived strains. Altogether, our study demonstrates that in silico and *in vitro* analysis of genetic associations across the human genome and the bacterial metagenome can provide functional insights into the reciprocal host-microbiome relationship. Furthermore, metabolic pathways located at SVs in gut bacteria may be used by them to interact with the host, influencing its health.

M173 - BioAutoML: end-to-end machine learning package for life sciences

Presenting Author – *Robson Bonidia, University Of Sao Paulo, Brazil*

Author/s – *Robson Bonidia, Anderson Avila-Santos, Breno Almeida, Peter Stadler, Ulisses Rocha, Danilo Sanches, André C. P. L. F. de Carvalho*

Abstract Content

Humanity has faced several challenges related to healthcare, epidemiological problems, climate change, energy consumption, and water resources. Consequently, with advances in sequencing, an increasing number of biological data have been generated in the post-genomic age, where approaches have been developed for genomics, transcriptomics, and proteomics problems. Due to this large amount of data, opportunities arise to change these challenging scenarios using Machine Learning (ML) algorithms. ML can extract useful and meaningful knowledge from biological data, reducing research expenses and increasing scientific efficiency. These advances benefit our society and economy, impacting people's lives in various areas, such as health care, the environment, pollution, and water treatment. Nevertheless, many studies usually neglected FAIR data principles for software development in ML. Furthermore, other challenges are that ML approaches applied to biological data also require quality steps related to feature engineering, algorithm selection, and hyperparameter tuning. These processes are manual and require extensive knowledge of ML. To address this concern, we developed BioAutoML, which automatically runs an end-to-end ML pipeline. To the best of our knowledge, our proposal automates the longest pipeline for biological sequence analysis, encompassing feature engineering, ML algorithm recommendation, and hyperparameter tuning. So far, we have achieved promising results on several problems, such as SARS-CoV-2, anticancer peptides, pro-inflammatory peptides, HIV-1 sequences, and phage virion proteins. BioAutoML lowers the barrier to applying feature engineering and metalearning in biological sequences for non-experts, democratizing ML in life sciences.

M175 - Evolutionary analysis of the adhesion systems of the human oral pathogen *Porphyromonas gingivalis*

Presenting Author – *Josefa Nuñez-Belmar, Center for Genomics and Bioinformatics, Chile*

Author/s – *Josefa Nuñez-Belmar, Mauricio Morales-Olavarria, J. Andrés Rivas-Pardo, Juan P. Cardenas*

Abstract Content

Porphyoromonas gingivalis is a keystone pathogen in periodontitis, due to its role in driving dysbiosis, dysregulating host immunity, and maintaining inflammation. The first step to invade the host is the initial adhesion to gingival epithelial cells, via a variety of adhesion systems (FimA and Mfa1), filamentous structures anchored to its external bacterial membrane, are responsible for adhesion to the host. Previous studies showed that genes from these adhesion systems contain different genotypes, suggesting a potential effect of those variants on *P. gingivalis* adhesion ability. Therefore, the study of the diversity and evolutionary properties of those adhesion systems could lead to a better understanding of their role in infection and their host specificity.

To perform phylogenetic and other evolutionary analyses of conserved genes involved in adhesion systems in *P. gingivalis*, the fimA nucleotide sequences were obtained from a set of 84 high-quality *P. gingivalis* genomes. Phylogenetic analyses for aligned sequences were performed by using IQTREE. Tajima D values were calculated using the pegas package from R. Evolutionary pressure among sites was calculated using the FEL and SLAC packages from the Datamoney Server.

The analyses suggested that FimA has just two positively selected sites ($p \leq 0.05$) and a set of 101 negatively selected sites (according to FEL). Tajima's D value was 0.89 ($p > 0.05$), suggesting that there is no excessive allele diversity detected in the population of fimA genes. The contrast between positively and negatively selected sites and the determinants from FimA different genotypes (I-V) are also discussed.

M176 - A close-up of ESBL/AmpC plasmid epidemiology in *Escherichia coli* from Dutch broiler farms using hybrid sequencing techniques

Presenting Author – *Ingrid Cardenas Rey, Wageningen University & Research, Netherlands*

Author/s – *Michael S.M. Brouwer, Arjan Visser de, Kees T. Veldman, Teresita Bello Gonzalez, Quillan Dijkstra, Gerwin Bouwhuis*

Abstract Content

Background: Bacterial plasmids play a crucial role in the spread of antimicrobial resistance (AMR) in all ecosystems. Besides conferring adaptability of its host, plasmids also transfer AMR genes to commensal and pathogenic bacteria, representing a significant risk to human and animal health. Detailed information on the spread of AMR bacteria and characterisation of AMR-associated plasmids is essential to sustain robust surveillance programs and develop intervention strategies to reduce AMR.

Objectives: The objectives of this study were: (i) to determine the genetic relatedness and spread of ESBL/AmpC-producing *Escherichia coli* in conventional Dutch broiler farms using whole genome sequencing and (ii) to apply hybrid sequencing techniques to improve the characterisation of the plasmids associated with the horizontal transmission of AMR genes.

Methods: Broiler caecal samples were consecutively collected at five (A-E) conventional Dutch farms. Selective culturing (MacConkey + 1 mg/L cefotaxime) was performed on each sample. Confirmed ESBL-*E. coli* isolates were used for Short (Illumina) and long-read (Oxford Nanopore Technologies) sequencing analyses. Hybrid sequence assembly was performed with Unicycler for further downstream analyses.

Results: Only samples from farm A resulted in the detection of ESBL-*E. coli* in consecutive time points. Isolates of ESBL-negative (n=66) and ESBL-producing *E. coli* (n=47) from farm A were selected for hybrid sequencing and downstream analysis. Multidrug resistance was observed in 85% of the isolates, from which 76 out of 113 were ESBL/AmpC producers. ESBL/AmpC plasmids were successfully characterised. Several lineages of related *E. coli* isolates with identical plasmids were detected, suggesting clonal spread between consecutive production rounds.

M177 - Investigating the role of mbtE analogs of *Mycobacterium abscessus* under iron-limited conditions

Presenting Author – *Mark Foreman, Faculty of Dental Medicine, Hebrew University of Jerusalem, Israel*

Author/s – *Mark Foreman, Daniel Barkan*

Abstract Content

Introduction: *Mycobacterium abscessus*is an emerging pathogen with an increasing prevalence over the past 2 decades. Iron acquisition is essential for most pathogenic bacteria and is closely regulated. Bacteria developed complex mechanisms to acquire iron, and often these mechanisms are essential for full virulence. In Mycobacteria the acquisition of iron is driven by siderophores called “Mycobactins” that are synthesized by the mbt gene cluster. However, the relative importance of each mbt gene, specifically the pivotal gene mbtE, is unknown. *M. abscessus* has two analogs of mbtE (MAB_2248c & MAB_2122). Their relative importance, if any, is undetermined.

Methods: We identified an *M. abscessus* Tn-mutant with disruption of the mbtE gene: MAB_2248c, and also constructed a targeted deletion mutant of the MAB_2122 gene. We then tested both mutants for growth characteristics, dependency on iron supplementation, and transcriptomic response compared to WT in iron-limiting conditions.

Results: The MAB_2248c Tn-mutant had substantial growth defect, especially in iron-limited media. Supplementation with Mycobactin-J, hemin, blood, or albumin salvaged the poor growth. Similarly, secreted mature mycobactins from WT bacteria rescued the MAB_2248c Tn-mutant during iron deprivation. Despite considerable homology, and in contrast to MAB_2248c, the ΔMAB_2122 mutant did not exhibit any appreciable growth retardation compared to WT.

Significance: Our results demonstrate the first characterization of the mbtE genes in *M. abscessus*. Understanding this pathway is essential to deciphering iron acquisition within hosts and its role in pathogenesis, potentially facilitating the development of anti-mycobacterial therapy.

M178 - Genomic dissection of an *Escherichia coli* strain isolated from bacteremia reveals insights into its hybrid pathogenic potential

Presenting Author – *Alejandra Migene Guzmán Del Carpio, Butantan Institute, Brazil*

Author/s – *Claudia Andrade Freire, Rosa Maria Silva, Eneas de Carvalho, Waldir Pereira Elias Junior*

Abstract Content

Background: In previous studies (Freire et al., 2021; Moraes et al, 2021), a collection of *Escherichia coli* isolated from bacteremia was analyzed identifying the strain EC092 harboring genes encoding virulence markers of enteroaggregative *E. coli* (EAEC) and four serine proteases autotransporters of Enterobacteriaceae (SPATEs), indicating its hybrid pathogenic potential.

Objectives: To analyze phenotypic and genotypic characteristics of *E. coli* EC092.

Methods: Secretion of SPATEs Pet, Pic, Sat and SepA was evaluated in culture supernatant by immunoassays. The whole genome sequence (WGS) was obtained using the Illumina platform and analyzed in silico.

Results: EC092 was able to secrete Pet, Pic, Sat and SepA. WGS analyses showed its classification into phylogroup B1, ST278, serotype O165:H4 and found EAEC virulence markers (aggR, aatA, aap, aaiA and aaiG) and SPATEs genes (pet, pic, sat, and sepA). These analyses also detected the presence of genes encoding fimbriae, toxins and iron uptake systems; while those defining extraintestinal or uropathogenic virulence potential were not detected. Phylogenetic analyses with strains of all *E. coli* pathotypes revealed that EC092 is related to phylogroup B1 EAEC strains isolated from feces. The relationship of EC092 with 98 EAEC genomes isolated from different geographic regions located it in a clade with four EAEC strains isolated from diarrhea in Kenya and in Egypt. Our data showed that strain EC092, isolated from bacteremia, is a hybrid-pathogenic strain with characteristics of extraintestinal pathogenic *E. coli* (ExPEC) and EAEC, presenting genetic virulence potential for its translocation from the intestine to the bloodstream.

M179 - Evolution of *Burkholderia multivorans* traits required for persistent infections of the cystic fibrosis lung

Presenting Author – *Sara Gomes, iBB - Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Portugal*

Author/s – *Sara C. Gomes, Mirela R. Ferreira, Zeyu Xiong, Janet S. Lee, Vaughn S. Cooper, Leonilde M. Moreira*

Abstract Content

Persistent infections caused by *B. multivorans* are important drivers of disease progression in the cystic fibrosis (CF) lung. Studying the interactions between bacteria and a/biotic factors in CF lung has given insights into how these influence antibiotic resistance and pathogenesis. Although changes in these phenotypes are caused by permanent exposure to antibiotics and the immune system, how *B. multivorans* evolves during chronic infections is unknown. This work aims to re-create *in vitro* the *in vivo* evolution of *B. multivorans* when exposed to tobramycin and RAW macrophages [1]. When grown for 15 days under biofilm conditions (bead-model) supplemented with tobramycin, the populations showed mixed colony morphologies. The smaller colonies presented a shorter lag phase (or inexistent) when compared to their ancestor, indicating that these evolved strains can present a better fitness. Smaller colonies were also obtained in a groundbreaking approach of evolving *B. multivorans* inside RAW macrophages. In this approach *B. multivorans* evolved for 5 passages after which the populations were collected for analysis. Whole populations were sequenced and mutation analysis to identify key genes implicated in adaptation will be presented. Taken together, the results demonstrate that *in vitro* evolution experiments can be an important way to study the evolution of bacteria and, consequently, its adaptation in the CF lung.

M180 - Status assessment of the implementation of molecular monitoring methods

Presenting Author – *Tiina Laamanen, University of Oulu, Finland*

Author/s – *Veera Norros, Kristian Meissner, Katharina Kujala, Kristiina Vuorio, Pirkko Kortelainen, Petteri Vihervaara, Lilja Nikula, Jacqueline Jerney, Mikko Tolkkinen, Afry Finland, Stefan Lambert*

Abstract Content

Molecular monitoring methods are revolutionizing the field of environmental monitoring of different organism groups such as microorganisms, insects, or fish. They offer accurate, repeatable, and cost-efficient species identification and show great potential for automatization. To assess the status of the implementation of molecular monitoring methods, we conducted an evaluation of the international readiness level of these methods through a systematic review of the scientific literature published within the past five years.

A search of the Web of Science database was performed using relevant search strings. Following the systematic review protocol implemented in the CADIMA tool (https://www.cadima.info), articles were screened against predetermined study selection criteria primarily based on the abstract but referring to the full text where necessary. These criteria included the requirement that the study discusses the topic of applying or implementing the adopted methodology in monitoring, and that at least some of the analysed samples were collected from an outdoor environment. Based on the extracted data, we assessed the Technology Readiness Level (TRL) of the method presented or applied in each paper from the point of view of its implementation in routine monitoring.

The internationally published scientific research within the last five years is heavily dominated by aquatic environments. Methods are broadly validated in small-scale field studies, but systematic large-scale demonstrations are still scarce. Among the well-represented taxa, the technology is the most developed in fish (TRL7) and other vertebrates (TRL6), while taxonomically universal methods remain the least ready (TRL5).

M181 - Clustered gene orientation bias on Gemmatimonadota chromosomes

Presenting Author – *Jürgen Tomasch, Institute of Microbiology of the Czech Academy of Sciences, Czech Republic*

Author/s – *Sahana Shivaramu, Karel Kopejtka, Izabela Mujakic, Michal Koblížek*

Abstract Content

Background: On most bacterial chromosomes, the majority of genes is coded on the leading strand. The extent of this gene orientation bias ranges from up to 85% in some Firmicutes to little more than 50% in some Proteobacteria. The understudied phylum Gemmatimonadota has currently only four closed genomes available that share a unique chromosome architecture: A conserved 600 kb region around the terminus of replication shows a pronounced gene orientation bias while the rest of the chromosome does not.

Objective: Here, we asked which genome properties can explain the clustered gene orientation bias in Gemmatimonadota and if its origin can be traced back to earlier branching, or is conserved in later branching phyla.

Methods: We determined the location of conserved genes and possible sources of instability such as transposons and repetitive DNA in the genomes of Gemmatimonadota and neighboring phyla. We also analyzed transcriptomes of two Gemmatimonas strains in order to test if this unusual genome architecture has an impact on gene expression.

Results: In Gemmatimonadota, the region of interest is poor in repetitive elements compared to the rest of the chromosome and harbors mostly core genes of this phylum. These genes show a consistently high expression level, while gene expression outside this region is more variable. Despite 'unusual' chromosome architectures in some representatives, neither earlier nor later branching phyla show a clustered gene orientation bias as found for Gemmatimonadota. This suggests a unique evolution of chromosome architecture in this phylum.

M182 - The influence of lateral transduction on bacterial genome content and structure

Presenting Author – *Rebecca Man, University of Edinburgh, United Kingdom*

Author/s – *Natalie Ring, Jamie Gorzynski, José R Penadés, J Ross Fitzgerald*

Abstract Content

Background: Lateral transduction (LT) may be the most powerful form of horizontal gene transfer discovered yet. Large fragments of the bacterial chromosome are packaged and transferred by phages at high frequencies via delayed prophage excision. *Staphylococcus aureus*, a major human and animal pathogen, has a strongly conserved genome structure but diverse accessory genome. The localisation of phage attachment (attB) sites and phage packaging orientations could enable around 60% of the *S. aureus* chromosome to undergo LT-related mobilisation, facilitating gene gain and loss and/or genome maintenance.

Objective: To investigate the influence of LT on the genome content and structure of *S. aureus*, using an array of comparative genomic approaches.

Methods: A dereplicated dataset of 211 complete *S. aureus* genomes was analysed. Based on the distribution of phage integration sites and direction of packaging, the genome was differentiated into two regions: R1, where LT activity is predicted to occur, and R2 which is predicted to be unaffected by LT. The frequency and location of recombination hotspots, pseudogenes, insertion sequences and essential genes in these two regions were compared using tools such as Gubbins, Pseudofinder and ISEScan.

Results & Conclusions: Recombination hotspots occur downstream of attB sites, corresponding to the phage packaging orientations. Significant differences were observed between the R1 and R2 regions: pseudogenes and insertion sequences were more densely distributed in R2, while essential genes were more frequently located in the attB-abundant region R1. Overall, these data are consistent with LT demonstrating a key influence on *S. aureus* genome organisation and diversity.

M183 - AsmA-like proteins are essential but redundant for growth and cell envelope integrity in *Pseudomonas aeruginosa*

Presenting Author – *Davide Sposato, University Roma Tre, Italy*

Author/s – *Jessica Mercolino, Luisa Torrini, Riccardo Alegiani, Francesco Imperi*

Abstract Content

Background: The outer membrane (OM) is an essential structure of diderm bacteria that protects them from large and/or hydrophobic toxic molecules, including many antibiotics. The OM is composed of phospholipids and lipopolysaccharide in the inner and outer leaflets, respectively, and hosts integral proteins (OMPs) and lipoproteins. While the systems responsible for translocation and insertion of lipopolysaccharide, lipoproteins and OMPs have been elucidated, the mechanism(s) mediating transport of phospholipids to the OM has remained elusive for decades. Very recently, studies in the model organism *Escherichia coli* have proposed a role for AsmA-like proteins in this process.

Objectives: To broaden the characterization of AsmA-like proteins by verifying their relevance for OM homeostasis and phospholipid transport in the human pathogen *Pseudomonas aeruginosa*.

Methods and Results: Homology search and Pfam analysis combined with structural predictions revealed that *P. aeruginosa* possesses seven AsmA-like proteins, six of which have properties compatible with phospholipid transport from the cytoplasmic membrane to the OM. Deletion of asmA-like genes in all possible combinations in the reference strain *P. aeruginosa* PAO1, followed by growth assays in the absence or presence of OM perturbing agents or antibiotics, revealed that four AsmA-like proteins are redundantly essential for growth and OM integrity. This evidence was confirmed by generating rhamnose-dependent conditional mutants. Notably, while three of these AsmA-like proteins are also present and important for OM homeostasis in *E. coli*, the other is specific to *Pseudomonas*, thus expanding the range of AsmA-like proteins that might play essential role(s) in diderm bacteria.

M184 - *Klebsiella pneumoniae*: Genomic insights into a priority pathogen

Presenting Author – *Aastha Kapoor, Indian Institute of Technology Jodhpur, India*

Author/s – *Lavanya Arora, Tamal Dey, Ardhendu Chakrabortty, Shankar Manoharan*

Abstract Content

Background: *Klebsiella pneumoniae* (Kpn), a non-motile bacterium, known to cause life-threatening and intractable infections in hospital and community settings. This is due to Kpn acquiring multidrug resistance as well as hypervirulence, thereby leading to various lethal infections, even in healthy individuals. Kpn encodes multiple virulence factors such as the capsule, fimbriae, hypermucoviscosity and high-affinity iron-scavenging siderophores like enterobactin, salmochelin, yersiniabactin and aerobactin.

Objectives: To investigate the distribution of antimicrobial resistance (AMR) and virulence determinants in Kpn genomes obtained from across the world and obtain insights into the region-wise variation of these determinants.

Methods: A global collection of Kpn genomes from multiple continents were analyzed for their virulence profile and AMR gene distribution.

Result: Genomic analysis revealed presence of wide-spread multidrug resistance (even to last-resort antibiotics) and multiple genes contributing to hypervirulence indicating a possibility of convergence of these traits. We show that Kpn strains circulating in different geographical zones across the world have varying antimicrobial resistance and virulence profiles.The Indian Kpn strains appeared to have higher prevalence of specific iron acquisition systems carried on specific genetic elements compared to Kpn strains from other geographical regions. The rapid emergence and spread of such strains around the globe requires further genomic surveillance to effectively contain an impending public health threat.

M185 - Mobile genetic elements within rare plasmids in a clinical *Acinetobacter baumannii* isolate

Presenting Author – *Tomas Liveikis, Vilnius University Life Sciences Centre, Lithuania*

Author/s – *Edita Sužiedėlienė, Laurita Klimkaitė, Kotryna Kvederavičiūtė, Aleksandras Konovalovas, Julija Armalytė*

Abstract Content

Background: *Acinetobacter baumannii* is a gram-negative, opportunistic pathogen that is notorious for its antimicrobial resistance (AMR). Numerous mobile genetic elements (MGEs) found in *A. baumannii* enable shuffling AMR genes among other bacteria, which in turn drives them to become multi-drug resistant. Analysis of *A. baumannii* plasmidome could help identify new and unique plasmids, which could hint at new emerging patterns of resistance acquisition.

Objective: To analyze the genomic features of plasmids, identified in a clinical *A. baumannii* isolate in order to identify unique MGEs and observe genome-wide events resulting from their activity.

Methods: The plasmids of *A. baumannii* isolate AB64 were typed according to Bertini et al. (2010) PBRT scheme. Size profiles were identified using PFGE method. The isolate was sequenced using Illumina and Nanopore New Generation Sequencing technologies. Obtained data was assembled with SPAdes (v3.15.5) and Unicycler (v0.4.9) and annotated using PROKKA (v1.14.6).

Results: Hybrid assemblies revealed two circular plasmids, sized 111 kb and 16 kb, belonging to replicon types GR24 and GR18, respectively. 111 kb plasmid contained multiple insertion sequences (ISs), of which two were novel, as well as multiple genes, typically found in *A. baumannii* chromosome, indicating a DNA exchange between plasmid and chromosome, without involving known mechanisms. The 16 kb plasmid features 5 pdif sites, single IS and a toxin-antitoxin system brnTA, whose function in *A. baumannii* is yet unknown. Additional tests are still required for deeper insight, however, current evidence implies that these transposition events are possibly beneficial to clinical isolates' survival in nosocomial environment.

M186 - Indications of transmission of mcr-1.26 IncX4 plasmids along the poultry food chain to humans

Presenting Author – *Ulrike Binsker, German Federal Institute For Risk Assessment, Germany*

Author/s – *Ulrike Binsker, Kathrin Oelgeschläger, Annemarie Käsbohrer, Jens A. Hammerl*

Abstract Content

Antimicrobial resistance is one of the major Global Health challenges. The drivers of emergence and evolution of antimicrobial resistances include antimicrobial use and abuse in human, animal and environmental sectors. Their interconnection can contribute to the spread of resistant bacteria and resistance determinants between sectors, inevitably affecting the health of these contiguous habitats. The association of resistance determinants with mobile genetic elements facilitates transmission across habitat boundaries further aggravating the problem. One such example is the transmissible plasmid-mediated colistin resistance (mcr) first discovered in 2016. Since then, the main determinant mcr-1 has been found in a vast variety of plasmid backbones in diverse bacterial species across all sectors. To date, 34 variants of mcr-1 have been described with varying prevalences. Whereas common variants, such as mcr-1.1 can be used for quantification of transmission events, rare variants allow for epidemiological tracing-back analysis to identify the origin and transmission dynamics of these genes. mcr-1.26 is rare and was detected in 2018 in an *E. coli* isolated from a hospitalized patient in Germany. We report on the presence of mcr-1.26 in 16 *E. coli* originating from poultry, such as feces and retail meat, already found in 2014. The mcr-1.26 was located on transmissible IncX4 plasmids highly similar to the plasmid reported for the human samples. Our study provides indications the emergence in and transmission of mcr-1.26-carrying IncX4 plasmids along the poultry food chain. Finally, our study indicates ongoing plasmid evolution of mcr-1.26 IncX4 by the acquisition of an additional beta-lactam resistance gene.

M187 - The open pan-genome and virulence landscape of *Renibacterium salmoninarum*

Presenting Author – *Rodrigo Pulgar, Universidad de Chile, Chile*

Author/s – *Ignacio Chávez, Dinka Mandakovic, Christian Hodar, Rodrigo Pulgar*

Abstract Content

Background: *Renibacterium salmoninarum* is the etiological agent of bacterial kidney disease (BKD), which considerably affects farmed salmonids worldwide. Although this bacterium is one of the oldest known fish pathogens, the functional characterization of its pangenome has not been fully characterized.

Objectives: The aim of this study was to construct the *Renibacterium salmoninarum* pangenome using the largest collection of genomes available and then predict and characterize its virulence factors.

Methods: First, we assembled and annotated the complete collection of *R. salmoninarum* genomes (n=94) from the European Bioinformatics Institute (EMBL-EBI) to construct its pangenome using the Roary pipeline. Then, we predicted its complete set of virulence factors (VF) using the virulence factor database (VFdb). Subsequently, they were classified and characterized using an interaction network approach via STRING and Cytoscape. Finally, we selected the most connected VFs in the network and evaluated their differential expression levels between *R. salmoninarum* exponential and stationary phases of growth.

Results: Our results indicate that despite the high similarity between the 94 compared genomes of *R. salmoninarum*, its pangenome (3796 orthologous groups (OG)) is still open. From these OGs, we identified 197 VFs, of which 194 belong to the coregenome and only three are part of the R. salmoninarum dispensable genome. These VFs were categorized into "Storage and Processing", "Defense" and "non-specific" processes and showed high heterogeneity in their connectivity degrees in the network. Interestingly, the most connected VFs showed significant differences in their expression levels between their growth phases, suggesting they are relevant in the infection process.

M188 - Isolation and genome sequencing of two furans-degrading *Pseudomonas* strains from Atlantic Salmon microbiota

Presenting Author – *Carla Gárate Castro, Universidad de Chile, Chile*

Author/s – *Mario Tello Reyes, Danilo Pérez-Pantoja*

Abstract Content

Farmed salmon can be exposed to furan derivatives, either by their incorporation in the diet or through the unwarranted administration of nitrofuran antibiotics. Remarkably, no studies have been conducted to characterize the presence of furans-degrading bacteria in the microbiota of Atlantic salmon. We hypothesized that the exposure to furans could favour the presence of bacteria that metabolize these compounds in the intestinal microbiota of fish. Therefore, the purpose of this work is to isolate and characterize furans-degrading bacteria from Salmon gut microbiota. Samples of Atlantic Salmon intestinal microbiota were cultured in mineral media supplemented with 3 mM of 2-furoic acid (FA) as the sole carbon and energy source for 5 days at 16°C or 30ºC. FA-degrading bacteria were enriched by successive re-inoculation in fresh media. Two strains having the ability to use FA as the sole carbon and energy source were obtained, named IAF-1 and SSA-FA1. The taxonomic identification of both strains was inferred by partial sequencing of the 16S rRNA gene, revealing its relatedness with the *Pseudomonas* genus. Whole genome sequencing allows the identification of hmf/psf genes involved in furans degradation in both strains, however cluster organization of the genes was slightly different. The ability to metabolize additional furan derivatives was assayed revealing that 5-hydroxymethylfurfural, 5-hydroxymethyl-2-furancarboxylic acid, 5-formylfuran carboxylic acid, and 2,5-furandicarboxylic acid are also growth substrates for both strains. These results suggest that the intestinal microbiota of Atlantic salmon harbor furans-degrading bacteria sharing similar genetic systems as those described in bacteria isolated from unrelated environments

M189 - Bacterial Taxonomic Identifier (BACTAX-ID) a new method for bacterial classification

Presenting Author – *Miguel D. Fernandez de Bobadilla, Instituto Ramón Y Cajal De Investigación Sanitaria (irycis), Spain*

Author/s – *Teresa M. Coque, Fernando Baquero, Val F. Lanza*

Abstract Content

Introduction: Accurate bacterial identification is essential for correct disease diagnosis, treatment of infection, and trace-back of disease outbreaks and antibiotic resistance transmission. Current bacterial typing schemes are based on the genomic analysis of a variable number of chromosomal alleles that result in a profile number known as “sequence type” (ST). Such methods (MLST, cgMLST, wgMLST) are sensitive to small changes (a single SNP can generate a new ST, discard the accessory genome, preclude the detection of hybrid lineages and more important, are only available for a few species of biomedical relevance.

Methods: BACTAX-ID is a novel method based on genomic distances provided by MASH. To create strain clusters, we use “pseudo-cliques”, a network in which its elements (genomes) share a high similarity (every genome must be connected to 80% of the network minimum). The system is organized in four hierarchical levels of different ANI (average nucleotide identity) values of 98%, 99%, 99.5% and 99,9%. When two strains are similar over a threshold value, both are part of the same pseudo-clique and they receive the same label for that level. Therefore, each genome has 4 hierarchical numbers, one for each of the levels, (i.e. 2.3.4.12 *Escherichia coli*). BACTAX-ID was trained and validated for all the species included in the WHO “watch lists” of priority pathogens for antibiotic resistance, all species for which more than 25 genomes are available in public databases. BACTAX IDs were compared with those given for widely accepted typing schemes if any. Metadata was considered when necessary.

Results: The method showed better specificity in the identification than the different schemes of MLST or cgMLST. It also allowed to identify hospital outbreaks and follow the path and evolution of different clonal lineages of different species across countries. The scalability of the scheme permits the identification of emergent novel clones of any species.

Conclusion: BACTAX-ID is a universal identification system, scalable and neutral which allows the classification at a subspecies level with a high degree of specificity. The simplicity of the nomenclature makes it suitable for microbial epidemiology, microbial ecology, and biogeography of known and “novel” species.

M190 - Genome-wide identification and expression analysis of AuTophagy Genes (ATG) in cotton and role in *Fusarium* and Verticillium wilt

Presenting Author – *Surendra Pratap Singh, Dayanand Anglo-Vedic (PG) College, Kanpur, India*

Abstract Content

Autophagy is an evolutionary conserved intracellular degradation process in which cytoplasmic contents are degraded in the lysosome/vacuole, and the resulting macromolecular constituents are recycled. More than 30 AuTophaGy (ATG) genes have been identified in yeast. They are yet not identified in cotton. Here, we identify 32, 34, 40, and 25 ATG genes in *Gossypium arboretum*, *G. herbaceum*, *G. hirsutum*, and *G. raimondii*, respectively, through genome-wide analysis. The phylogenetic analysis revealed that these cotton ATGs were quite similar to *Arabidopsis* ATGs and divided into 6 groups: WD-40 has 2 ATGs, ATG27/like contains 21 ATGs, ATG9/like contains 23 ATGs, ATG_N/_C contains 16 ATGs, ATG-like contains 30ATGs, and ATG8-like has 40 ATGs in 2 sub-groups of 17 and 23 ATGs respectively. Further, physical properties analysis, evolutionary studies (Ka/Ks analysis), gene structure, chromosomal localization, and motif analysis were done in ATG members of Gossypium sp. The expression analysis shows the unique GhATG expression under the flower, leaf, root, stem, and anther. Further, qRT-PCR was performed to quantify the expression of ATGs in the *G. hirsutum* infected with cotton wilt-causing fungus *Fusarium oxysporum* and *Verticillium dahliae*. The elevated expression of unique ATGs showed their active involvement under fungal virulence. The RNA-seq was performed in combination with autophagy inhibitors/activators exogenously treated to the infected cotton plant. The DEGs are used to study the GO, pathway analysis, and MAPMAN. Overall, this study indicates that ATGs expression initially enhances resistance. Moreover, this can be used in developing new genes/strategies for the control of fungal pathogenesis in cotton.

M191 - Microgem pipeline: Tiered comparative genomic analysis of bacterial isolates

Presenting Author – *Rasmus L. Marvig, Rigshospitalet, Denmark*

Author/s – *Maria A. Misiakou*

Abstract Content

Background: Technological advancements have led to the implementation bacterial genome sequencing as a routine method in clinical microbiology laboratories, and comparison of genomic sequences generated from bacterial isolates are useful for epidemiological investigations.

Objectives: Nonetheless, while isolates from clinical samples comprise a wide range of species and levels of genomic diversity, there is a lack of bioinformatics pipelines tailored for a wide range of species to efficiently and accurately compare both distantly and closely related genomes.

Methods: Here, we present our Microgem pipeline built with a workflow manager to characterize and compare bacterial isolate genomes.

Results: From a single executable pointing to short-read sequence reads, Microgem first performs steps common to other pipelines: read trimming, genome assembly, species identification, multilocus sequence typing, identification of resistance and other determinants, plasmid prediction, and quality control. Secondly, Microgem performs a two-tier comparative genomics analysis to identify the genetic relationships and distances to relatives of a given isolate: (tier 1) assembled genomes of all samples of the same species and sequence type are compared using a maximum-likelihood phylogenetic method tailored to handle many genomes with potentially large genetic diversity; (tier 2) sequence reads from only closest genetic relatives from the prior analysis are aligned to a reference genome to get a more accurate analysis for closely related isolates.

We have weekly run Microgem over 38 weeks to analyze the genomes of total 8,708 bacterial isolates from a tertiary hospital, and the results from the two-tiered approach have sufficiently provided genomic information for epidemiological investigations.

M192 - Fusion/Fission protein families identification in Archaea

Presenting Author – *Anastasiia Padalko, University of Vienna, Austria*

Author/s – *Govind Nair, Filipa L. Sousa*

Abstract Content

The majority of new archaeal lineages remain without a cultivated representative, but the scarce experimental data from the cultivated organisms shows they harbor distinct functional repertoire. To unveil the ecological as well as evolutionary impact of Archaea from metagenomics we need a set of computational methods and in-depth analysis. The genome-wide fusion protein screening is meant to aid protein functional annotation. A fusion protein is defined as a protein consisting of two or more domain originally encoded by different genes. If the gene splits, then the process is called fission. Fusion identification is required for proper phylogenetic reconstruction and metabolic pathway completeness assessment. Functional mappings between fused and unfused proteins might partially fill the gaps in the metabolic models.

In the archaeal genome-wide screening we identified 1927 fusion/fission protein clusters in both new and well-studied lineages. The identified fusion/fission families belong to all types of metabolism, genetic and cellular processes. In our approach, several experimentally validated proteins known to have undergone fussion/fission events were also identified. However, more than a half of the protein families identified remains poorly characterised on either fused or unfused side. To distinguish fusion from fission events, we implemented the assignment based on a combination of numeric and taxonomic features. As a result, we retrieved more than 300 high-confidence fusion clusters with enrichment in energy metabolism and cofactor biosynthesis, while fissions prevailed in genetic information processing and central carbon metabolism. After bacterial mappings, out of the identified 1927 clusters, 150 (<8%) families turned out to be unique for archaea, corresponding to events that occurred at different depths of the archaeal tree.

M193 - Emergence and genomic characterization of IMP-producing multi-drug resistant *Pseudomonas aeruginosa* in Bulgaria

Presenting Author – *Ivan Ivanov, National Center Of Infectious And Parasitic Diseases, Bulgaria*

Author/s – *Ivan Stoikov, Deyan Donchev, Elina Dobreva, Rumyana Hristova, Stefana Sabtcheva*

Abstract Content

Background: Multidrug-resistant (MDR) *Pseudomonas aeruginosa* infections represent major public health concern and require comprehensive understanding of their genetic make-up.

Objectives: This study aimed to characterize two blaIMP- carbapenemase producing MDR *P. aeruginosa* strains.

Methods: Antimicrobial susceptibility testing was according to EUCAST. Carbapenemase activity was studied by CarbaNP and PCR. Biofilm formation was quantified by the crystal violet assay. Carbapenemase transmissibility was studied through mating experiments, whereas AMR gene expression was quantified by qRT-PCR. Short and long read NGS data were applied for genome assembly and inference of AMR, virulence determinants and MLST.

Results: Both PA3541 and PA3796A strains were categorized as MDR. PA3541 was susceptible to colistin, meropenem-vaborbactam, aztreonam, and cefiderocol while PA3796A only to amikacin and colistin. Two closely related blaIMP variants were detected: blaIMP-13 and blaIMP-84 and mating experiments suggested their chromosomal localization characteristic for the detected widespread clone ST621. In addition both strains had blaOXA-914, aph(3')-IIb, aac(6')-Ib4 as well as identical substitutions in gyrA(T83I) and parC(S87L) contributing to the MDR phenotype. PA3796A carried also aph(6)-Id, aph(3')-VIb, blaPER-1, while PA3541 harboured only aac(6')-29. BlaIMP cassettes were situated in In320 integron inserted in Tn5051-like transposon. Elevated expression of MexX multi-drug efflux operon was observed in both isolates. Due to an identical 10 amino acid indel preventing the primer binding, both strains showed negative oprD expression. A variety of virulence factors associated with adhesion, antiphagocytosis, iron uptake, quorum-sensing, as well as secretion systems, toxins, and proteases were confirmed, suggesting significant pathogenic potential consistent with the observed strong biofilm formation.

M194 - The effect of compensatory evolution in the emergence and transmission of rifampicin-resistant *Mycobacterium tuberculosis*

Presenting Author – *Galo Goig, Swiss Tropical and Public Health Institute, Switzerland*

Author/s – *Sebastien Gagneux*

Abstract Content

Background: The role of bacterial factors in the emergence and transmission of drug-resistant *Mycobacterium tuberculosis* remains unclear. Experimental data show that drug resistance-conferring mutations are often associated with a decrease in replicative fitness of bacteria *in vitro*, and that this fitness cost can be mitigated by compensatory mutations. However, the role of compensatory evolution in clinical settings has been controversial.

Objectives: To assess whether compensatory evolution is associated with amplification of drug resistance and a higher transmission probability of rifampicin-resistant tuberculosis.

Methods: We carried out a genomic epidemiological study by analyzing available *M. tuberculosis* isolates and their associated clinical data from patients routinely diagnosed with rifampicin-resistant tuberculosis. We applied Bayesian reconstruction of transmission trees and phylogenetic multivariable regression analysis to identify patient and bacterial factors associated with transmission of rifampicin-resistant *M. tuberculosis* strains.

Results: Compensatory evolution was associated with smear-positive pulmonary disease (aOR=1.49; CI=1.08-2.06) and a higher number of drug resistance-conferring mutations (IRR=1.38; CI=1.28-1.48). Compensatory evolution was also associated with increased transmission between patients (aOR=1.55; CI=1.13-2.12), independently of other patient and bacterial factors. Our findings suggest that compensatory evolution enhances the *in vivo* fitness of drug-resistant *M. tuberculosis* genotypes, both within and between patients.

M195 - Microorganisms and the Nagoya Protocol

Presenting Author – *Elke Brockmann, Chr. Hansen A/S, Denmark*

Abstract Content

The Nagoya Protocol, a supplementary agreement to the Convention on Biological Diversity (CBD) of 1992, seeks to facilitate the protection of biodiversity globally. In April 2023, 139 countries were parties to the Nagoya protocol.

The CBD recognizes sovereign rights of countries over the genetic resources in their territory. Incentive and eventually financial contribution to the necessary investments for the biodiversity conservation are intended to be created for the membership countries by partaking in benefits arising from utilization of genetic resources. The Nagoya protocol further enforces the sharing of the benefits arising from utilization of genetic resources: Countries, that are party to the Nagoya Protocol have the obligation to monitor the compliant use of genetic resources by researchers, companies and in general users of genetic resources located in their jurisdiction. The approaches taken for the implementation of access and benefit sharing principles in legislation and the bureaucratic system can differ considerably in the different member countries.

While the Nagoya protocol primarily is designed for animal and plant genetic resources, no distinction is made between animal/plant genetic resources and microorganisms in most national legislations. The inclusion of microorganisms is discussed controversially due to the ubiquitous nature and the widespread existence of niches allowing for the proliferation of many microorganism species.

Challenges and implications for users of microbial genetic resources, for research progress and commercial/institutional collaborations will be discussed.

M196 - The IS91-encoded Orf121 negatively influences IS mobility

Presenting Author – *Aurélien Fauconnier, INSERM, University of Limoges, CHU Limoges, RESINFIT, UMR1092, France*

Author/s – *Aurélien Fauconnier, Margaux Gaschet, Thomas Jové, Marie-Cécile Ploy, Cécile Pasternak*

Abstract Content

Prokaryotic insertion sequences (IS) contribute to bacterial multidrug resistance. IS91 is usually associated with virulence and antibiotic-resistance genes, but its contribution to their dissemination is unclear. IS91 belongs to an atypical IS family with a HUH transposase mediating transposition events using a rolling circle transposition mechanism. This IS family displays two functionally distinct ends: transposition initiates at the so-called oriIS end and terminates at the terIS end. However, terIS may not be recognized, resulting in the mobilization of an adjacent DNA fragment ("one-ended transposition"). Unlike the other family members, IS91 carries a small ORF potentially encoding a 121 amino acids polypeptide, Orf121, upstream of the tnpA transposase gene.

We investigated the role of Orf121 in the *in vivo* transposition of IS91 using a genetic system based on the mating-out procedure in *Escherichia coli*.

We showed that co-expressing Orf121 (in cis or trans), together with the transposase gene, strongly decreased both the IS91 transposition frequency and the one-ended transposition rate; these results indicate the Orf121 polypeptide may exert a negative effect on the *in-vivo*-transposition of IS91 and that it may be required for accurate recognition and cleavage of the terIS end. Then, we mapped more than 1,000 *in vivo* IS91 insertion sequences and showed that the target site specificity of IS91 does not depend on Orf121. Finally, we showed that the activity of the Porf121 promoter is significantly higher than that of the PtnpA promoter.

M197 - Horizontal gene transfer network in gut microbiome

Presenting Author – *Darina Cejkova, Brno University of Technology, Czech Republic*

Author/s – *Jana Schwarzerova, Ivan Rychlik*

Abstract Content –

Selection pressure on animal gut commensal bacteria due to antibiotics overuse in farming for decades has consequences in global dissemination of antibiotic resistant genes. Animal gut represents an unexplored reservoir of antibiotic resistant genes, which can be mobilized with other cargo genes via horizontal gene transfer across bacteria and disseminated to other niches.

Traits of horizontal gene transfer in genomes of gut microbiota were analysed using data mining, computational and network analyses. We established a bacterial culture collection of 452 isolates originated from healthy chicken and porcine gut samples. Extracted gDNAs were sequenced on Illumina platform, assembled by SPAdes, annotated by PROKKA and characterised by eggNOG-mapper. A semi-automated pipeline to search for nearly identical genes (> 99 % id over 99 % length) co-shared by different genera has been designed and statistically evaluated.

Altogether over 6000 genes suspected to horizontal gene transfer were identified. Genes associated with intracellular trafficking and secretion and DNA repair were enriched, also genes of unknown function were dominant. Main reservoirs of genes associated with horizontal gene transfer were found in *Phocaeicola* sp. (Bacteroidaceae) and UBA9475 sp. (early Pseudoflavonifractor, Oscillospiraceae). Only several genes were co-shared between Gram-positive and Gram-negative bacteria, mostly genes directly associated with mobilome and antibiotic resistance. On the contrary, main gene transfer was detected between different genera of the same phylum. Therefore, we suggest that strong selection pressure on gene alleles exist at this level. More importantly, hypothetical genes represent important yet an unknown section of resistome and/or mobilome.

M198 - A previously uncharacterised hydrogenase dominates fermentation in the human gastrointestinal tract

Presenting Author – *Caitlin Welsh, Monash University, Australia*

Author/s – *Caitlin Welsh, Chris Greening, Samuel Forster, Rachael Lappan, Dena Lyras, Gustav Berggren, Jodee Gould, Remy Young, Emily Gulliver, Tom Watts, Nhu Quynh Doan*

Abstract Content

Molecular hydrogen (H_2) is an important metabolite cycled by microorganisms within the human gastrointestinal tract (GIT) with key roles in human nutrition and health. H_2 is produced during fermentation by various bacteria and consumed as an energy source by other bacteria, including acetogens, methanogens, and sulphate reducers. H_2 has traditionally been used as an indicator of gut dysbiosis through breath tests and the disruption of H_2 cycling is associated with colorectal cancer, IBS and other GIT disorders. Despite strong links to human health, the microorganisms, pathways, and enzymes responsible for gastrointestinal H_2 production remain unresolved. Here we show that a previously uncharacterised enzyme, the Group B [FeFe]-hydrogenase, encoded by all four dominant gut phyla, primarily mediates fermentative H_2 production. Leveraging a dataset of 300 stool and biopsy metagenomes, 78 metatranscriptomes, and 801 gut bacterial isolate genomes, we show the genes for this enzyme are abundant, highly expressed, and widely distributed in the human GIT. Based on transcriptomic and gas chromatography analysis of 16 taxonomically diverse gut isolates, the Group B [FeFe]-hydrogenases mediate rapid H_2 production during fermentative growth. Furthermore, *Bacteroides*, a genus previously unknown to be hydrogenogenic, are dominant H_2 producers in this environment. Additional biochemical characterisation confirmed that the Group B [FeFe]-hydrogenase is catalytically active and binds a diiron centre. This combination of culture-dependent and independent analysis provides new insights into how H_2 is produced within the human GIT, and identifies the key groups involved, enhancing our ever-growing understanding of the impacts of the gut microbiota on human health.

M199 - A genomic profile of *Acinetobacter calcoaceticus-Acinetobacter baumannii* (Acb) complex in South Korean agricultural envrionments

Presenting Author – *So Yun Jhang, eGnome Inc., Republic of Korea*

Author/s – *So Yun Jhang, Sojin Ahn, Jaewoong Yu*

Abstract Content

Acinetobacter is a genus of opportunistic pathogens in the Proteobacteria group, species of which are widespread in the environment, such as soil, water, and sewage. The rapid emergence of antibiotic-resistant strains poses a significant threat to public health and demands urgent attention. While numerous studies have investigated *Acinetobacter* strains in hospital environments, limited information is available on the role of different *Acinetobacter* species and antimicrobial resistance profile in agriculture environments. In order to understand the genetic mechanisms underlying resistance, we provide new data and insights regarding evolutionary dynamics and the content of genetic diversity on 11 whole-genome sequences (WGS) of streptomycin-resistant *Acinetobacter calcoaceticus-Acinetobacter baumannii* (Acb) complex that was isolated from different environments and locations in South Korea. Long-reads were sequenced with the MinION sequencer (Oxford Nanopore Technologies), followed by genome assembly and genome completeness using Flye and BUSCO, respectively. Afterwards, pan-genome analysis was performed to characterize the genomic compositions among different *Acinetobacter* species as well as focus on the phylogenetic relationships, and evolutionary trends of *Acinetobacter*. As a result, the size of 11 complete genomes were varied from 3.8Mbp to 4Mbp with GC content of 38% to 41%. The presence of mobile genetic elements, including insertion sequences and transposons, may contribute to the dissemination of antibiotic resistance genes. Importantly, we identified numerous multi-resistant genes, including aminoglycoside antibiotic genes, indicating the potential for the rapid spread of resistance in agricultural settings. Therefore, our study provides further insights into the occurrence and resistance profile of Acb complex of agriculture environment origin.

M200 - Whole genome sequence of *Treponema paraluisleporidarum* ecovar Lepus, strain V3603-13

Presenting Author – *David Šmajs, Masaryk University, Czech Republic*

Author/s – *Petra Pospíšilová, Darina Čejková, Pavla Havlíčková, Pavla Fedrová, Lenka Paštěková, David Najt, Linda Hisgen, Simone Lueert, Friedrich-Loeffler*

Abstract Content

Background: Lagomorph syphilis is caused by *Treponema paraluisleporidarum* ecovar Cuniculus (TPeC) and ecovar Lepus (TPeL), infecting rabbits and hares, respectively. This pathogen is closely related to the human pathogen *Treponema pallidum*, causig syphilis (ssp. *pallidum*), yaws (ssp. *pertenue*), and bejel (ssp. *endemicum*).

Objectives: The main objective of this study is to determine the first complete genome sequence of TPeL and compare it to available treponemal sequences.

Methods: The complete genome sequence of TPeL, isolate V3603-13, from an infected mountain hare (Lepus timidus) has been determined with previously invented pooled segment genome sequencing approach.

Results: The TPeL V3603-13 genome revealed an overall gene synteny with the TPeC Cuniculi A genome and with the human pathogen *T. pallidum*. Compared to the TPeC Cuniculi A genome, TPeL V3603-13 contained four insertions and 11 deletions larger than three nucleotides (ranging between 6–2,932 nt). In addition, there were 25 indels one to three nucleotides long, altogether spanning 36 nt. Moreover, the nucleotide variants between the TPeC Cuniculi A genome and TPeL V3603-13 included single nucleotide variants (SNVs, n=293) and double nucleotide differences (n=8, covering 16 nucleotides). Major proteome differences between TPeL and TPeC were found to be encoded by the tpr gene family and by genes encoding outer membrane proteins, which suggests that these components are essential for host adaptation of lagomorph syphilis agent.

M201 - Investigation of nasopharyngeal microbiota profiles of COVID-19 patients

Presenting Author – *Hatice Turk Dagi, Selçuk University, Turkey*

Author/s – *Ekin Eryilmaz, Ecem Narin Copur, Dilek Ergün, Salih Macin, Ugur Arslan*

Abstract Content

The coronavirus epidemic started in China. February 2020, cases of new coronavirus-related pneumonia were named as Coronavirus Disease 2019 (COVID-19) by the World Health Organization. Microbiota is major player in mediating immune response and can also affect viral infectivity. In this study, we aimed to analyze the changes in the nasopharyngeal microbiota profiles by comparing COVID-19 patients and healthy controls.

Thirty two patients admitted to Selcuk University Medical Faculty Hospital between 1 November 2020 and 1 February 2021 and 16 healthy controls were included to the study. The hypervariable regions of 16S rDNA genes (V2, V4, V8, V3-6, V7-9) were sequenced from nasopharyngeal swabs using the Ion Semi-conductor method on the Ion 5S (Ion Torrent™, TermoFisher, USA) device, and the data were transferred to the Torrent suite and Ion Reporter (ThermoFisher, USA).

No significant difference was between the nasopharyngeal microbial alpha diversity of patients and healthy controls ($p>.05$). Firmicutes phylum was significantly higher ($p=.037$), while Bacteriodetes ($p=.006$) and Proteobacteria ($p=.032$) phylums were lower in patients. Prevotellaceae was lower in patiens ($p=.023$). Neisseria genus was 60% lower ($p<.001$), Rothia mucilaginosa 87% higher ($p=.010$) and Veilonella atypica 46% lower ($p<.001$) in patients.

In conclusion, *Firmicutes* was higher, *Bacteriodetes* and *Proteobacteria* lower, *Neisseria* lower and *R. mucilaginosa* higher in patiens. Determining the nasopharyngeal microbiota profile of COVID-19 patients can help both to detect biomarkers to assess the severity of the disease and to develop therapeutic/prophylactic strategies. Further studies are needed on the importance and function of the nasopharyngeal microbiota in viral infections.

M202 - Integrative metagenome and transcriptome analysis reveals a host-microbe interaction of KGMB04 as a potential therapeutic target

Presenting Author – *JIHWAN PARK, Korea Research Institute of Bioscience and Biotechnology, Korea, Republic of*

Author/s – *Eunsol Um, Hyun-Ahm Sohn, Hanyong Go, Yang-Ji Shin, Jung-Sook Lee, Mirang Kim*

Abstract Content

There are still unmet needs for the development of therapeutics for non-alcoholic steatohepatitis (NASH), which involves the accumulation of fat and inflammation in the liver without significant alcohol consumption. Based upon the previous findings that the gut microbiome can contribute to the alleviation of NASH via a gut-liver axis, here, we applied a systems approach to the gut microbiome in a NASH mouse model. We performed a comparative metagenome analysis of the large intestine from the mice with normal chow or NASH diet and identified differentially abundant species, showing a significant change in microbial abundance between the two conditions. We then selected KGMB04 among the species by applying a co-occurrence network analysis. To examine the therapeutic potential of KGMB04, we compared the transcriptome profiles of NASH diet mice in the absence or presence of KGMB04 treatment. We showed that the genes associated with immune response and fibrosis were significantly downregulated, whereas the genes associated with glucose and lipid metabolism were upregulated, suggesting that KGMB04 may play an important role in both reduction of lipid levels and resolution of inflammation in the liver. Furthermore, a molecular network analysis revealed that the key metabolic pathways regulated by the treatment of KGMB04, which can be the potential mechanism for alleviation of NASH. Therefore, our integrative metagenome and transcriptome analysis of the NASH mouse model revealed a therapeutic potential of KGMB04 and its host-microbe interaction in NASH treatment.

M203 - The Nagoya Protocol on access and benefit-sharing (ABS): does it matter for me?

Presenting Author – *Melania Munoz-Garcia, Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures GmbH, Germany*

Author/s – *Amber Scholz*

Abstract Content

The Nagoya what? The Nagoya Protocol was established to ensure that benefits arising from the utilization of genetic resources are shared with biodiversity holders. Microbiology study results can contribute to implement innovative solutions that support conservation and sustainable use of biodiversity locally. That is the ABS principle. Based on that, many countries around the world regulate access to biological material for research purposes. That means, researchers must be aware of how to legally access genetic resources and the implications of non-compliance.

The German Nagoya Protocol HuB project (GNP-HuB) is here to help! The project aims to support the academic sector in Germany (and beyond) to understand ABS and their obligations.

What do we do? The GNP-HuB focuses on developing user-friendly and interactive tools to communicate about ABS to make it accessible and understandable by the research community.

What have we developed? The GNP-HuB has established a platform for exchange, discussion and support. Our website provides easy to digest information about ABS and compliance in the European Union. The HuB network is where German scientists can share and learn from others' experiences. The project supports information and awareness raising sessions at different levels and manages a help desk platform to address specific cases.

Upcoming issues: A multilateral system for benefit-sharing from digital sequence information (or DSI, a policy term that refers broadly to genomic sequence data) is under development. Why should scientists be aware of this discussion? The results could impact the way genetic research is performed nowadays.

M204 - Shock toxic toxin gene (tst) frequency and analysis of virulence by whole genomic sequencing of ten *Staphylococcus aureus* strain

Presenting Author – *Carmen Aravena, University of Valparaiso, Chile*

Author/s – *Javier Tognarelli, Constanza Toloza, Camila Quintana*

Abstract Content

Background: *Staphylococcus aureus* (SA) is part of the human microbiota that causes different diseases pneumonia, bacteriemia, food poisoning, and shock toxic syndrome, encoded multiple virulence factors; however, SA has different genetic determinants, so we don't know the virulome of circulant strains in Valparaiso.

Objectives: To study the frequency of shock toxic syndrome gene (tst) and characterize the virulome of clinical and carriers SA strains.

Methods: sts gene was detected by PCR in 146 strains (47 clinical, nasal carriage: 41 elderly people, and 58 from nursing and medicine students). Six carriers' strains and four clinical strains were selected for WGS (Illumina MiSeq). The genetic characterization of each strain was analyzed using bioinformatics tools https://www.genomicepidemiology.org/.

Results: tst frequency was higher in students than elderly people or clinical isolates (13,8% vs 4,0%). Clinical strains were MRSA1 (SCCmecII2A-ST225-CC5, MRSA13 (SCCmecIB-ST5-CC5), MRSA7 (SCCmecIVC-pvl+ST8-CC8), and MSSA14 (ST2267-CC30). The carriers` isolates MRSA2 and MRSA52AM were SCCmecIB-ST5-CC5. MSSA strains 63e, 73AM, 181e and 189AM were ST109-CC1, ST398, ST30-CC30 and ST45-CC45 respectively. All strains encoded hemolysin genes, 8/10 positives for staphylokinase, 7/10 encoded the complement inhibitor (scn), lukD/lukE genes were in all MRSA strains, lukF/lukS in MRSA7-ST8, and ACME only in MSSA189AM-ST398.

Discussion and Conclusions: SA isolated from infection and carriers encoded different pathogenic potential, probably related to their genetic lineage. We describe the virulome of Chilean/Cordobes clone in clinical and carriers' strains, clones of global circulation (USA100, USA300), and clonal complexes described in carriers so CC45 and ST398 are for the first time reported in Chile.

M205 - ABRomics - an open access online platform on antimicrobial resistance to store, integrate, analyze and share multi-omics data

Presenting Author – *Philippe Glaser, Institut Pasteur, France*

Author/s – *Julie Lao, Pierre Marin, Romain Dallet, Fabien Mareuil, Etienne Ruppé, Claudine Médigue, Alix De Toisy, Kenzo-Hugo Hillion, Aurélien Birer, Nadia Goué, Richard Bonnet*

Abstract Content – Antibiotic resistance (ABR) is a major public health issue prioritized for mitigation by international institutions. Multidrug resistant bacteria (MDRB) and Antibiotic Resistance Genes (ARGs) carried by mobile genetic elements spread between the human, animal, and environmental sectors. Whole Genome Sequencing (WGS) is used for molecular typing purposes at the highest resolution. It provides identification of ARGs and their genetic supports as well as mutations leading to a decrease in antibiotic susceptibility. Epidemiological and WGS data are used for tracking MDRB in hospital outbreaks but also across the animal and environmental sectors. Sharing and interoperability of high-quality data (sequence and metadata) are key requirements for addressing the spatio-temporal dissemination of MDRB. To this aim, the French Priority Plan on ABR has funded the development of an online, open platform dedicated to antibiotic resistance.

We are establishing a repository of structured, interoperable, standardized, and well-annotated multi-omics data with tailored mathematical and bioinformatics tools to answer generic and specific research questions related to ABR. The ABRomics platform includes standardized pipelines to run ABR analyses of WGS from pathogenic strains supported with integrated databases (ARG, sequence types [ST], virulence factors [VF]). Uploading data, launching pipelines, viewing and cross-referencing enriched results will be achieved through easy-to-use web interfaces. ABRomics ß-version integrating the ABR detection genomic pipeline and other markers such as ST, and VF will be available in summer 2023. Core-genome multi-locus sequence typing, relationships between strains and metagenomics pipelines will next be made available.

M206 - Bioinformatic analysis of genomic islands in whole genomes of *Piscirickettsia salmonis* strains

Presenting Author – *Jaime Figueroa, Universidad Andres Bello, Chile*

Author/s – *Genaro Soto-Rauch, Guillermo Nourdin-Galindo, Denise Haussmann, Jaime Figueroa*

Abstract Content – Piscirickettsiosis is a systemic infection generating great losses in salmonids in Chile, and in that sense the outbreaks of the causative agent *Piscirickettsia salmonis* have been controlled with antibiotics and vaccines; however, the bacterium has exhibited a variety of evasive strategies including the formation of biofilm. The bacterium shows genomic islands as clusters of relevant genes that move within and between genomes with various elements of their own. This work was focused on analyzing the relationship between the number of pathogenic islands and their virulence genes in complete genomes from different strains of EM and LF genogroups, as well as on the search for genomic islands related to non-virulent factors with metabolic islands and fitness. Genomic island conservation and variation between LF and EM genogroups from different strains was evaluated, considering LF-89 and Psal-001 as reference strains. The genomes were analyzed with GIPSy and IslandViewer bioinformatics tools. About 13 pathogenic islands per strain were quantified, with 1-47 loci per island with behaviors unique to each genogroup. When comparing islands with respect to genomes, a higher conservation rate was observed in the EM genogroup in contrast with oscillating values within members of the LF genogroup. Regarding non-virulent factors, genes related to metabolic processes were found. From an in-silico perspective, the EM genogroup showed greater convergence in pathogens islands when compared to LF genomes. Finally, different types of transposases were found to support a high genomic plasticity.

M207 - Phenotypes, virulence factors and vesicles associated with clinical strains of *Pseudomonas aeruginosa*

Presenting Author – *Tania Henriquez, University of Siena, Italy*

Author/s – *Tania Henriquez, Francesco Santoro, Donata Medaglini, Lucia Pallecchi, Massimiliano Marvasi, Eugenio Paccagnini, Mariangela Gentile, Pietro Lupetti, Chiara Falciani*

Abstract Content

Background: *Pseudomonas aeruginosa* has become a major health concern, as it was reported as the sixth pathogen responsible for resistance-associated deaths in 2019. Several mechanisms have been linked to the ability of this microorganism to colonize the host, including virulence factors, resistance mechanisms and, more recently, extracellular vesicles.

Objective: The aim of this work was to characterize the phenotype, virulence factors and vesicles associated with clinical strains of *P. aeruginosa*.

Methods: We tested a total of 15 *P. aeruginosa* strains, 8 collected from clinical samples, 5 from cystic fibrosis patients and 2 reference strains. The isolates were characterized for pigment/siderophore production, hemolysis, growth phenotype, susceptibility to antibiotics, among others. From them, 4 strains (including reference strain *P. aeruginosa* PAO1) were selected according to their virulence factors/phenotype and used for extracellular vesicles purification and characterization.

Results: Our analysis indicated that 5 isolates were sensitive to antibiotics (including 3 strains with intermediate results), while 3 strains were resistant to all the tested antibiotics. The other 5 strains had different combinations of resistance to carbapenems, cephems, aminoglycosides, and fluoroquinolones. Also, significant differences in growth behavior were observed as well as in pigment production. Two of the most resistant strains (a fast- and a slow-growing strain) together with a mucoid/pyorubin-producing strain were selected for vesicle purification. Altogether, our results support previous reports of highly diverse phenotypes among clinical *P. aeruginosa* strains and highlight the importance of studying their virulence factors and vesicles to better understand their pathogenicity.

M208 - Identification of *in vivo*-expressed proteins in the biofilm matrix and outer membrane vesicles of *Histophilus somni*

Presenting Author – *Thomas Inzana, Long Island University, College Of Veterinary Medicine, United States*

Author/s – *Yue-Jia Lee, Mohd Abdullah, Yung-Fu Chang*

Abstract Content

Background: There is limited efficacy in vaccines currently available to prevent some bacterial diseases, such as bovine respiratory disease (BRD) due to *Histophilus somni*, which is an excellent model for biofilm-related infections. Vaccine efficacy can potentially be improved based on inclusion of bacterial antigens expressed in the host, rather than those expressed only during *in vitro* growth.

Objectives: During *H. somni* infection in the bovine host, biofilms become well established, and essential iron is restricted. Half of the *H. somni* genome is differentially expressed during biofilm formation, compared to planktonic growth, and novel iron-binding proteins are expressed only *in vivo*. Therefore, the protein composition of spontaneously released outer membrane vesicles (OMVs) was examined during iron-sufficient and iron-restricted growth, and in the biofilm matrix compared to planktonic cells.

Methods and Results: Mass spectrometry-bioinformatic proteome analysis revealed a dramatic physiological change for *H. somni* as it transitioned from the planktonic to the biofilm mode of growth. For example, many proteins associated with quorum-sensing signaling were detected only in the biofilm matrix, supporting the link between quorum-sensing and biofilm formation. Transferrin-binding proteins (Tbps) were detected in the OMVs, suggesting that OMVs participated in iron acquisition. However, at least two TbpA-like proteins were present in OMVs only when iron was restricted, indicating expression of these Tbps was differentially regulated. Based on an immuno-informatic analysis, potential vaccine candidates were predicted, with protein CBN71009 selected as a top vaccine candidate. These results provide supportive information for developing non-traditional vaccines to prevent diseases due to bacterial pathogens.

M209 - Two adjacent genes of *Vibrio vulnificus* promote neutrophil evasion in soft tissue.

Presenting Author – *Takashige Kashimoto, Kitasato University, Japan*

Author/s – *Takashige Kashimoto, Takehiro Kado, Kohei Yamazaki, Ykihiro Akeda, Toshio Kodama, Shunji Ueno*

Abstract Content

Vibrio vulnificus causes severe disease outcome for immunocompromised patient through consumption of contaminated foods, or for healthy host through open wound. Regardless of route of infection, symptoms were developing in a short duration. These facts suggest that innate immunity is the main defense for *V. vulnificus* and this organism defeat the host defense system. Despite that, how host-innate immunity and *V. vulnificus* confront each other is still unveiled. Here, we established the new method ISLAP (Identification of Specific genes using a Library of Avirulent Phenotypes), which is modified the signature tagged transposon basis mutagenesis (STM). ISLAP identified 40 mutants out of over 3,000 mutants obtained by STM that failed to evade neutrophils in the wound infection model. For unknown genes identified by ISLAP, we focused on the potential lipoprotein transporters which genes are localizing next each other in the chromosome. The bacterial burdens of deletion mutants of each transporter in muscle tissue under injection of bacteria were not decreased compared with that of Wt, whereas these were significantly decrease in the spleen. Pull down assay indicated the possibility that those transporters related to the acylation of lipid A, a core of LPS. As a result of LPS analysis by silver-staining, the patterns of LPS fragments altered upon deletion of those unknow transporters. Those data suggest that potential new role of LPS in neutrophil evasion in *V. vulnificus* and this finding expands our understanding on the bacterial evasion mechanisms of innate immunity.

M210 - Deciphering the regulatory network of irp high-pathogenicity island (irp-HPI) encoding siderophore piscibactin

Presenting Author – *Marta Lages, Universidade de Santiago de Compostela, Spain*

Author/s – *Manuel L. Lemos, Miguel Balado*

Abstract Content

The piscibactin siderophore system is widespread among Vibrionaceae and has been recognized as a key virulence factor in numerous fish and mollusk pathogens such as *Vibrio anguillarum*, *V. neptunius* or *Photobacterium damselae* subsp. *piscicida*. Piscibactin is encoded in the irp high-pathogenicity island (irp-HPI) and, in *V. anguillarum*, its expression has a dual requirement of low iron-availability and temperature (<18-20°C). Nonetheless, not much is known about its regulatory mechanisms. In this work, in-frame deletion mutants of the two putative AraC-like transcriptional regulators encoded in irp-HPI, AraC1 and AraC2, were constructed and their role in the expression of the piscibactin genes analyzed at cold (15 °C) and warm (25 °C) temperature. The role of the conserved global regulators H-NS and ToxR-S was also studied. The results showed that AraC2 does not modulate irp-HPI gene expression, and that HNS and ToxR-S would have a secondary role. More notable, the inactivation of araC1 (renamed pbtA) disables the expression of piscibactin biosynthesis and uptake genes, which results in a significant decrease in virulence. All results taken together suggest that, although the main regulator of the irp-HPI island is PbtA, other uncharacterized regulators may play a role in temperature-dependent modulation of virulence factors in *V. anguillarum*.

M211 - The master regulator PbtA, encoded in the irp high-pathogenicity island (irp-HPI), controls a plethora of virulence-related gene

Presenting Author – *Miguel Balado, Universidade de Santiago de Compostela, Spain*

Author/s – *Marta A Lages, Manuel L Lemos*

Abstract Content

DNA acquired through horizontal gene transfer usually encodes factors that ensure its own expression. This is the case with the irp-HPI island and the piscibactin activator PbtA. We hypothesized that PbtA could also modulate the expression of other functions encoded elsewhere in the genome. Thus, the main aim of this work was to study the interconnection of the irp-HPI genomic island and the core genome of *Vibrio anguillarum*. To this purpose, an RNAseq analysis was used to identify genes that were differentially expressed after the inactivation of pbtA in the *V. anguillarum* RV22 genetic background. Results showed that PbtA modulates the expression of genes related to arginine metabolism, sulfur and nitrogen metabolism, siderophore production, T6SS, LPS, MARTX toxin, etc. The direct interaction of PbtA with some promoter regions was confirmed by electrophoretic mobility shift assays. Interestingly, PbtA may act as a transcriptional activator or repressor depending on the target promoter. Our results greatly suggest that the high virulence and temperature plasticity of the *V. anguillarum* strains harbouring the irp-HPI element is likely due not only to the acquisition of the siderophore piscibactin, but also to the changes in the transcriptome of the whole genome mediated by master regulator PbtA.

M213 - Gut colonization of the sulphidogenic *Bilophila wadsworthia* under a high-fat diet

Presenting Author – *Lizbeth Sayavedra, Quadram Institute, United Kingdom*

Author/s – *Muhammed Yasir, Andrew Goldson, Arlaine Brion, Mar Moreno Gonzalez, Keith Turner, Naiara Beraza, Gwenaelle Le Gall, Tianqi Li, Annalisa Altera, Arjan Narbad*

Abstract Content

High-fat diets alter the gut microbiota composition and stimulate the proliferation of the sulfidogenic bacterium *Bilophila wadsworthia* (Bw). Bw expansion is linked to gut inflammation and dysfunction of the intestinal barrier and bile acid metabolism. The genetic basis for its colonization in the gut remains largely unknown.

In this study, we used a genome-wide transposon mutagenesis approach for Bw, TraDIS-Xpress, to identify genes essential for gut colonization of mice under a high-fat diet with or without a simplified humanized microbial consortium (SIHUMI). The effect of the microbiota on host health was also determined. Compared to Bw alone, the combination of Bw with SIHUMI caused a lower weight increase, higher gut permeability and abundance of the pro-inflammatory cytokines IL-1a and IFN-G. Comparison of the mutants present in culture, against the mutants in the gut, revealed that 82 genes were not-essential in culture but beneficial for gut colonization. These included genes for respiration and microcompartment formation, which allow Bw to efficiently respire taurine and isethionate. A higher number of genes was required by Bw for gut colonization when together with the SIHUMI consortia as compared to monoculture, including the synthesis of nucleotides and histidine.

Our results suggest that Bw uses microcompartments for competitive metabolism that allows it to thrive in the gut, similar to enteric pathogens. The exacerbated detrimental effect of Bw with SIHUMI, suggests that the microbial composition plays a key role in the modulation of the activity of this pathobiont.

M214 - Metabolites and metabolic pathways in human macrophages infected with non-tuberculous Mycobacteria

Presenting Author – *Han Sang Yoo, Seoul National University, Republic of Korea*

Author/s – *Suji Kim, Hye Jin Eom, Su Min Kyung, Jun Ho Lee, Eun-Seo Lee*

Abstract Content

Non-tuberculous mycobacteria (NTM) are opportunistic pathogens that can cause pulmonary disease in immunocompromised hosts. Non-tuberculous mycobacterial lung disease (NTM-LD) has been received attention due to the global increase of the diseases. Especially the rapid-growing mycobacteria (RGM) group, known as *Mycobacterium abscessus*, and the slow-growing mycobacteria (SGM) group, known as Mycobacterium avium complex (MAC), are main causes of the pulmonary infections. Following inhalation or aspiration of NTM, macrophages act as the first defense line to inhibit the infection. The pathogenic success of NTM is tightly linked to its ability to recalibrate host metabolic processes in infected host macrophages. To define the metabolic impact of NTM infection, global metabolic profiling of NTM-infected macrophages was investigated. In total, 275 differentially accumulated metabolites in NTM-infected macrophages were identified by CE-TOFMS and LC-TOFMS analyses. Macrophages infected with NTM present a relative decrease in glucosinolate biosynthesis, calcium signaling pathway, and ABC transporters. Cellular metabolomics revealed that the SGM infection induced a distinct metabolic profile compared to the RGM infection. The SGM infection resulted in elevated intracellular levels of cysteic acid and decreased the level of Gin, Pelargonic acid, and Val compared to the RGM infection group. Specifically, Butanoate metabolism and Carbohydrate digestion pathways were inhibited in the SGM-infected macrophages following infection. These findings demonstrate precise modulation of host macrophage metabolic pathways by NTM infection. In addition, these metabolites might be biomarkers for diagnosing NTM infection using clinical samples.

M215 - Multiresistant bacteria in Austria during the COVID-19 pandemic

Presenting Author – *Gernot Zarfel, Medical University Of Graz, Austria*

Author/s – *Andrea J. Grisold, Julia Schmidt, Josefa Luxner, Lena Gruber, Yasmin Mandl*

Abstract Content

Multidrug-resistant bacteria (MRB) represent an increasing problem in the hospital and in the outpatient area. The grampositive methicillin resistant *Staphylococcus aureus* (MRSA) is one of the most feared representatives of MRB, the gram negative counterpart are multiresistant Enterobacteria (especially last line carbapenem-resistant Enterobacteria; CRE). The COVID-19 pandemic has resulted in the disruption of healthcare systems, with an possible influence on the spread of MRB and their genetic background.

The aim of the study was to compare the occurrence of MRSA and CRE in the years before the COVID-19 pandemic and in the years during the pandemic. Genetic and phenotypic analyses of MRSA and CRE isolated during this period were used for the comparison.

MRSA decreased from 237 (2019) to 180 (2020). The proportion of MRSA that are typical of a hospital (HA-MRSA) fell from 62% (147 of 237 isolates) to 51.1% (89 of 180 isolates). This also represents the lowest proportion of HA-MRSA documented in one year for Austria.

The changes in CRE presented a reduction of cases by 50% (although the number of cases was low) and carbapenem-resistant Enterobacter completely disappeared during2020 and 2021.

The Covid pandemic had an impact on the spread of MRBs in Austria. Reduced contacts and improved hygiene measures showed their effect. However, the sharp decline in CRE can probably also be explained by the reduced disease activity. This shows that the diversity of CRE was very high in all the years studied and that nosocomial outbreaks played a minor role as a cause.

M216 - The F-box type III effector XopI from *Xanthomonas* opens stomata and interacts with plant ubiquitin ligase complexes

Presenting Author – *Daniela Büttner, Martin Luther University Halle-Wittenberg, Germany*

Author/s – *Oliver Nagel, Ulla Bonas*

Abstract Content

Xanthomonas euvesicatoria, causal agent of bacterial spot disease on pepper and tomato, is a non-vascular pathogen and enters the intercellular spaces of the plant tissue via stomata. Pathogenicity depends on type III effectors (T3E) which are translocated into plant cells and manipulate cellular processes to the pathogen's benefit.

Here, we characterized the function of the T3E XopI by infection studies and microscopy after transient or stable in planta expression of xopI. We also identified and analysed XopI interaction partners using yeast two-hybrid assays, pull-down experiments and coimmunoprecipitation. Our data revealed that XopI contributes to disease symptoms and counteracts stomatal closure during plant defense and after induction by the phytohormone abscisic acid. Furthermore, XopI suppresses the increase in reactive oxygen species after recognition of microbe-associated molecular patterns (MAMPs) and complements a *Pseudomonas syringae* coronatine mutant for stomata opening in *Arabidopsis*. XopI is the only known T3E with an F-box motif, suggesting a role in ubiquitination. Interaction studies revealed that XopI interacts via the F-box motif with SKP1 (S-phase kinase-associated protein 1)-like proteins which are components of SCF (SKP1[S-phase kinase-associated protein 1]/CULLIN1/F-box protein)-type E3 ubiquitin ligases. Our data suggest that XopI acts as adaptor between SKP1 and plant proteins and leads to their degradation by the proteasome.

M217 - Evaluation of copper alloys for reducing infection by methicillin resistant *Staphylococcus aureus* and vancomycin resistant *Enterococcus faecium*

Presenting Author – *Mee Soo Chang, Seoul National University, Republic of Korea*

Author/s – *Jun Hee Woo, Yong Pil Chung, Mee Soo Chang*

Abstract Content

Backgrounds: Multi-drug resistant pathogens are increasing among healthcare-associated infections. It is well known that copper and copper alloys have antimicrobial activity.

Objectives: We evaluated the activity of copper against bacteria in a hospital setting in Korea.

Methods: Methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *Enterococcus faecium* (VRE) were inoculated onto copper, copper alloy and stainless steel plates in a laboratory and medical intensive care unit (ICU). After 24 hours of incubation, colony-forming units (CFU) were counted in the laboratory. Two similar rooms were chosen in the ICU; one room had copper-containing surfaces, and the other room contained items with a stainless steel surfaces. Items were sampled weekly for 8 weeks when the rooms were not crowded and when the rooms were busier with healthcare workers or visitors.

Results: *In vitro* time-kill curves showed copper or, a copper alloy yielded a significant reduction in MRSA and VRE CFUs over 15 minutes. Upon exposure to stainless steel plates, CFUs were slowly reduced for 24 hours. *In vivo*, MRSA CFUs were lower in rooms with copper-containing surfaces compared with controls, both after cleaning and after patients had received visitors (p < 0.05). Analysis of VRE revealed similar results, but VRE CFUs from copper-containing surfaces of drug carts in the ICU did not decrease significantly.

Conclusions: Copper has antimicrobial activity and appears to reduce the number of multi-drug resistant microorganisms in a hospital environment. This finding suggests the potential of the use of copper fittings, instruments and surfaces in hospital.

M218 - Identification of *Arcobacter butzleri* genes important for adhesion and/or invasion of human tissue culture cells

Presenting Author – *Itsaso Baztarrika, University of the Basque Country UPV/EHU, Spain*

Author/s – *Marc M.S.M Wösten, Rodrigo Alonso, Ilargi Martinez-Ballesteros, Itrati Martinez-Malaxetxebarria*

Abstract Content

Arcobacter butzleri is a foodborne pathogen that mainly causes enteritis in humans, but the number of cases of bacteraemia has increased in the recent years. However, there is still limited knowledge on the pathogenic mechanisms of this bacterium. To investigate how *A. butzleri* causes disease, single knockout mutants in the cadF, cj1349, ciaB and flaAB genes, which might be involved in adhesion and invasion properties, were constructed. These constructed mutants and isogenic wild-type were then tested for their ability to adhere and invade human Caco-2 and HT29-MTX cells. Adhering and invading *A. butzleri* strains were also visualized by EVOS M5000 fluorescent microscope. The fibronectin binding protein deficient cj1349 mutant showed reduced adhesion but similar invasion properties compared to the wild-type strain. Both flaAB and ciaB mutants were less able to adhere and to invade the tissue culture cells than the wild-type strain; and the cadF mutant was virtually unable to invade. These results suggest that the flagellum may promote *A. butzleri* adhesion, but is not required for invasion. In contrast to a functional flagellum the fibronectin binding protein CadF appears to be essential for *A. butzleri* to invade. This is the first time genes involved the adhesion and invasion processes of A. butzleri have been identified.

M219 - High throughput single cell-derived growth analysis in isogenic *Staphylococcus aureus* populations

Presenting Author – *Jonathan Hira, UiT The Arctic University Of Norway, Norway*

Author/s – *Jonathan Hira, Bhupender Singh, Tirthankar Halder, Anel Mahmutovic, Mona Johannessen, Christian Lentz, Clement Ajayi, Sk. Arif Ahmed*

Abstract Content

Background: It has become increasingly evident that genetically identical populations of bacterial pathogens such as *Staphylococcus aureus* contain subpopulations with different function, e.g., persister cells with reduced susceptibility to antibiotic killing that have been associated with chronic infections. These phenotypes are not stable and therefore escape routine clinical diagnostics (Bär et al., 2022). There is a need for approaches that can rapidly determine clinically relevant parameters phenotypically rather than genetically directly from clinical specimens.

Objective: To improve our understanding on the scope and relevance of phenotypic heterogeneity in bacterial cell populations, we intended to develop a workflow to systematically detect phenotypic heterogeneity and enable a separate functional characterization of phenotypically different cells.

Methods: We have developed a high-throughput pipeline that integrates cellular phenotypic profiling of bacterial populations using chemical probes and fluorescent reporter strains, flow cytometry-based analysis and sorting of single bacteria coupled to downstream single cell-derived real-time monitoring of growth on agar and in liquid culture.

Results: In a proof-of-concept study we have applied this pipeline to study growth-related phenotypic heterogeneity in isogenic *S. aureus* populations. We observed high cell-to-cell variability in both growth delay and generation time that were in part correlated with the fluorescence labelling profile during sorting. Under infection-mimicking conditions at low pH, the assay readily detected dormant growth variants, such as clinically relevant ´non-stable small colonies´. The pipeline is easily adaptable to investigate purified single cells or subpopulations of diverse origin in growth and non-growth assays.

M220 - Regulation of TolCV1 efflux pump in *Vibrio vulnificus*

Presenting Author – *Young Ran Kim,Chonnam National University, Korea, Republic of*

Author/s – *Yue Gong, Young Ran Kim*

Abstract Content

Vibrio vulnificus infection, frequently resulting in fatal septicemia, has become a growing health concern worldwide because of the global warming and the rapid emergence of multidrug-resistant strains. RtxA1 toxin is essential for *Vibrio vulnificus* cytotoxicity and necrotic cell death. An efflux pump TolCV1 system in *Vibrio vulnificus* is employed for the secretion of RtxA1 toxin and antimicrobial resistance. We found that the expression of *V. vulnificus* TolCV1 protein was increased time-dependently, which was downregulated in an rpoS deletion mutation. The expression of TolCV1 was increased after treatment of the host signal bile salt and the growth of tolCV1 mutant was totally abolished in the presence of bile salt. A tolCV1 mutation resulted in significant reduction of *V. vulnificus* induced-virulence in mice. A library of drugs approved by Food and Drug Administration was screened for efficacy against *Vibrio vulnificus* wild type and TolCV1 mutant strain using antimicrobial assays. We found that some drugs showing down-regulation on TolCV1 expression decreased *Vibrio vulnificus* cytotoxicity.

M221 - The influence of intracellular location on antibiotics susceptibility of *Mycoplasma hominis* endosymbiont of *Trichomonas vaginali*

Presenting Author – *Valentina Margarita, University of Sassari, Italy*

Author/s – *Valentina Margarita, Gavino Carboni, Nicia Diaz, Pier Luigi Fiori, Paola Rappelli*

Abstract Content

Background: *Mycoplasma hominis*, an opportunistic pathogen of the human lower urogenital tract, is correlated with important sequelae in pregnant women such as preterm birth, postpartum infection, and spontaneous abortion. It is characterized by small size and the lack of rigid cells wall, rendering it resistant to b-lactam antibiotics. Infection is mainly treated with tetracycline, while macrolides are recommended for pregnant women, neonates, and children. *M. hominis* can survive and replicate in the protozoon *Trichomonas vaginalis* cells, establishing an endosymbiotic relationship. The intracellular location may represent a way for the bacteria to escape the immune system and a protection from antimicrobial activities.

Objectives: To investigate the possible influence of the symbiosis with *T. vaginalis* on the antibiotic susceptibility of *M. hominis*

Methods: Sensitivity to antibiotics in *M. hominis* isolated in association with *T. vaginalis* was compared with that of mycoplasmas not associated with protists. We assessed the minimal inhibitory (MIC) and lethal concentrations (MLC) of tetracycline on *M. hominis* strains after 30 days of *in vitro* cultivation both alone and in association with *T. vaginalis*.

Results: The incidence rate of *M. hominis* resistant to C14 and C15 macrolide members was higher in strains associated with *T. vaginalis* than those isolated from women not affected by trichomoniasis, while sensitivity to tetracycline and quinolone was similar in the two groups.

In vitro experiments showed higher MIC and MLC values for *M. hominis* strains grown in association with *T. vaginalis*, compared with bacteria cultivated alone, suggesting that the intracellular localization of bacteria in trichomonad cells could interfere with antibiotic susceptibility.

M222 - Interactions between nanofibrous materials and pathogenic bacteria

Presenting Author – *Simona Lencová, University Of Chemistry And Technology Prague, Czech Republic*

Author/s – *Kamila Zdeňková, Hana Stiborová, Kateřina Demnerová*

Abstract Content

Nanofibrous materials (NMs) have huge potential in medical and food industry applications, for which is essential that the NMs are microbiologically safe. To ensure that, NMs are often functionalized with antimicrobial substances. However, this can lead to the development of antimicrobial resistance, which is considered one of the global biggest threats to human health. Therefore, new technologies are being intensively sought. Current research shows that reducing or even eliminating microbial risks may be possible by modifying the morphology of NMs. In our study, nonfunctionalized polyamide (PA) NMs differing in fiber diameter and surface density were prepared and their interactions with bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Escherichia coli*), specifically biofilm formation and cell retention, were examined. PA NMs functionalized with AgNO3 or chlorhexidine served as controls. Biofilm formation was evaluated by CFU enumeration, resazurin staining, and scanning electron microscopy (SEM). Retention was tested by filtration of bacterial suspension through an NM filter followed by CFU enumeration and SEM. PAs morphology proved to be important in both types of interactions. Fiber diameter was confirmed as a crucial factor influencing biofilm formation ($p \leq 0.01$); the correlations between air permeability and surface density and fiber diameter were revealed. Surface density was determined to be a crucial factor influencing bacterial cell retention ($p \leq 0.01$), while no effect of fiber diameter was found. The results suggest that by morphology adjustment, risks of infections and intoxications associated with contaminated NMs could be reduced.

M223 - Population structure and core genome phylogeny of *Serratia marcescens*

Presenting Author – *Chao-Jung Wu, Taipei Medical University, Taiwan*

Author/s – *Jun-Ning Yang, Yin-Tai Tsai, Yi-Yuan Yang, Yi-Lin Liang*

Abstract Content

Serratia marcescens is a Gram-negative opportunistic pathogen that causes a full spectrum of diseases in immunocompromised patients and is one of the most commonly isolated organisms in human infections. Due to the lack of an easy and cheap molecular typing method such as multi-locus sequence typing, the population structure of *S. marcescens* was less studied and the transmission of highly-virulent strains was untraceable. In this study, our objective was to analyze the core genome and determine the population structure of a large collection of S. marcescens. Five-hundred *S. marcescens* genomes were randomly selected and downloaded from GenBank followed by genome annotation and pangenome analysis. The genes present in all isolates were defined as the core genome. Ontology analysis was performed to discover the functional groups of the proteins that translated from the core genome. A phylogenetic tree with the neighbor-joining method and 100 bootstrap replicates was constructed based on the core genome sequences of the 500 isolates. The genomes we analyzed had an average size of 5.2 million bases and a total of 44865 genes were identified. With the minimum blastp identity of 95%, 588 genes were predicted in all 500 *S. marcescens*. The proteins translated from the core genome participated in biological processes including cell wall and phospholipid biosynthesis, cell division, translation, and transportation. Five clades with one outlier strain were identified in the core genome phylogenetic tree. The linkage between strain characteristics and core genome phylogeny will be further investigated.

M224 - Antibody response against the type VI secretion system of *Pseudomonas aeruginosa* in cystic fibrosis patients

Presenting Author – *Veronica Chaves Vargas, Max Planck Institute for Evolutionary Biology, Germany*

Author/s – *Steffi Jimmy, Holger Sondermann, Carsten Schwarz, Petra Bacher, Daniel Unterweger*

Abstract Content

Bacterial pathogens are often targeted by the host's immune system. This interaction between bacteria and the immune system is particularly relevant for patients with cystic fibrosis (CF) that are chronically colonized with *P. aeruginosa* and unable to clear the infection from their lungs. Which bacterial proteins are targeted by the adaptive immune system during infection is not fully understood. Existing reports indicate that the type VI secretion system (T6SS), a molecular nanomachine for the translocation of effector proteins, is a target of the adaptive immune response. Here, we (i) characterize the T6SSs of *P. aeruginosa* isolates and (ii) determine the adaptive immune response to the T6SS of *P. aeruginosa* in one and the same patient to gain a better mechanistic understanding of T6SS-mediated immune activation.

P. aeruginosa colonies were directly isolated from the patient's sputum sample. The isolates were confirmed by PCR using species-specific primers, and subjected to whole genome sequencing. All of the isolates harbor hcp1 in their genomes, which encodes one of the most abundant structural proteins of the H1-T6SS. One of the isolates expresses the protein under laboratory conditions, demonstrating phenotypic diversity among the isolates.

An ELISA was designed to determine the titer of anti-Hcp1 antibodies in the patient. We observed a robust and specific response against Hcp1. This response is considerably higher compared to the response against two cytoplasmatic *P. aeruginosa* proteins. These findings support previous data and advance our understanding of a patient with a strong anti-T6SS antibody response.

M225 - Role of the unique *Legionella longbeachae* capsule in virulence and infection

Presenting Author – *Silke Schmidt, Institut Pasteur, France*

Author/s – *Sonia Mondino, Laura Gomez-Valero, Pedro Escoll, Maryse Moya-Nilges, Martin Sachse, Thierry Fontaine, Augusto Goncalves, Dario Zamboni, Carmen Buchrieser*

Abstract Content

Legionella longbeachae (Llo) are facultative intracellular bacteria that can cause Legionnaires' disease (LD), a severe form of pneumonia. In contrast to most *Legionella* species that are found in aquatic environments, Llo was isolated from contaminated potting soils and it is mostly prevalent in Southeast Asia, Australia, and New Zealand, where it causes between 50-60% of LD cases. In recent years, clinical cases of Llo infection have also been reported in Europe and America.

Previously, our group identified a locus in the Llo genome coding for a putative capsule that is unique as compared to all other Legionella species characterized to date. Llo is highly virulent *in vivo*. Yet, Llo poorly stimulates pro-inflammatory cytokines in macrophages *in vitro*. We thus hypothesize that the capsule may represent a novel virulence feature of Llo. We show by electron microscopy that Llo indeed expresses a capsule and that a deletion mutant in the capsule transporter operon is not encapsulated. In a murine infection model, the capsule mutant is completely avirulent and virulence can partially be restored using a complementation plasmid. Similarly, the capsule mutant is impaired in growth in the environmental host Acanthamoeba castellanii. Our results suggest that the capsule has an essential function in the Llo infection cycle.

We currently seek to characterize the capsule composition and to investigate the role of the capsule in the environmental host *Acanthamoeba castellanii*. We hope to gain insights into how the environmental bacterium Llo can cause disease and what determines its virulence at the molecular level.

M226 - Photodynamic Inactivation of Chlorophyllin and Polyethylenimine on *Pseudomonas aeruginosa* Biofilm

Presenting Author – *Mona Mahmoud, Universitätsklinikum Erlangen - Friedrich Alexander Universität Erlangen Nürnberg, Germany*

Author/s – *Mona Mahmoud, Peter Richter, Michael Lebert, Andreas Burkovski*

Abstract Content

Antimicrobial resistance (AMR) remains a global challenge. One of the leading pathogens contributing to the burden of AMR in 2019 and the priority target for the development of alternative antimicrobial agents is the opportunistic pathogen *Pseudomonas aeruginosa*

Here, we use antimicrobial photodynamic inactivation (aPDI) as a promising antimicrobial approach that may not develop bacterial resistance in the near future3. We study the effect of a plant-based photosensitizer (chlorophyllin) and a cationic permeabilizer polyethylenimine (PEI), exposed to red light-emitting diode (LED) on *P. aeruginosa* free-living planktonic cells and biofilm.

The broth microdilution checkerboard method was used to test antimicrobial susceptibility to determine bactericidal concentrations. As a substrate for biofilms, the Calgary biofilm device was applied.

No antibacterial effect of PEI alone has been found; however, 100 µg/ml PEI potentiated lower concentrations of the illuminated chlorophyllin (125 µg/ml) to completely eradicate the planktonic cells. Synergistically, the illuminated chlorophyllin (125 µg/ml) and PEI (100 µg/ml) dislodged the established biofilm by 76%. More than 500 µg/ml illuminated chlorophyllin and 100 µg/ml PEI are required to completely eradicate bacteria in colony biofilm.

Our findings reveal that PEI ameliorates the antimicrobial activity of the illuminated chlorophyllin against *P. aeruginosa* planktonic and biofilm states and the concentration required to eradicate the bacteria in the biofilm is more than fourfold that is required to eradicate planktonic cells. These data suggest that the tested combination is promising for the eradication of *P. aeruginosa* biofilm and could be a good candidate for industrial applications.

M227 - *Aeromonas salmonicida* plasmid transfer to *Chlamydia suis* is achieved only by modification of the vector with chlamydial DNA

Presenting Author – *Hanna Marti, University of Zurich, Switzerland*

Author/s – *Hanna Marti, Michael Biggel, Kensuke Shima, Delia Onorini, Jan Rupp, Steve J. Charette, Nicole Borel, University of Zurich, Zurich, Switzerland*

Abstract Content

Background: The obligate intracellular *Chlamydia* (C.) genus comprises several species responsible for disease in humans and animals. The Chlamydiae are part of a phylogenetically isolated phylum generally not prone to acquire antibiotic resistance genes. Thus, recently acquired tetA(C)-conferred tetracycline resistance in porcine *C. suis* is of particular interest to identify risk factors responsible for acquisition of foreign DNA into the conserved *Chlamydia* genus. Previous studies have shown that tetA(C) and neighboring genes are part of a genomic island on the *C. suis* chromosome. This Tet-island shares a high similarity with pRAS3-3432, a mobilizable plasmid detected in the fish pathogen *Aeromonas salmonicida* ssp. *salmonicida* (Ass).

Here, we investigated the conditions under which pRAS3-3432 may be transferred from Ass to *C. suis*.

Methods: We used internalization and culturing assays to investigate whether mesophilic *C. suis* and psychrophilic Ass can at least temporarily occupy the same niche. Additionally, we focused on the pRAS3-3432 plasmid and modified it with assembly cloning methods to construct vectors capable of transformation into *C. suis*.

Results: Considering the low internalization rate of Ass and the different growth requirements between the two species, it is highly unlikely that pRAS3-3432 is transferred from Ass to *C. suis* following co-infection. *Chlamydia suis* is not naturally competent under normal growth conditions and does not take up pRAS3-3432 without modification. Transformation of *C. suis* was only possible with vectors that contained *C. suis*-specific plasmid or chromosomal DNA.

M229 - ATO transporters as essential elements of cellular function and homeostasis in *Candida albicans* pathogenesis

Presenting Author – *Rosana Alves, University of Minho, Portugal*

Author/s – *Cláudia Barata-Antunes, Faezeh Faezeh, Vitor Fernandes, Alexandra Gonçalves, Margarida Casal, Alistair Brown, Patrick Van Dijck*

Abstract Content

Background: *Candida albicans* is an opportunistic pathogen that uses carboxylic acids as nutrients to survive and successfully thrive in different environmental niches. Surprisingly, the carboxylate ATO family is greatly expanded in this pathogen when compared with other fungi, however, its precise function is still unclear.

Objectives: Here, we cover the function and regulation of the 10 members of the ATO family, as well as their contribution for *C. albicans* virulence.

Methods: We addressed state-of-the-art approaches to elucidate how ATO transporters allow *C. albicans* adaptation to different host niches and environmental factors. These strategies included 1) efficient CRISPR-Cas9 technologies for multiple gene targeting, 2) phenotypic analyses and heterologous expression studies to confirm transporter specificity, 3) fluorescence microscopy imaging to assess membrane localization and protein dynamics and 4) *in vivo* assays using phagocytes to study host-pathogen interactions.

Results: We present for the first time a detailed study on each member of the ATO family. Understanding the function and regulation of plasma membrane transporters in pathogenic fungi is now of utmost importance, having the potential to reveal novel molecular players of virulence pathways that ultimately can be used for the development of new antifungals and diagnostic approaches.

M231 - *Pseudomonas syringae* biofilm development alterations due to external factors by the ATR FT-IR spectroscopy in a flow chamber

Presenting Author – *Jakub Budil, Charles University, Czech Republic*

Author/s – *Radovan Fišer, Alexander Kromka, Petra Lišková*

Abstract Content

In this work, we have focused on the impact of alterations of cultivation conditions during the inoculation and the growth of *P. syringae* pathovar morsprunorum (Psm) biofilms on ZnSe prism in a custom-made flow chamber that was constantly irrigated with fresh diluted Luria Bertani medium and monitored by the attenuated total reflection Fourier transformation infrared (ATR FT-IR) spectroscopy. The effect of the initial bacterial concentration and the duration of the inoculation phase, the composition of the supply medium, and the fluid flow rate was investigated with respect to the ATR FT-IR spectra kinetics and the biofilm morphology. Decreasing the initial bacterial concentration (10× lower) caused a delay in the biofilm development without noticeably affecting the general biofilm architecture with its mushroom-shaped microcolonies. Increasing the flow rate 2× improved the biofilm coverage of the ZnSe prism more strongly than 2× higher medium concentration, suggesting that the Psm biofilm in our flow chamber could be limited mostly by the oxygen rather than by other nutrients.

Additionally, the interaction of Psm biofilm with antibacterial agents has been investigated in a flow chamber by ATR-FTIR for the first time. The copper sulfate ($CuSO_4$) represents widely used copper-based treatments of plants [1], copper nanoparticles represent a more novel approach [2], while novel synthetic pore-forming antibiotics lipophosphonoxins represent macromolecular anti-infectives [3]. The early biofilm (4h) exposed to sublethal $CuSO_4$ concentrations (0.015mM) formed a compact biofilm without any of the previously observed [4] mushroom-shaped microcolonies possibly due to restrictions of the motile subpopulation.

M232 - Identification of residues involved in posttranslational modification of RTX toxins of Gram-negative pathogens

Presenting Author – *Michaela Grobarcikova, Czech Academy of Sciences, Czech Republic*

Author/s – *Adriana Osickova, Sarka Knoblochova, David Jurnecka, Peter Sebo, Jiri Masin, Jiri Cerny*

Abstract Content

Bordetella pertussis adenylate cyclase toxin (CyaA) and *Escherichia coli* α-hemolysin (HlyA) belong to the Repeats in ToXin (RTX) family that play key roles in the virulence of numerous Gram-negative pathogens. CyaA translocates unique N-terminal AC domain into the cytosol of phagocytes and undermines their bactericidal functions by unregulated conversion of ATP to cAMP. Both CyaA and HlyA then permeabilize the membrane of eukaryotic cells by forming cation-selective pores. The toxins bind preferentially to cells expressing β2 integrins but can also penetrate the membrane of cells not expressing β2 integrins. CyaA and HlyA are synthesized as protoxins and are activated by covalent posttranslational acylation that is catalyzed by the dedicated acyltransferases CyaC and HlyC, respectively. The acyls are linked at α-amino groups of two lysine residues located within conserved acylation sites, namely Lys860 and Lys983 in CyaA and Lys564 and Lys690 in HlyA. Site-directed mutagenesis defined the residues involved in the recognition of the toxin acylation sites by the acyltransferases. We show that substitution of Tyr990 and Arg991 in CyaA and of Tyr697 and Arg698 in HlyA reduces the extent of acylation of Lys983 in CyaA and of Lys690 in HlyA, respectively. We further show that these substitutions reduce the cytotoxic and cytolytic capacity of both toxins towards model sheep erythrocytes and human macrophage THP-1 cells. Using AlphaFold prediction and homologous modeling of CyaC on the known structure of ApxIC, we aim to identify residues directly engaged in the interaction between the acyltransferase and the RTX toxin.

M233 - Genetic characterization of pirlimycin resistant MRSA from a German dairy farm

Presenting Author – *Tobias Lienen, German Federal Institute For Risk Assessment, Germany*

Author/s – *Tobias Lienen, Mirka Wörmann, Anja Gretzschel, Mirjam Grobbel, Sven Maurischat, Bernd-Alois Tenhagen*

Abstract Content

Background: Mastitis caused by MRSA in dairy herds is associated with occupational health risks for farm staff. MRSA are resistant against virtual all beta lactam antibiotics. Treatment with the lincosamide antibiotic pirlimycin is licensed for use in dairy cows and might be a promising approach to treating MRSA infections.

Objectives: This study aimed at monitoring pirlimycin resistance and its genetic background in MRSA from a dairy farm with a history of MRSA detection, in which pirlimycin was used prior to dry period.

Methods: Quarter milk samples (QMS) of previously MRSA affected cows were tested for antimicrobial resistant staphylococci over a one-year period. Isolates were characterized by susceptibility testing against 19 antimicrobials and whole-genome sequencing.

Results: Four of 143 MRSA isolates retrieved from 82 cows were resistant to clindamycin and pirlimycin. All four isolates originated from different cows and expressed an eight-fold multidrug-resistant phenotype. The isolates differed genomically and were associated to the spa types t034 (3/4) and t588 (1/4). Pirlimycin resistance was associated with the genes erm(C) (3/4) or lnu(B) (1/4). One *Staphylococcus epidermidis* isolate, which was additionally retrieved during the monitoring, also exhibited clindamycin resistance and harbored the erm(C) gene indicating a possible inter-species transmission of the erm(C) gene on the dairy farm.

Conclusions: The use of pirlimycin prior to the dry period might be a promising approach to support control of MRSA in dairy herds. However, pirlimycin resistance may lead to treatment failures. Continuous monitoring of antimicrobial resistance is important to evaluate the situation on farm level.

M234 - Deciphering the *Staphylococcus aureus* SprF1 antitoxin RNA targetome to understand its contribution to antibiotic persistence

Presenting Author – *Emeline Ostyn, University of Rennes 1, France*

Author/s – *Yoann Augagneur, Marie-Laure Pinel-Marie*

Abstract Content

Persister cells are a subpopulation of transiently antibiotic-tolerant bacteria associated with antibiotic treatment failures and relapsing infections. Among others, type I toxin-antitoxin (TA) systems have been linked to persister cell formation. They are composed of a peptide toxin whose overexpression confers growth stasis or cell death, and of an RNA antitoxin that base-pairs with the toxin mRNA to inhibit its translation. We recently demonstrated that SprF1 is an RNA antitoxin that belongs to the SprG1/SprF1 type I TA system in *Staphylococcus aureus*, and that also binds ribosomes to inhibit global translation and promote persister cell formation. Based on these innovative results, the aim of this project was to identify the RNA targetome of SprF1 using MAPS (MS2-affinity purification coupled with RNA sequencing) to better understand the role of SprF1 in *S. aureus* antibiotic persistence. This approach led to the identification of 11 novel RNA targets able to directly interact with the SprF1 antitoxin. Among them, we selected the rpmE2 mRNA encoding the ribosomal protein L31, and the yidC mRNA encoding a protein insertase because of their potential role in antibiotic persistence. The interaction of SprF1 with these two targets was precised *in silico* using the IntaRNA software and confirmed *in vitro* by gel retardation assays. Upcoming investigations will focus on deciphering the link between SprF1, these two new mRNA targets and the formation of persister cells. Overall, this work could lead to the identification of novel therapeutic targets to fight against recurrent *S. aureus* infections.

M235 - Structural comparison of β-glucans by NMR and FT-IR in solid state

Presenting Author – *Ruslan Bikmurzin, Vilnius University of Applied Sciences, Lithuania*

Author/s – *Ruslan Ruslan, Rimantė Bandzevičiūtė, Arūnas Maršalka, Andrius Maneikis, Lilija Kalėdienė*

Abstract Content

β-glucans are one of the major components of the yeast cell walls and are highly abundant in crops such as oat and barley. Biological activity of β-glucans depends on: source, molecular weight, branching degree and length as well as extraction method and solubility. Analysis of β-glucans by nuclear magnetic resonance (NMR) and Fourier transform infrared (FT-IR) spectroscopy in solid state has an advantage revealing preserved structure of the extracted β-glucan molecules.

Yeast β-glucans were extracted from *Saccharomyces cerevisiae* and *Candida lusitaniae* by hot water and alkali-acidic methods. Oat and barley β-glucans were extracted with sodium hydroxide and ethanol. Structural analysis was by carried out in solid-state by 13C NMR and FT-IR spectroscopy.

Yeast β-glucans exhibited characteristic structure of β-1,3/1,6-linked glucans. Both alkali and hot water extracted β-glucans possessed proteins, chitin and other impurities. Higher protein and chitin content were found in hot water extracted yeast β-glucans. α-glucan levels were higher in alkali extracted samples. Extraction methods resulted in chemical shift differences between yeast β-glucan fractions.

Oat and barley β-glucans has characteristic structure of β-1,3/1,4-linked glucans. Samples from crops might contain some levels of α-glucans, proteins and other impurities.

Species-specific structure of analyzed β-glucans is only observed when comparing yeast β glucans with oat and barley β-glucans. However, comparison between *S. cerevisiae* and *C. lusitaniae* β-glucans require further research. Also, structural analysis of high molecular weight β glucans in solid state by FT-IR spectroscopy possessed difficulties or was limited due to band intensity changes and overlapping originating from different molecules.

M236 - Dephosphocholination by *Legionella* effector Lem3 functions through remodelling of the switch II region of Rab1b

Presenting Author – *Marietta Kaspers, Universitätsklinikum Hamburg-Eppendorf, Germany*

Author/s – *Vivian Pogenberg, Aymelt Itzen*

Abstract Content

Bacterial pathogens often make use of post-translational modifications (PTMs) to manipulate host cells. E.g. *Legionella pneumophila*, the causative agent of Legionnaires disease, secretes the enzyme AnkX that uses cytidine diphosphate (CDP)-choline to post-translationally modify the human small G-Protein Rab1 with a phosphocholine moiety at Ser76. Later in the infection, the *Legionella* enzyme Lem3 acts as a dephosphocholinase that removes the phosphocholine through hydrolytic cleavage. While the molecular mechanism for Rab1 phosphocholination by AnkX has recently been studied in detail, structural insights into the activity of Lem3 remained elusive.

We stabilized the transient Lem3:Rab1b complex by substrate mediated covalent capture. Thereby, we provide detailed characterisation of Lem3's unique catalytic mechanism through the crystal structures of Lem3 in the apo-form and in complex with Rab1b. Lem3 shares high structural similarity with metal-dependent protein (PPM) phosphatases and acts on Rab1 by locally unfolding the functionally important regulatory switch II region. In addition, Lem3:Rab1b complex structure provides first insight into the recognition of protein substrates by PPM phosphatases.

M237 - Characterization of the type III secretion system sorting platform from the plant pathogen *Xanthomonas euvesicatoria*

Presenting Author – *Christian Otten, Martin Luther University Halle-Wittenberg, Germany*

Author/s – *Daniela Büttner*

Abstract Content

Many Gram-negative bacterial pathogens use a type III secretion (T3S) system to deliver effector proteins into eukaryotic cells. T3S systems are conserved in plant- and animal-pathogenic bacteria and consist of at least nine conserved structural core components, which are designated Sct (secretion and cellular translocation) in animal-pathogenic bacteria. Sct proteins are involved in the assembly of the membrane-spanning secretion apparatus which is associated with an extracellular needle structure and a cytoplasmatic sorting platform. Recent developments in cryo-electron microscopy revealed structures of T3S system components in the context of assembled secretion systems from animal pathogens. In contrast, the structure and composition of T3S systems in plant-pathogenic bacteria is still unknown.

One of the model organisms to study T3S in plant-pathogenic bacteria is *Xanthomonas euvesicatoria*, which is the causal agent of bacterial spot disease in tomato and pepper plants. Structure predictions using AlphaFold suggest that the T3S system of *X. euvesicatoria* shares similarities with T3S systems from animal pathogens. In the present study, we investigated the contribution of structural domains in the cytoplasmic sorting platform to the assembly of the T3S system from *X. euvesicatoria*. *In vivo* interactions studies confirmed the importance of domains for interactions between components of the sorting platform, which were previously predicted by AlphaFold. Fluorescence microscopy revealed that the sorting platform associates with the core complex of the T3S system upon activation of T3S, which is trigged by a pH shift.

M238 - AMT2 gene plays an important role in transmigration process of *Cryptococcus neoformans* across the Blood-Brain Barrier

Presenting Author – *Mantana Jamklang, Suranaree University of Technology, Thailand*

Author/s – *Sainamthip Rangdist, Natchaya Pakdeesiriwong, Ekkasit Kanklang*

Abstract Content

Background: *Cryptococcus neoformans* is an encapsulated yeast causing a life-threatening infection of the central nervous system in immunocompromised individuals. Our cryptococcal transcriptome demonstrated that exposure of *C. neoformans* to endothelium upregulated the amt family ammonium transporter (AMT2) and previous studies elsewhere revealed that AMT2 induces an invasive phenotype expressing with pseudohyphal growth.

Objective: The aim of this study was to investigate the role of the AMT2 gene in transmigration process as well as determine the relevant function in morphological changes of *C. neoformans*.

Methods: The *in vitro* model of BBB was used to determine the transmigration ability of *C. neoformans* lacking AMT2 (amt2Δ) across the BBB compared to the wildtype (WT) strain, H99. Other biochemical tests were used to determine the ability of the cryptococcal strains to produce virulence factors.

Results: The mutant strain (amt2Δ) demonstrated a significantly defect in transmigration ability across the BBB. The pseudohyphal growth in both strains were not observed under the *in vitro* condition. The ability of the mutant strain in polysaccharide capsule and melanin production, urease and phospholipase activity, was comparable to the WT. The mutant strain has no defect on the growth under low concentration of ammonium.

In conclusion, AMT2 plays a significant role in transmigration process of *C. neoformans* across the BBB with no association with other virulence factors. Our future direction is to explore how AMT2 gene play roles in the transmigration. Such knowledge has a potential to improve drug development for prevention of individuals suffering from cryptococcal meningitis.

M239 - Probiotic *LimosiLactobacillus reuteri* KUB-AC5 against multidrug-resistant uropathogenic *Escherichia coli* infection with the contribution of host cell Toll-like receptor 2 (TLR2)

Presenting Author – *Parameth Thiennimitr, Chiang Mai University, Thailand*

Abstract Content

Background: The rising of multidrug-resistant (MDR) uropathogenic *Escherichia coli* (UPEC) strains is a significant threat to medical care. The recurrent bacterial urinary tract infection (UTI) caused by UPEC is a globally common infectious disease. Probiotic bacteria have been reported as an alternative way to combat MDR UTI. Lactic-acid-producing bacteria (LAB) in the genus *LimosiLactobacillus* is one of the most studied and used probiotics. However, the strain-specific effect plays a critical role in the outcomes. *L. reuteri* KUB-AC5 (AC5), isolated from the chicken gut, conferred the anti-*Salmonella* effect by attenuating gut inflammation in mice. However, the anti-UPEC effect of AC5 has never been explored.

Objectives: To determine both direct and indirect (immunomodulatory) effects of viable and non-viable probiotic AC5 on the proliferation of UPEC strains (UTI89, CFT073, and clinically MDR isolates). Together with the contribution of host cell Toll-like receptor 2 (TLR2) in the AC5 anti-UPEC action.

Methods: Live and/or dead AC5 was used in spot-on lawn, agar well-diffusion, and co-culture assay. Human urothelium cell line (UMUC-3) and murine macrophage (RAW264.7) were pretreated with AC5 before being infected with UPEC in a cell invasion and phagocytic killing assay with a multiplicity of infection (MOI) 20. Then, recovered UPEC (CFU/mL) were enumerated. The fold change of proinflammatory gene (NOS2, KC, MIP-2, IL-6, and TNF-alpha) expressions were detected by quantitative polymerase chain reaction. TLR2 knockout of UMUC-3 and RAW264.7 also were used.

Results: LAB probiotic AC5 directly inhibits and activates host immune cells against UPEC with the contribution of TLR2 activation.

M240 - Evaluation of heterologous prime-boost vaccine strategy against *Burkholderia* spp.

Presenting Author – *David DeShazer, A.v.topchiev Institute Of Petrochemical Synthesis, Ras, United States*

Author/s – *David DeShazer, Sergei Biryukov, Jennifer Dankmeyer, Zander Hedrick, Nathaniel Rill, Caitlyn Orne, Christopher Klimko, Jennifer Shoe, Melissa Hunter, Yuli Talyanski, Ju Qiu*

Abstract Content

Background: *Burkholderia pseudomallei* is a gram-negative saprophytic bacillus that is the causative agents of melioidosis that affects both humans and animals that reside predominantly in tropical climates such as those found in Southeast Asia and northern Australia. Development of a safe and effective vaccine would mitigate the growing threat to global health from this disease. Objectives. Here we employ a heterologous prime-boost vaccine strategy, utilizing *B. thailandensis* E555 ΔilvI, a non-pathogenic strain that harbors a deletion of the acetolactate synthase (ilvI) gene required for synthesis of branched chain amino acids. C57BL/6 mice were administered two doses, 28 days apart, of E555 ΔilvI in combination with various permutations of subunit vaccines composed of hemolysin co-regulated protein 1 (Hcp1), O-polysaccharide (OPS) and/or capsular polysaccharide (CPS) conjugated to the highly immunogenic CRM197 protein carrier in the presence of Alhydrogel (Alu) and CpG2006.

Methods: Tissues and blood were collected for immunoassays 28 days after vaccination for cytokine and antibody titer analyses. The mice were exposed to ~3.4 LD50 equivalents of aerosolized *B. pseudomallei* K96243 at 29 days after the last vaccination and observed for 60 days.

Results: The most protection was conferred by a CPS-CRM197 + CpG/Alu prime followed by a E555 ΔilvI boost. This heterologous vaccination approach also conferred the greatest number of downregulated cytokines in splenocytes from vaccinated mice following Hcp1, CPS and OPS restimulation, which has been associated with protective vaccination strategies in past iterations. This novel vaccine approach may offer a more robust and diverse protective response against *B. pseudomallei*.

M241 - Modularization and characterization of a type II secretion system from the plant pathogen *Xanthomonas euvesicatoria*

Presenting Author – *Samuel Goll, Martin Luther University Halle-Wittenberg, Germany*

Author/s – *Samuel Goll, Patrick Martin, Daniela Büttner*

Abstract Content

The Gram-negative plant-pathogenic bacterium *Xanthomonas euvesicatoria* causes bacterial spot disease on pepper and tomato plants. Essential for pathogenicity is the type III secretion system which delivers bacterial effector proteins into the plant cells to suppress plant immunity. Additionally, *X. euvesicatoria* employs an Xps type II secretion (T2S) system which secretes various degradative enzymes to the plant apoplast during infection, including xylanases, proteases and a lipase, and contributes to virulence. The T2S apparatus is a complex nanomachine consisting of a cytoplasmic ATPase, inner membrane proteins, periplasmic pseudopilins and an outer membrane secretin. T2S systems are conserved in many Gram-negative bacteria and have been intensively studied in animal-pathogenic bacteria. In plant pathogens, however, the function as well as assembly mechanisms of T2S components are largely unknown. We, therefore, generated a modular expression construct consisting of the 11 xps genes from *X. euvesicatoria* for functional studies. Deletion and complementation studies showed that all but one xps gene are essential for T2S in *X. euvesicatoria*. Localization studies with fluorescent reporter fusions, in addition to *in vitro* and *in vivo* interaction studies, provided first insights into the architecture and assembly mechanisms of the Xps T2S system.

M242 - Hypermucoviscous *Klebsiella pneumoniae* strains from ST25 infect intestinal epithelial cells and stimulate inflammatory response

Presenting Author – *Sudeb Saha, Tohoku university, Japan*

Author/s – *Sudeb Saha, Stefania Dentice Maidana, Mariano Elean, Kohtaro Fukuyama, Juan Martin Vargas, Rodrigo Exequiel Serda, María Ángela Jure, Haruki Kitazawa*

Abstract Content

The prevalence of hypermucoviscous carbapenem-resistant *Klebsiella pneumoniae* with sequence type 25 (ST25) is increasing in South America. In this work, the infectivity and the inflammatory response triggered two *K. pneumoniae* ST25 strains (LABACER01 and LABACER27) were evaluated in human intestinal epithelial cells. Caco-2 cells (7×105 cells/well) were challenged with LABACER01 or LABACER27 (104 cells/well) and at different hours pots-infection (2, 6 and 12) the cytotoxic effect, adhesion, and invasion rate, as well as the expression of tight junctions and inflammatory cytokines were investigated. LPS challenge was used as control for inflammation. LPS and both *K. pneumoniae* strains significantly reduced cell viability and increased LDH release, indicating cell damage. However, the effects of bacteria were lower than LPS ($p < 0.05$). No differences in cell viability or LDH activity were detected between the LABACER strains. Both *K. pneumoniae* strains adhered to and invaded Caco-2 cells, with no significant differences between them ($p < 0.05$). LPS and LABACER strains significantly reduced the expression of occludin, ZO-1, and claudin, increased the expression of the inflammatory factors COX-2, iNOS, and enhanced the mRNA and protein levels of TNF-α, IL-6, MCP-1, and IL-8. LABACER01 and LABACER27 showed a lower ability to stimulate inflammatory response than LPS and no significant differences were observed between the two bacteria. Here, the virulence and inflammatory profile of hypermucoviscous carbapenem-resistant *K. pneumoniae* ST25 was explored for the first time in human intestinal epithelial cells. Results expand the knowledge of the biology of these emerging multiresistant *K. pneumoniae* clones.

M243 - Tailored liposomal nanotraps for the treatment of streptococcal infections

Presenting Author – *Hervé Besançon, University Of Bern, Switzerland*

Author/s – *Yu Larpin, Lucy J. Hathaway, Parham Sendi, Annette Draeger, Eduard Babiychuk*

Abstract Content

In view of the alarming increase in severe infections caused by antibiotic-resistant pathogens, the development of novel therapeutic approaches is crucial. Bactericidal treatment increases the likelihood of positive selection for drug-resistant mutants; hence, the benefit of agents that do not directly target the pathogen is evident. Virulence factors, such as exotoxins, play an important role in the fitness of pathogens; yet, they are not essential for bacterial survival. Secreted exotoxins kill or modify the behavior of host cells allowing bacteria to erode epithelial barriers and to evade host immune responses.

Recently we have shown that empty liposomes composed exclusively of naturally occurring lipids can be used to neutralize a variety of bacterial exotoxins. Here we tailored the liposomal lipid composition to selectively neutralize toxins secreted by Group A Streptococci (*Streptococcus pyogenes*, GAS) and Group G Streptococci (*Streptococcus dysgalactiae* subspecies *equisimilis*, GGS). Both GAS and GGS produce two potent pore-forming exotoxins, streptolysin O (SLO) and streptolysin S (SLS). SLO and SLS are key virulence factors that allow the establishment of successful streptococcal infections.

Here we show that SLO binds to cholesterol (Ch)-containing liposomes, whereas SLS preferentially interacts with liposomal sphingomyelin (Sm). We show that despite group-specific and strain-specific expression of exotoxins, the whole palette of streptococcal hemolysins/cytolysins can be completely neutralized by liposomes composed of Ch:Sm. Moreover, the efficiency of liposomal nanotraps can be further enhanced by adapting the liposomal formulation to sequester individual toxins. These compounds constitute new therapeutic tools for for patients with streptococcal infections.

M245 - Susceptibility of human oral bacteria to roseoflavin, a vitamin B2 analogue

Presenting Author – *Nadja Schwendenmann, Furtwangen University Of Applied Sciences, Germany*

Author/s – *Leonie Steiner, Lars Seufert, Matthias Mack, Markus Egert*

Abstract Content – A recent study showed, that *Streptococcus mutans* from the human oral cavity is auxotrophic for riboflavin (RF, vitamin B2). This finding might open up new therapeutic options against caries. In soil, *Streptomyces davaonensis* produces the antivitamin Roseoflavin (RoF). RoF is a structural RF analogue and competitive inhibitor of RF and has the potential to negatively affect growth of microbial competitors. Hence, the oral application of RoF might be a novel approach to ameliorate the cariogenic potential of the human oral microbiota. However, little is known about the antimicrobial efficacy of RoF against human oral bacteria. Here, we obtained bacterial pure cultures from saliva samples of 25 healthy human volunteers and identified them by using Matrix Assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF-MS). So far, 27 different species were obtained, all representing well-known human oral bacteria, and tested for their susceptibility to RoF using the disk diffusion method. While all gram-negative species (12) were found resistant, 10 out of 15 gram-positive species were clearly inhibited by RoF. These data suggest that RoF-treatment might indeed be suitable to modulate the human oral microbiota. Ongoing research aims at increasing the number of tested bacterial strains and at characterizing the inhibited species and the biochemistry of the underlying inhibition processes in more detail.

M246 - DnaJ-mediated Expression of IL-1beta is under the Control of ERK and p38

Presenting Author – *DaeKyum Kim, Korea University, Republic of Korea*

Author/s – *DaeKyum Kim, Jaehoo Lee, Yeji Lee, Un-Hwan Ha*

Abstract Content

Backgrounds: *Pseudomonas aeruginosanosa* (*P. aeruginosa*) is an opportunistic bacterial pathogen that possesses diverse virulence factors. Heat shock proteins (HSPs) are recognized as their roles in modulating innate immune responses. Recently, we found that DnaJ (a homolog of HSP40) derived from *P. aeruginosa* induces the expression of IL-1beta, which plays a role in stimulating anti-microbial defense responses.

Objectives: The signaling mechanisms related to the DnaJ-mediated IL-1beta expression remain unclear.

Methods: We measured mRNA and protein levels of IL-1beta by applying real-time Q-PCR, immunoblot analysis, and ELISA.

Results: To verify the effect of DnaJ in the increase of IL-1beta expression, we pretreated recombinant DnaJ (rDnaJ) with proteinase K, known as an effective enzyme to degrade proteins. As a result, the expression was completely diminished by the pretreatment, and it was induced in a dose-dependent manner, implying the action of rDnaJ protein for the expression. Next, we applied various chemical inhibitors, which block the activation of essential signaling mediators, to examine the related signaling pathways involved in the induction. It was shown that the IL-1beta expression is under the reciprocal control of p38 and ERK signaling pathways. Furthermore, the reciprocal actions of MAPKs was also involved in the activation of inflammasome-related molecules, including vimentin, NLRP3, caspase-1 and GSDMD, which are required for the maturation of IL-1beta. Thus, these results support that rDnaJ induces the production of IL-1beta through the reciprocal actions of two important MAPKs, ERK and p38.

M247 - A microfluidic study of the role of fibrinogen in the development of *Staphylococcus aureus* biofilms

Presenting Author – *Alessia Di Claudio, Humanitas University, Italy*

Author/s – *Luca Pellegrino, Raffaella Parente, Maria Rita Fumagalli, Eleonora Secchi, Andrea Doni, Roberto Rusconi*

Abstract Content

Background: *Staphylococcus aureus* is a clinically pathogen that plays an important role in establishing chronic infections in host tissues and medical implants. Many infections, such as the nosocomial ones, occur when *S. aureus* has easy access to the bloodstream (bacteriemia) and can metastasize reaching distal organs, eventually leading to the development of sepsis (1).

Objectives: Despite major advances in this field, the influence of plasma proteins on biofilm formation from *S. aureus* remains unclear (2). Therefore, we aim at studying this phenomenon under controlled environmental and physiological conditions.

Methods: We used microfluidics in order to perform real-time observations of bacterial adhesion and early biofilm development with high spatial and temporal resolution (3). Moreover, we exploited a newly developed microfluidic platform – composed by isolated micropillars – which triggers the formation of suspended, filamentous biofilms (aka streamers) and allows the in-situ analysis of the streamers' biochemical and rheological properties (4,5).

Results: We found that the presence of fibrinogen in the blood plasma increases bacterial adhesion and promotes a stronger and more compact biofilm architecture. Starting from comparing normal human plasma (NHP) and serum, we observed different phenotypes in terms of shape and cell density. Since NHP differs from serum in fibrinogen content (6), we then examined NHP in depleted conditions, confirming the crucial role played by fibrinogen in driving biofilm formation from *S. aureus*. Additional experiments on biofilm streamers have supported this finding and will further allow us to expand these results on a broad range of environmental settings.

M248 - G-quadruplex DNA motifs in *Helicobacter pylori* genome and their potential role as a drug target

Presenting Author – *Monika Kumari, Central University Of Haryana, India*

Author/s – *Saumya Jaiswal, Uma Shankar, Sharad Gupta, Amit Kumar, Vikas Yadav, Puja Yadav,*

Abstract Content

Background: *Helicobacter pylori* infect human stomach and cause different gastric disorders. With the increase in antibiotic resistance, *H. pylori* infection is becoming inadequate for the treatment purpose. Recently, several studies have shown the presence of DNA secondary structures, G-quadruplexes in many organisms that plays a vital role in various biological processes and pathogenesis. However, these secondary structures have not been explored much in pathogens.

Objectives: Present work aimed to characterize the presence of putative G-quadruplex (PGQs) motifs within the *H. pylori* genome. The study also determined the role of these secondary structures in *H. pylori* pathogenesis and biological processes.

Methods: In this study QGRS mapper, CD spectroscopy and NMR was used to identify stable PGQs in *H. pylori*. And bacterial growth was evaluated to study the effect of G-quadruplex specific ligand. Moreover, G-quadruplex structures association in replication process was determined by qPCR stop assay.

Results: The study revealed PGQs presence in 23 genes through QGRS mapper algorithm with possible association of 11/23 PGQs in virulence. CD spectroscopy and NMR confirmed the formation of G4 motifs in screened PGQs. These PGQs were interrupted by substitution mutation Guanine to Adenine. Point mutation consistently reduced the G4 formation in these PGQs *in vitro* revealing the effectiveness of G4 mutation in their stability. Following, the cell viability assay result showed that G4 binding ligand inhibited the growth of *H. pylori* with an IC50 at 0.015µM. Consequently, study includes that there is significant effect of G-quadruplex in *H. pylori* pathogenesis and biological processes.

M249 - Prevalence and molecular characterization of tick-borne *Rickettsia* species in South Korea

Presenting Author – *So Youn Youn, Animal And Plant Quarantine Agency, Republic of Korea*

Author/s – *Ji yeon Lim, Mi Sun Yoo, Se Jeong An, A Tai Truong, Soon Seek Yoon, Yun Sang Cho*

Abstract Content

Ticks are great menace to pets, livestock, and human health. They serve as vectors to all pets, livestock, and human pathogens including Rickettsiae.

Tick-borne rickettsioses are considered important emerging from mild to life threatening that impact on medical and veterinary health worldwide, but their etiological agents remain incompletely characterized in South Korea. Therefore, we determined the molecular characteristics of Rickettsia spp. infections in ticks in this area.

A total of 29547 ticks (2291 adults, 1769 nymphs and 327 larvae) were collected by dragging and flagging method from March to July in South Korea. Ticks were sorted into 4387 pools according to collection date, location, species developmental stage, and sex. Each pool consisted of adults (1), nymphs (1-10) or larvae (1-50). DNA preparation of ticks were assayed for rickettsiae by citrate synthase gene (gltA) and resulting amplicons sequence to determine the identity and prevalence of spotted fever group rickettsiae (SFGR).

Haemaphysalis longicornis (3557; 2008 adults and 1549 nymphs) were the most commonly collected ticks, followed by *H. flava*. The minimum field infection rate was 12.5 % for the gltA gene PCR assays. The gltA gene sequences from positive pools of H. longicornis and H. flava were similar to R. japonica and *R. monacensis*. SFGR were detected in two species of ixodid ticks collected in South Korea during 2022 March-October. Results from this study demonstrate the need to conduct longitudinal investigations to identify tick-borne rickettsiae and better understand their potential impact on medical and veterinary health.

M250 - CbpA is involved in *LigiLactobacillus salivarius* FFIG58 ability to modulate TLR3-medieted intestinal antiviral immunity *in vivo*

Presenting Author – *Haruki Kitazawa, Tohoku university, Japan*

Author/s – *Hikari Yamamuro, Yoshiya Imamura, Lorena Arce, Mikado Tomokiyo, Binghui Zhou, Sudeb Saha, Fu Namai, Aramaki Aza Aoba, Aoba-ku, Wakako Ikeda-Ohtsubo*

Abstract Content

Previous studies demonstrated that *LigiLactobacillus salivarius* FFIG58 adhered to porcine intestinal epithelial (PIE) cells and beneficially modulated the antiviral immune response. PIE cells treated with the FFIG58 strain had reduced expression of rotavirus proteins VP6 and NSP5 and increased expression of antiviral factors after the challenge with the pathogen. Considering that the surface-layer protein choline-binding protein A (CbpA) is involved in the ability of *L. salivarius* strains to adhere and colonize the intestinal tract, in this work, we advanced in the characterization of the FFIG58 strain by developing a CbpA mutant (ΔCbpA) strain and evaluating its immunomodulatory activities *in vivo*. Infant BALB/c mice (3 weeks-old) were orally treated with FFIG58 strain or the ΔCbpA mutant for 5 days (108 cells/day/mouse) and then received an intraperitoneal injection of poly(I:C) to induce intestinal inflammation. Non-lactobacilli treated mice were used as controls. The wild-type FFIG58 strain significantly reduced the inflammatory-mediated intestinal damage, the increase of inflammatory intraepithelial lymphocytes (CD3+CD8aa+NKG2D+ cells) and the up-regulation of pro-inflammatory mediators (TNF-α, IL-1β, IFN-γ, IL-15, RAE1, IL-8) (p<0.05) triggered by TLR3 activation in the intestine. Mice treated with the ΔCbpA mutant showed no differences with untreated controls when the inflammatory factors were evaluated. The results show that the CbpA protein is involved in the ability of the FFIG58 strain to beneficially modulate TLR3-mediated intestinal inflammation *in vivo*. This is a step forward in the understanding of the cellular mechanisms involved in the antiviral capabilities of *L. salivarius* FFIG58.

M251 - Phage host range specificity switch

Presenting Author – *Hui Yi Tay, National University of Singapore, Singapore*

Author/s – *John Chen*

Abstract Content

Bacteriophages (phages) play a critical role in the evolution of the bacterial genomic landscape. Phages serve as a reservoir for antibiotic resistance genes and can act as highly efficient transducing agents that drive the transmission of genetic elements such as plasmids and pathogenicity islands. Here, we observed a rapid switch in Φ80a phage host range specificity upon replication in the clinical *S. aureus* isolate C12. We first determined that the observed change in host specificity was not due to interference by other endogenous phages in C12. An analysis of the C12 genome pointed to a lack of endogenous prophages. SOS induction of C12 also indicated that plasmids and other mobile genetic elements could not be transduced out, further indicating the lack of resident transducing phages in C12. Next, we determined if the host range specificity switch was a general mechanism to any incoming phage(s) or was only specific to Φ80a. Currently, we are screening through the host genome to identify *S. aureus* encoded determinants that account for the switch in phage host range specificity. Results from this study could have far-reaching implications for bacterial evolution and host-encoded phage host range determinants identified from this study could also be potentially manipulated for the purpose of phage therapy.

M252 - Instability of antimicrobial susceptibility in negative controls during biocide exposure studies

Presenting Author – *Thomas Willmott, University of Manchester, United Kingdom*

Author/s – *Paul Kelly, Gavin Humphreys, Andrew McBain*

Abstract Content

Risk assessment of biocide exposure frequently relies on experiments where pure cultures of bacteria are taken through a rapid succession of passages in the presence of aqueous solutions of biocides. In many cases, controls, where the test bacteria are similarly passaged in the absence of biocides, are not included. Here, we aimed to examine the instability of antimicrobial susceptibility in negative control samples. We have passaged 10 microbial species (*Pseudomonas aeruginosanosa*, *Staphylococcus aureus*, *Escherichia coli* (DSM 682 and K12), *Enterococcus hirae*, *Klebsiella pneumoniae*, *Mycobacterium smegmatis*, *Bacillus subtilis*, *Candida albicans* and *Salmonella enterica*) 20 times in biocide-free media and tested susceptibility to five biocides and 10 antibiotics by MIC/MBC and disk diffusion. Passaged microbial strains (10/10) exhibited decreased susceptibility to biocides and both increased (9/10) and decreased (9/10) antibiotic susceptibilities. *S. aureus* showed the greatest susceptibility decreases to gentamycin, ceftazidime, tetracycline and cephalothin. Fold changes in antibiotic susceptibility ranged between -1; with decreased *E. hirae* sensitivity to kanamycin, and 0.75; with increased *P. aeruginosa* sensitivity to tetracycline. We suggest that changes in antimicrobial susceptibility during rapid and repeated microbial passage should be considered in biocide risk assessment.

M253 - Effects of chitin on *Listeria monocytogenes* pathogenicity and host responses

Presenting Author – *Monica Cazzaniga, University College Cork, Ireland*

Author/s – *Miguel Villoria Recio, Cormac Gahan*

Abstract Content

Background: Chitin contributes to a healthy gut microbial population, modulates the immune response, and attenuates virulence gene expression in *Listeria monocytogenes*. However, little research is available on the effects of chitin in host-pathogen interactions. Here, we explored the polymer in the context of *L. monocytogenes* infection.

Objectives: Our studies investigated the modulatory properties of chitin in both host and pathogen and suggest that chitin is an excellent candidate for dietary inhibition of *Listeria* infection.

Methods: Different virulence-repressing concentrations of chitin were tested on HT29-MTX-E12, C2BBe1, and RAW 264.7 cells. Viability, adhesion/invasion, and proliferation assays were performed under the influence of chitin and a lux-reporter strain was used to assess virulence gene expression upon infection. ELISA assays were conducted to measure cytokine expression on LPS- and *Listeria monocytogenes*-induced macrophages during chitin treatment. The permeability of epithelial cells in the presence of chitin was evaluated with a TEER assay.

Results: Biologically relevant concentrations of chitin did not reduce viability of treated cells or change permeability of epithelial cells after 24h treatment. However, adhesion/invasion of epithelial cells with either 0.05% or 0.1% chitin pre-treated bacteria was significantly reduced. Chitin effectively downregulates the virulence genes of *L. monocytogenes* in situ and influences the ability to adhere to and invade epithelial cells. In macrophages chitin exerted immunomodulatory properties, reducing LPS-activated IL-6 but inducing a TNF-α response. The data suggest that chitin may provide a potential dietary intervention to inhibit *L. monocytogenes* infection in the gut and murine experiments are ongoing to further test this hypothesis.

M254 - Deconstructing the intracellular pathogen effector network of *Citrobacter rodentium*

Presenting Author – *Priyanka Biswas, Imperial College London, United Kingdom*

Author/s – *Priyanka Biswas, Lucrecia Alberdi, Julia Sanchez-Garrido, Gad Frankel*

Abstract Content

Citrobacter rodentium, an attaching/effacing pathogen, injects 31 type effectors into the host cell cytoplasm via a type III secretion system. These effectors interact with host cell proteins to hijack diverse processes. It has recently been shown that the effectors form a robust network that can sustain significant contractions (>60%) and still maintain pathogenicity. This suggests that rather than working individually, effectors form a robust and flexible network and exhibit functional synergy, antagonism, or redundancy. To assess the minimum cohort of effectors required for pathogenesis, we sequentially deleted effector genes from *C. rodentium*; the mutant strains were used to orally infect C57BL/6 mice. We quantified the impact on pathogen-host interaction by enumerating faecal bacterial shedding and lipocalin-2 and analysing histological section and neutrophil recruitment. Contraction of *C. rodentium* effector network from distinct starting points led to the generation of CRl21, CRp20, CRpb20, and CRi17 strains, retaining only 10, 11, 11, and 13 effectors respectively. The four strains colonized mice, triggered low-grade inflammation, and triggered protective immunity. This project revealed that the effector NleG8 exhibits novel context-dependent effector essentiality (i.e., it is essential in some networks but not others). Interestingly, 12 effectors are missing from all 4 strains, suggesting that they are redundant for pathogenesis. Indeed, a mutant missing the shared 12 effectors (CRm12) colonized mice similarly to the wild type. Intriguing questions for future studies are: how does the host respond to infection with CRm12 and what is the selective pressure that maintains these 12 effectors in *C. rodentium*?

M257 - YgfB increases β-lactam resistance in *Pseudomonas aeruginosanosa* by counteracting AlpA-mediated *ampDh3* expression

Presenting Author – *Ole Eggers, University Of Tübingen, Germany*

Author/s – *Fabian Renschler, Lydia Anita Michalek, Noelle Wackler, Elias Walter, Fabian Smollich, Kristina Klein, Michael Sonnabend, Valentin Egle*

Abstract Content

The uncharacterized gene *ygfB* contributes to β-lactam resistance in multidrug-resistant (MDR) *Pseudomonas aeruginosanosa* (*Pa)* strains by increasing expression of the cephalosporinase AmpC, which mediates resistance to broad-spectrum cephalosporins and other classes of β-lactams. AmpC production is activated by 1,6-anhydro-N-acetylmuramyl-L-alanyl-D-glutamyl-meso-diaminopimelic-acid peptides (anhMurNAc-peptides) arising from the peptidoglycan pathway upon binding to the transcriptional regulator AmpR.

By transcriptomic, expression and promoter activity analysis as well as antibiotic susceptibility testing, LC-MS analysis of peptidoglycan precursors and protein-protein/protein-DNA interaction assays, we unraveled the mechanism how *YgfB* contributes to AmpC overproduction and β-lactam resistance.

YgfB inhibits expression of the amidase AmpDh3, which degrades the AmpR activating anhMurNAc-peptides in the cytoplasm, resulting in increased AmpC production. *YgfB* does so by binding to AlpA, an essential transcriptional activator of AmpDh3. Since AlpA production can be induced by DNA-damage, e.g. by treatment with ciprofloxacin (CIP), we investigated the impact on the combination of ciprofloxacin and β-lactams in the MDR strain ID40. Checkerboard assays showed, that *ygfB* prevents an effective combination of CIP and several β-lactam antibiotics via the AlpA-AmpDh3 pathway, while in the *ygfB* deletion mutant the combination could inhibit growth in therapeutic concentrations.

We identified a novel contributor and mechanism in the complex regulation network of MDR in *Pa*. Differences in the regulatory network modulating a*mpDh3* expression in various *Pa* strains might explain the strain-by-strain variations of the effectiveness of a ciprofloxacin/β-lactam combination. Given the plasticity of these regulation networks causing MDR, we think that this is an important step towards better understanding MDR in *Pa*.

M258 - DnaJ-induced miRNA-146a negatively regulates the Expression of IL-8

Presenting Author – *Jaehoo Lee, Korea University, Republic of Korea*

Author/s – *Jaehoo Lee, Daekyum Kim, Yeji Lee, Un-Hwan Ha*

Abstract Content

Backgrounds: As a member of damage-associated molecular patterns, heat shock proteins (HSPs) are well-known for their roles in initiating innate immune responses. Recently, we found that *Pseudomonas aeruginosanosa* (*P. aeruginosa*)-derived DnaJ (a homolog of HSP40) increases the expression of IL-8, which is an important chemokine to recruit immune cells, thereby contributing as an immune modulator to development of anti-microbial responses.

Objectives: The signaling mechanisms related to the IL-8 expression in response to DnaJ remain unclear.

Methods: We measured expression levels of IL-8 and miR-146a by applying real-time Q-PCR.

Results: To verify the marked effect of DnaJ for the expression of IL-8, we compared its effect with the other three HSP homologs, DnaK, GroEL, and HtpG. As a result, recombinant DnaJ (rDnaJ) appeared for its strong inducing effect. The expression of miR-146a was also highly increased by the treatment with rDnaJ. Next, it was found that the treatment with heat-killed *P. aeruginosa* (Hk Pa) markedly induced the expression of IL-8. However, the degree of induction in response to the post-treatment with Hk Pa was notably reduced half by the pretreatment with rDnaJ for 12 h, implying the possible involvement of miRNA-146a in the Hk Pa-mediated induction of IL-8 expression. To examine the negative effect of miRNA-146a, we transfected the miR-146a inhibitor into cells, resulting in recovering the reduced expression. Thus, these results suggest that DnaJ-induced miR-146a might reduce the excessive expression of IL-8 during microbial infections.

M259 – How order of adminstration of Ciprofloxacin, Caspofungin and Bacteriophages influence *Staphylococcus aureus*/*Candida albicans* DU

Presenting Author – *Marta Gliźniewicz, Pomeranian Medical University in Szczecin, Poland*

Author/s – *Marta Gliźniewicz, Barbara Dołęgowska, Bartłomiej Grygorcewicz*

Abstract Content

Alternative therapies against drug-resistant microorganisms are still widely searched. One of the approaches to solve this problem is phage-antibiotic synergy (PAS) therapy. However, we must extend knowledge of how bacteriophages and antibiotics cooperate in the human body environment. Also, the dosage of antibiotics and phages and the time and order of administration need to be optimized.

This study aimed to examine how the order of drugs and phages administration influences *S. aureus*/*C. albicans* biofilm in the human urine model. To do so, 24h-old *S. aureus*/*C. albicans* biofilm was formed on 96-well plates. Then biofilm was suspended in human urine and antibiotics (CIP 8 mg/L (1x MBEC80); CASP 0,2 mg/L (1×MBEC80) and phage cocktail (AD) approx. 1×107 PFU/mL were added simultaneously or in 6-hour or 24-hours intervals. After incubation, viability assay and biofilm biomass assay were performed and compared to the control (LB medium).

Results show that there is more than one favorable combination, and the optimal treatment is changeable depending on the time of intervals and the environment of treatment. When limited components were applied, biofilm eradication was less efficient on LB and human urine models.

In conclusion, the order of administration of antibiotics and phages is critical for successfully eradicating *S. aureus*/*C. albicans* biofilm in the human urine model. This aspect should be considered when planning alternative therapies based on the synergistic action of drugs and bacteriophages.

M260 - *Lantana camara* L. water extract modulates phagocytotic and bactericidal activities of macrophages against bacterial pathogens

Presenting Author – *Franklin Wang-Ngai CHOW, The Hong Kong Polytechnic University, Hong Kong*

Author/s – *Patrick Pak-Ting HAU, Carsten Tsun-Ka Kwok, Ray Chun-Wai Yu, Fiona Wong, Sai-Wang Seto*

Abstract Content

Background: The global emergence of multidrug-resistant pathogens poses serious threats to human health. Ethanol extract of the flowering plant *Lantana camara* Linn was demonstrated to inhibit bacteria effectively. However, it is highly toxic and cannot be studied further in cell-culture models. While the water extract of *L. camara* L. (LWB) is non-toxic and understudied, we therefore investigate its effect on host-cells during multidrug-resistant bacteria infections.

Objectives: This study aims to examine the anti-microbial and anti-inflammation effects of LWB in a mouse macrophage model during bacterial infections.

Methods: The cytotoxicity of LWB was examined by MTT-assay, the minimal-inhibiting-concentration (MIC) of LWB against bacteria (*Staphylococcus aureus* (SA) and *Escherichia coli* (EC)) was determined by broth-dilutions. Phagocytotic and bactericidal activities of RAW264.7 macrophages against bacteria were determined by measurement of colony-forming-units (CFU) and nitric-oxide(NO) level by Griess reagent post-infection. Immune genes regulated by LWB in cells were studied using qRT-PCR.

Results: MTT assay suggested that 2mg/ml of LWB was not cytotoxic. While 2mg/ml of LWB cannot inhibit bacteria growth, phagocytotic rate of 2mg/ml LWB-treated macrophages against SA and EC were reduced by 4 and 1.49-fold respectively. Moreover, LWB treatment stimulated NO-production by 4-fold during SA-infection but reduced NO-production by 4.83-fold during EC-infection. Furthermore, LWB is likely to suppress the LPS-induced pro-inflammatory responses via inhibiting the NF-κB signalling pathway.

Together, these findings suggest that the non-toxic LWB maybe a therapeutic option that orchestrates the immune system against multidrug-resistant bacterial infections and potentially prevents drastic immune responses.

M262 - Functional characterization of VirB- and Icm/Dot-like type IV secretion systems from the plant pathogen *X. euvesicatoria*

Presenting Author – *Sabine Drehkopf, Martin Luther University Halle-Wittenberg, Germany*

Author/s – *Daniela Büttner*

Abstract Content

The Gram-negative bacterium *Xanthomonas euvesicatoria* causes bacterial spot disease on pepper and tomato plants and depends on a type III secretion system to translocate bacterial effector proteins into plant cells. *X. euvesicatoria* also contains two putative type IV secretion (T4S) systems with homology to the VirB T4S system from Agrobacterium and the Icm/Dot T4S system from the human pathogen Legionella pneumophila. In the present study, we investigated a contribution of T4S genes to the interaction of *X. euvesicatoria* with its host plants or with other bacteria. The analysis of reporter fusions and protein-protein interaction studies revealed that T4S genes are expressed and that the corresponding gene products are likely involved in the assembly of oligomeric protein complexes corresponding to known interactions of T4S system components. Mutant studies suggest that both T4S system are likely involved in plasmid transfer and do not significantly contribute to bacterial virulence. Bioinformatic analyses and *in vitro* secretion assays led to the identification of T4S substrates which are targeted to both T4S systems, suggesting a broad substrate specificity of VirB-type and Icm/Dot T4S systems from *X. euvesicatoria*. Protein modeling revealed that T4S substrates contain a predicted C-terminal structural motif that was identified in T4S effectors from *X. citri* as part of the T4S signal and binding site for T4S-ATPase VirD4. Notably, however, in *X. euvesicatoria* a similar structural motif is not sufficient for T4S, suggesting the presence of additional targeting mechanisms.

M263 - Influence of glycan structure on colonization of *Streptococcus pneumoniae* on human respiratory epithelial cells

Presenting Author – *Ye Yu Chun, National University of Singapore, Singapore*

Author/s – *Kai Sen Tan, Lisa Yu, Michelle Pang, Millie Wong, Rei Nakamoto, Wan Zhen Chua, Amanda Huee-Ping Wong, Zhe Zhang Ryan Lew, Hsiao Hui Ong, Vincent T. Chow, Thai Tran, De Yun Wang, and Lok-To Sham*

Abstract Content

The capsular polysaccharide (CPS) of *Streptococcus pneumoniae* encases the cell envelope, shielding the bacteria from host immunity. Depending on the CPS produced, the outer surface charge of the cell may vary. To date, approximately 100 CPS types (serotypes) have been identified. Yet, how the extensive composition and configuration of CPS influence interactions with host remain enigmatic. We constructed 258 isogenic capsule-switch mutants representing 84 serotypes in *S. pneumoniae*. The mutants were chromosomally tagged to allow abundance of each strain to be quantified by amplicon sequencing. This collection enables systematic measurement of the affinity of structurally related CPSs to primary human nasal and bronchial epithelial cells. Contrary to the paradigm, surface charge did not significantly affect epithelial cell binding. We also elucidated structural features of CPS that may affect adhesion to respiratory cells, such as rhamnose sugar residues or human-like glycomotifs. Finally, pneumococcal CPS production generally reduced the innate immunity of the epithelial cells, as observed by the lower levels of IL-6, GM-CSF, and MCP-1 cytokines. Our results establish the importance of surface CPS structure to colonization on the human airway.

M264 - Congruency of genotypic and phenotypic antimicrobial resistance in udder isolates

Presenting Author – *Julia Anna Schwenker, Christian-albrechts-universität Zu Kiel, Germany*

Author/s – *Christina Hölzel*

Abstract Content

The increased incidence of antibiotic resistance in mastitis pathogens is a challenge in efficient and successful mastitis therapy. Many of these resistances are encoded by known antibiotic resistance genes (ARGs), showing regional differences in prevalence. Prevalence of ARGs in mastitis pathogens is poorly studied, and less is known about the correlation between ARG occurrence and the actual expressed – i.e. phenotypic – resistance.

The objective of this study was to correlate genotypic to phenotypic resistance in udder isolates. Therefore, isolates were obtained from quarter milk samples (QMS, n=34, quarters with previous mastitis history). Moreover, 48 isolates from routine mastitis diagnostics were included. Isolates were identified at species level by MALDI-ToF; major pathogens were confirmed by species-specific PCR. ARG-PCR testing included mecA, blaZ, ermA, ermB, U-blaCTXM, sul1, sul2, strA and strB, depending on pathogen. Phenotypic resistance was assessed by microdilution. Susceptibility was recorded as minimum inhibitory concentrations, MICs, and categorized by CLSI-vet breakpoints where available.

First results indicate phenotypic and genotypic differences between quarter milk samples and isolates from routine mastitis diagnostics. Pretreated animals were positive for mecA in 7 out of 34 quarters. In routine mastitis isolates, mecA was not detected; however, five of eight *S. aureus*-isolates were positive for blaZ. Penicillin MICs showed moderate agreement: in two cases, blaZ was apparently not expressed (MIC was wildtype, but blaZ was present). In one case, MIC was increased (0.5) but neither blaZ nor mecA were detected. These and other results will be discussed.

M265 - The F-box type III effector XopI from *Xanthomonas* opens stomata and targets regulators of abscisic acid signaling and plant immu

Presenting Author – *Oliver Nagel, Martin Luther University Halle-Wittenberg, Germany*

Author/s – *Daniela Büttner, Ulla Bonas*

Abstract Content

The non-vascular pathogen *Xanthomonas euvesicatoria* (Xe) enters the plant tissue via stomata and causes bacterial spot disease in pepper and tomato. Bacterial multiplication in the plant tissue depends on translocated type III effectors (T3Es) which counteract basal defense responses. The F-box T3E XopI suppresses the microbe-associated molecular pattern (MAMP)-triggered generation of reactive oxygen species (ROS) and interferes with stomatal closure in response to MAMPs and the phytohormone abscisic acid (ABA). The virulence function of XopI depends on the F-box motif which is required for the interaction of XopI with SKP1, a component of SCF (SKP1[S-phase kinase-associated protein 1]/CULLIN1/F-box protein)-type E3 ubiquitin ligases. Protein interaction studies suggest that XopI acts as adaptor between SKP1 and plant proteins. Here, we identified plant interactors of XopI using yeast two-hybrid assays, pull-down and coimmunoprecipitation experiments, and analysed their contribution to the virulence function of XopI by virus-induced gene silencing. Our experiments revealed that XopI interacts with an ATP binding cassette (ABC) protein, which contributes to MAMP-triggered stomatal closure. Additional XopI interactors include the catalase CAT2 and a RAF-like kinase involved in ABA signaling. ABCF4 and the RAF-like kinase, but not CAT2 are destabilized in the presence of XopI, suggesting that XopI triggers the degradation of some but not all of its plant interactors.

M266 - Prevalence of *Neisseria meningitidis* carriage among university students in Lithuania and characteristics of carriage isolates

Presenting Author – *Aistė Bulavaitė, Vilnius University, Lithuania*

Author/s – *Silvija Kiverytė, Rimvydas Ivaškevičius, Inga Ivaškevičienė, Milda Plečkaitytė*

Abstract Content

Background: *Neisseria meningitidis* causes invasive meningococcal disease (IMD) associated with significant morbidity and mortality. The incidence of IMD in Lithuania was among the highest in Europe during 2009–2019 reaching an average of 2.24 cases/100,000 population. In 2020–2022, the incidence decreased resulting in 0.4 cases/100,000 population. Oropharyngeal carriage of *N. meningitidis* is considered a prerequisite for the development of IMD, however no carriage studies have been performed in Lithuania.

Objectives: We sought to determine the prevalence of meningococcal throat carriage among university students (18–25 years aged) and to perform molecular characterization of carriage isolates.

Methods: Oropharyngeal swabs (n=401) were collected between December 2021 and February 2023. N. meningitidis was detected by culture and swab PCR targeting porA and ctrA genes.

All isolates were characterized by multilocus sequence typing (MLST) and genotyped analyzing vaccine-related antigens to evaluate their coverage by 4CMenB and MenB-Fhbp vaccines using the Meningococcal Deduced Vaccine Antigen Reactivity (MenDeVAR) Index method.

Results: An overall carriage prevalence was 5% (20/401). Among 15 carriage isolates, capsule null locus cnl) accounted for 46.7%, followed by genogroups B (26.7%), Y (13.3%), and non groupable (13.3%). Genogroup B was compatible with that implicated in IMD in Lithuania. About 50% of carriage isolates have invasive potential. Invasive isolates of serogroup Y were not very common in Lithuania. Detecting serogroup Y among carriage isolates indicates that cases of IMD caused by it are likely to increase. Five of 15 isolates were potentially covered by the MenB-Fhbp and one isolate by the 4CMenB vaccine.

M267 - Biocidal action of Zn and syndet against Gram-positive bacteria

Presenting Author – *Dimitrina Foteva, , Bulgaria*

Author/s – *Dimitrina Foteva, Ivayla Pantcheva, Slavka Tcholakova, Svetoslav Anachkov*

Abstract Content

In 2019, around 2% of deaths were caused by *Staphylococcus aureus* infections [1]. Although numerous antimicrobial agents have been developed to fight pathogens, there is a great demand for mild cleansing formulations with antibacterial properties [2]. Synthetic detergents (syndets) are commonly used in cleansing formulations and should kill most bacteria without irritating the skin. However, in many cases, they are ineffective, especially if they contain zwitterionic surfactants. In our study, we investigated the biostatic and biocidal activity of zinc (Zn^{2+}), anionic and zwitterionic syndets against *S. aureus*. By the agar diffusion method, we determined the minimum inhibitory concentrations (MIC) of the tested biocides and their mixtures. Using the plate-count method, we found that anionic and zwitterionic syndet mixtures with zinc exhibit synergistic biocidal action at short exposure times. This effect stems from the increased cell permeability by syndet, resulting in higher zinc uptake and enhanced antibacterial properties. Moreover, the said synergism is robust in the presence of essential oils, cationic co-surfactants, or preservatives, which are typical additives in personal care formulations

M268 - Growth phase-dependent regulation of aagR and aaf genes by dispersin in enteroaggregative *Escherichia coli*

Presenting Author – *Jorge Giron, Translational Genomics Research Institute, United States*

Author/s – *Miguel Ares, Diana Rodriguez-Velarde, Jorge Soria-Bustos, Miguel De la Cruz, James Nataro, Jorge Giron, Gabriela Hernández-Martínez, Javier Torres, María L. Cedillo*

Abstract Content

Background: Enteroaggregative *Escherichia coli* (EAEC) is an enteric pathogen responsible for causing persistent diarrhea in the pediatric population and in immunocompromised patients. EAEC produces robust biofilms and adheres to the colonic mucosa where is causes an inflammatory response. The Aggregative Adherence Fimbriae (AAF) encoded on the large virulence plasmid (pAA) are responsible for the stickiness of EAEC strains and for inducing inflammation. Dispersin is a small protein also encoded on the pAA, which was shown to be secreted and displayed on the bacterial surface. It was proposed that dispersin regulates the mode that the AAF/II fimbriae are displayed on the bacterial surface consequently regulating their function in adherence the auto-aggregation of EAEC. Both the AAF and Dispersin are regulated by the master regulator AggR,

Objective and Methods: We investigated the role of dispersin in regulating transcription of aggR and fimbrial genes aafA and aafB using qRT-PCR.

Results: We show that dispersin affects the transcription of the AggR master regulator and consequently the expression of the AAF/II fimbriae, acting as a positive or negative signaling element at exponential or stationary phase, respectively. The comparison of cell adherence and biofilm formation of EAEC strain 042 versus the 042 aap mutant substantiated the dual growth phase-dependent effect of dispersin on the transcription of the aggR and aafA genes. These data unveil a heretofore unforeseen role of dispersin as a growth phase-dependent regulatory protein and provide insights into the transcriptional networks that control the expression of the main virulence factors during EAEC infection.

M269 - The secretome of the fish pathogen *Tenacibaculum maritimum* includes virulence-related proteins and outer membrane vesicles

Presenting Author – *M. Pilar Escribano, Universidade de Santiago de Compostela, Spain*

Author/s – *M. Pilar Escribano, Miguel Balado, Alicia E. Toranzo, Manuel L. Lemos, Beatriz Magariños*

Abstract Content

Tenacibaculum maritimum, the etiological agent of tenacibaculosis in marine fish, constitutively secretes extracellular products (ECPs). However, their protein content and their role in pathogenesis are unknown. In this study, a collection of 64 *T. maritimum* strains was used to analyze the prevalence of extracellular proteolytic and lipolytic activities related to virulence. Results suggested that the ECPs of *T. maritimum* contain outer membrane vesicles (OMVs), which were characterized by electron microscopy and purified. Total protein content and the proteins associated to OMVs and soluble fraction of the ECPs (S-ECPs) were identified by nLC-TIMS-QTOF. 641 proteins were identified including some virulence factors. Outer membrane proteins such as TonB-dependent transporters and the T9SS-related proteins appeared to be mainly associated with OMVs. By contrast, putative virulence factors such as sialidase, chondroitinase, sphingomyelinase, ceramidase and collagenase were found only in the S-ECPs. The final *in vitro* and *in vivo* evaluation of the fractions showed that both contribute significantly to the enzymatic activities, haemolysis, biofilm production, and cytotoxicity of *T. maritimum*. This work provides information on the composition of the *T. maritimum* secretome and constitutes a basis for future studies about the role of OMVs in its ecology and pathogenesis.

M270 - Construction and analysis of the T-DNA insertion mutant library of *Ustilago esculenta*

Presenting Author – *Jintian Tang, China Jiliang University, China*

Author/s – *Jintian Tang, Furong Yang, Yafen Zhang, Wenqiang Xia, Haifeng Cui, Zihong Ye*

Abstract Content

Agrobacterium tumefaciens-mediated transformation (ATMT) system was optimized and T-DNA insertion mutant library of *Ustilago esculenta* was constructed. Mutants defective in fusion and dikaryotic hyphal formation were screened and the T-DNA insertion sites were analyzed for further research on molecular mechanism of dikaryotic hyphal formation of *U. esculenta*. An artificial modified strain (TSP) with the ability of self-mating and a vector containing resistance gene (neo) of G418 were used for library construction. When the concentration of G418 was 75 μg/mL, the growth of TSP was completely repressed. AS concentration of 100 μg/mL, co-cultivation time of 24 h, spore concentration of 1×105 spores/mL, and A. tumefaciens concentration of OD600=0.3, were the optimal ATMT conditions for *U. esculenta*. Genetic stability of T-DNA in mutant was tested by PCR with the detection of neo target. Mating assay was performed on mutants to test the ability of fusion and dikaryotic hyphal formation. Genome of defective mutants formed in fusion and dikaryotic hyphal formation was sequenced for T-DNA insertion site analysis. Observations showed five mutants had defects in dikaryotic hyphal formation. Whole genome resequencing of two of them (TSP-1 and TSP-23) showed T-DNA inserted in the exon of mfa2.1 a loci (GenBank: MK097140.1) (TSP-1) and intergenic regions of two hypothetical proteins (TSP-23) respectively. In summary, this study optimized the ATMT system and constructed ATMT mutant library of *U. esculenta*, and dikaryotic hyphal formation defective mutants were screened and the T-DNA insertion sites were analyzed by genome resequencing.

M271 - Molecular cross-talk between Sa3int phages and their *Staphylococcus aureus* host

Presenting Author – *Ronja Dobritz, Interfaculty Institute for Microbiology and Infection-medicine Tübingen (IMIT), Germany*

Author/s – *Carina Rohmer, Christiane Wolz*

Abstract Content

As a major opportunistic pathogen of human and animals *Staphylococcus aureus* asymptomatically colonizes the nasal cavity, but is also a leading cause of life-threatening acute and chronic infections. More than 90% of the human nasal isolates of *S. aureus* were found to carry Sa3int phages, which integrate as prophages into the bacterial hlb gene thus disrupting the expression of an important virulence factor. The virulence factor-encoding genes carried by the Sa3-phages are all highly human-specific and probably essential for bacterial survival in the human host. Thus, both insertion and excision of the prophage could potentially confer a fitness advantage to *S. aureus*. However, how the *S. aureus* host modulates the life cycle of its temperate phages remains largely unknown. Our data suggest that this regulation is strain specific, with certain *S. aureus* strains being more prone than others to support either a lysogenic or a lytic life cycle. We constructed *S. aureus* single lysogens with integrated Sa3int prophages and found significant differences in phage transfer rates between different strains. Based on this finding, strains were grouped into low and high transfer strains. To get a more precise picture of the regulatory circuits we constructed replication deficient mutants, performed differential RNAseq to determine the transcriptional units and analysed a set of mutant strains. By transcriptional start site prediction we identified promoter-regions that are differentially active in high and low transfer strains and are a tool to identify regulators of the host by measuring promoter-fusion constructs in different mutant backgrounds.

M272 - Biocidal action of Ag and soap against *Staphylococcus aureus*

Presenting Author – *Gergana Georgieva, Sofia University, Bulgaria*

Author/s – *Dimitrina Foteva, Nikolay Avramov, Tsvetelina Paunova-Krasteva, Prem Chandar, Joseph Carnali, Svetoslav Anachkov*

Abstract Content

Microbial pathogens (bacteria, viruses, and fungi), which can cause infections and diseases in humans, are one of the leading causes of death worldwide, namely one-fourth of global deaths annually. Therefore, the control and prevention of microbial infections are of utmost importance. To fight with pathogens, many antimicrobial agents have been developed, such as antibiotics, disinfectants, and antiseptics. However, due to mutations, new strains of antimicrobial-resistant microorganisms are emerging, thus making the search of new biocides a substantial challenge.

In our study, we have tested the biocidal action of soaps and silver (Ag+) against planktonic and adherent *S. aureus*. Soaps are commonly used in hand-wash formulations and should be able to kill most bacteria within 30-60 s without damaging the skin, however, in many cases they are not very effective alone. We found that the antibacterial properties of soaps improve when their hydrophobicity increases up to 12 carbon atoms. Moreover, when soaps are combined with Ag+, we observed a synergistic effect: increased cell permeability due to soap, resulting into higher silver uptake and enhanced biocidal action. Furthermore, using SEM and TEM, we detected the external and internal defects due to the treatment, thus elucidating the mechanism of biocidal action.

M273 - Activity-Based Protein Profiling identifies *Klebsiella pneumoniae* Serine Hydrolases With Potential Roles in Host-Pathogen Intera

Presenting Author – *Md Jalal Uddin, UiT The Arctic University Of Norway, Norway*

Author/s – *Marco C. Viveen, Janetta Top, Mona Johannessen, Marcel R. de Zoete, Rob J. L. Willems, Christian S. Lentz*

Abstract Content

Klebsiella pneumoniae, a member of the Enterobacteriaceae family, is an opportunistic pathogen that naturally resides in the gastrointestinal tract microbiome of healthy humans and animals but can cause various extra-intestinal infections, including urinary tract, bloodstream, and lung infections. In the light of increasing levels of antimicrobial resistance of this pathogen, understanding the molecular factors that contribute to colonization and infection and validating their potential as drug targets is important.

Here, we aimed to identify and functionally characterize *K. pneumoniae* serine hydrolases (SH), one of the largest and most diverse and specifically druggable enzyme families in nature, that is not well studied in this pathogen.

We used activity-based protein profiling, a chemical proteomics technique that uses active site-directed, functionalized probes, to detect active SHs in live bacteria. After initial profiling of SH activities under different growth conditions using a fluorescent SH-reactive flurophosphonate probe and a gel-based read-out, we labeled active enzymes with a biotinylated probe analog and identified them using liquid chromatography-mass spectrometry after streptavidin-enrichment. Preliminary target validation was done using transposon mutant strains in colonization/infection assays with HT29-MTX and human-derived 2D colon organoids.

A total number of seventeen SHs were identified *K. pneumoniae* with unknown or predicted functions as esterases, proteases, and lipases. Several SH-deficient mutants that did not show any growth deficiency in *in vitro* media, showed reduced fitness in human-derived organoid co-culture studies. Our data suggest that these SHs are likely have important roles at the host-pathogen interface in the gut and are subject to further investigation.

M274 - Algae *Prototheca* as a causative agent of bovine mastitis

Presenting Author – *Magdalena Crhanova, Veterinary Research Institute, Brno, Czech Republic, Czech Republic*

Author/s – *Magdaléna Crhánová, Monika Beinhauerová, Vladimír Babák, Oto Hanuš, Růžena Seydlová, Ivana Kucharovičová, Petr Roubal, Marcela Klimešová*

Abstract Content

Background: The unicellular achlorophyllous algae *Prototheca* is one of the non-bacterial agents of bovine mastitis, and its prevalence in dairy cattle is increasing worldwide. Mastitis caused by Prototheca and other non-bacterial agents (yeasts or fungi) is not treatable with antibiotics, and the only defense against it is consistent prevention in dairy farms.

Objectives: Non-bacterial mastitis agents are not routinely diagnosed, so there is a lack of experience in their eradication. Our work aimed to map the non-bacterial mastitis agents with a focus on the prevalence of *Prototheca* in the Czech Republic to propose methods of detecting these agents and measures to prevent their spread.

Methods: A total of 1051 bulk tank milk samples were collected from 21 farms in the Czech Republic. For each sample, somatic cell count (SCC) was determined to indicate possible mammary gland inflammation. The presence and number of bacteria, fungi, yeasts, and *Prototheca* algae were determined using standard culture methods. Samples suspicious of the presence of Prototheca were identified using a two-stage real-time PCR system.

Results: The average SCC was (236±139) x103/ml, and the total number of microorganisms was (129±203) x103 ml. A total of 70 (6.66%) of all bulk tank milk samples tested were found to contain at least one species of mold. At least one yeast species was present in 1013 bulk tank milk samples out of 1051, i.e., in 96.3% of cases. In 46 samples (4.37 %), one of the *Prototheca* species was detected, and the most prevalent was *Prototheca bovis*.

M275 - Extracellular vimentin enhances phagocytosis of *Escherichia coli* and *Candida albicans* by human neutrophils

Presenting Author – *Lukasz Suprewicz, Medical University Of Bialystok, Poland*

Author/s – *Karol Skłodowski, Magdalena Zakrzewska, Krzysztof Fiedoruk, Robert Bucki*

Abstract Content

Background: Phagocytosis of invading pathogens is a primary innate immune function ascribed to neutrophils. Vimentin is a cytoskeletal protein that maintains cellular structure and integrity. Recent studies have shown an increased blood level of its secretory form - extracellular vimentin (eVim), during infections. However, the role of eVim in the innate immune response of human neutrophils toward bacterial and fungal infections remains poorly understood.

Objectives: This study aimed to investigate the effects and mechanism of eVim on the phagocytic activities of human neutrophils during the *Escherichia coli* and *Candida albicans* challenge. We also aimed to explore the molecular mechanisms underlying the effects of eVim on neutrophil function.

Methods: Human neutrophils were isolated from healthy volunteers, treated with eVIM, and incubated with bacteria or fungus. The effects of eVim on phagocytosis and netosis in human neutrophils were assessed using Western blot, qPCR, and confocal imaging. The expression of interleukin-8 (IL-8) was measured using multiplex ELISA.

Results: We found that eVim stimulated phagocytosis and netosis in human neutrophils with a simultaneous reduction in the intracellular survival of bacteria and fungi. qPCR gene expression screening showed that eVim exploited the FC-gamma R pathway to stimulate phagocytosis. eVim also stimulated IL-8 expression in human neutrophils. These findings provide new insights into the role of eVim in the innate immune response and may have implications for developing novel therapeutic strategies for infectious diseases..

M276 - Genomic and transcriptomic analyses uncover the influence of T6SS on *Klebsiella pneumoniae*

Presenting Author – *Wanzhen Li, Institute of Antibiotics, Huashan Hospital, Fudan University, China*

Abstract Content

Background: Gram-negative bacteria use type VI secretion systems (T6SSs) to deliver toxin effectors to interact with neighboring cells for niche advantage. *Klebsiella pneumoniae* (*K. pneumoniae*) is an opportunistic nosocomial pathogen that often carriers multiple cope T6SSs, but the function of its T6SS has not yet elucidated.

Objectives: Our study aimed to better understand the T6SS potential of *K. pneumoniae*.

Methods: We conducted a genomic analysis on the evolution, T6SS, virulence and antimicrobial resistance of 65 *K. pneumoniae* in patients with different infections. We combined transcriptome analysis after deletion of key genes hcp and vgrG in T6SS of this species. Then the vital role of T6SS in *K. pneumoniae* was verified by competitive growth assays.

Results: Genes encoding a T6SS cluster were present in all *K. pneumoniae* in this study, and there is no correlation was found between T6SS cluster and carbapenem resistance and virulence genes. Differentially expressed genes (DEGs) including 1298 co-upregulated and 1714 co-downregulated were identified after hcp and vgrG deletion. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analysis have demonstrated common changes in quorum sensing, propionate metabolism and other pathways. We found that the deletion of hcp and vgrG genes up-regulated of beta-lactam (blaKPC-2) and other resistance genes. Interbacterial competition experiments showed that hcp and vgrG are essential genes for T6SS competitive ability of ST11 *K. pneumoniae*. In summary, this study suggested that T6SS is associated with drug resistance, and its key genes hcp and vgrG are critical for the interspecies competition of *K. pneumoniae*.

M277 - *Mycolicibacterium komossense* as model organism for investigating rifampicin resistance in *Mycobacterium tuberculosis*

Presenting Author – *Joanna Rivas, University of St Andrews, United Kingdom*

Author/s – *Joanna Esther Rivas, Robert Hammond*

Abstract Content

Background: Tuberculosis (TB) is a bacterial infection caused by *Mycobacterium tuberculosis* (Mtb) a fastidious, and highly pathogenic organism. Antibiotic resistant Mtb strains are threatening progress in containing the global tuberculosis epidemic. Although there are different mycobacteria used as model organisms for Mtb research, these mycobacteria cannot mimic all the characteristics found in Mtb. The need to find a new rapidly growing and non-pathogenic Mycobacterial species that better reflects the antibiotic resistance characteristics of Mtb against rifampicin is crucial for better modelling Mtb resistance.

Objective: Characterise the resistance profile of *Mycolicibacterium komossense* (*M. komossense*) as a new model organism for rifampicin resistance studies.

Methods: Growth experiments were performed at different temperatures and the generation time of M. komossense was calculated. The minimum inhibitory concentrations (MIC) of *M. komossense* against different antitubercular drugs using the broth microdilution method was also estimated. The rate of spontaneous rifampicin resistance development and its physiological cost on *M. komossense* was assessed.

Results: Preliminary analysis reveals that *M. komossense* is a fast-growing Mycolicibacterium that grows equally well at 30 and 37°C producing a bright yellow pigment. However, its growth is inhibited at 42°C. *M. komossense* has a doubling time of 8 hours and has a similar MIC to those of Mtb when exposed to rifampicin, ethambutol and bedaquiline. However, *M. komossense* shows an intrinsic resistance against pyrazinamide and petromanid. This study extends our knowledge about *M. komossense* and it might encourage its use as a surrogate for the highly pathogenic Mtb.

M278 - Late stages of the intracellular life cycle of *Piscirickettsia salmonis* are required for the pathogen infectivity

Presenting Author – *Pamela Aravena, Universidad de Chile, Chile*

Author/s – *Javiera Ortiz-Severin, Veronica Cambiazo*

Abstract Content

Piscirickettsia salmonis is a facultative intracellular pathogen and the causative agent of piscirickettsiosis, a systemic infection of salmonid fish. *P. salmonis* actively replicates in fish macrophages, and its ability to enter and multiply within host cells is essential for its pathogenesis. Using an *in vitro* infection model, we report that at late stages of the intracellular life cycle *P. salmonis* triggers a transcriptional response to down-regulate translation and energy metabolism. At the same time the expression of virulence factors along with components of the nutrient starvation and stress resistance responses are up-regulated, suggesting the differentiation of *P. salmonis* into a form more virulent and potentially more resistant to environmental stresses. Our results indicate that bacteria extracted from infected cells at late stages of their intracellular life (10 dpi) significantly increase their replication in new target cells (up to 100 times) and also their cytopathogenicity (up to 55%) when compared with bacteria extracted at early infection time or with extracellular *P. salmonis* grown in broth. By confocal microscopy, recruitment of Lamp-1 and Rab-7 proteins into the *P. salmonis* containing vacuole was detected, suggesting that at this late stage of infection, *P. salmonis* resides within a phagosome-like compartment. These results provide insights into the regulatory mechanisms induced during the intracellular life cycle of *P. salmonis* that confer the bacterium the ability to leave the host cell and survive in hostile environmental conditions. We propose that the capability of *P. salmonis* to differentiate into a more resilient form constitute an important virulence trait.

M279 - Regulation of secondary metabolites biosynthesis by zinc in *Aspergillus fumigatus*

Presenting Author – *Cheol-Won Yun, Korea University, Republic of Korea*

Author/s – *Cheol-Won Yun, Hyewon Seo*

Abstract Content

Aspergillus fumigatus is a representative opportunistic fungal pathogen and causes various lung diseases, such as asthma and cystic fibrosis. Many strategies by which fungi protect themselves from the host defense system have been reported, and the secretion of secondary metabolites is one mechanism. To date, many studies on genetic mechanisms have shown the mechanisms involved in secondary metabolite expression, and some response factors to micronutrient stimuli have been reported. Recently, we found that zinc regulates gliotoxin and pseurotin A biosynthesis via ZafA, which is a zinc-responsive transcriptional activator. Furthermore, we found that the biosynthesis of gliotoxin and pseurotin A are regulated in opposite ways by zinc utilization and that each secondary metabolite is synthesized when the synthesis of another secondary metabolite fails to protect it against the defense system of the host. Here, we report the relationships between ZafA and the expression of secondary metabolites, including gliotoxin, pseurotin A, and fumagillin.

M281 - The catalytic and regulatory subunits of protein kinase A have divergent roles in growth and adherence in *Candida glabrata*

Presenting Author – *Chi-Jan Lin, National Chung Hsing University, Taiwan*

Author/s – *Nai-Jia Zheng, Yu-Shan Chang*

Abstract Content

Candida glabrata is an opportunistic human fungal pathogen and the third prevalent causative agents of candidiasis. The intrinsic tolerance to antifungal drugs and oxidative stress and the ability of adherence and biofilm formation are important virulence factors for *C. glabrata*. In pathogenic fungi, cyclic AMP dependent protein kinase A (PKA) signaling pathway and its downstream transcription factors have essential roles in growth, adherence and biofilm formation. However, studies of PKA in *C. glabrata* are limited. In this study, we identified the catalytic subunits Tpk1 and Tpk2, and the regulatory subunit Bcy1 of PKA in *C. glabrata*. The tpk1 and tpk2 mutants exhibited normal growth compared with the wild type, whereas the bcy1 mutant showed impaired growth in *C. glabrata*. Intriguingly, the bcy1 tpk1 double mutant exhibited severe growth defects than that of the bcy1 mutant at 37°C. In addition, the tpk1 mutant exhibited increased biofilm formation while the tpk2 and bcy1 mutants showed reduced biofilm formation in *C. glabrata*. The loss of biofilm formation in the bcy1 mutant was restored by complementation of BCY1 or further deletion of TPK1 gene. Furthermore, our data showed that tpk2 and bcy1 mutants had increased agar invasion whereas the tpk1 and bcy1 tpk1 double mutants exhibited reduced and abolished agar invasion, respectively. In conclusion, the catalytic and regulatory subunits of PKA have divergent functions on growth and adherence in *C. glabrata*.

M282 - Metagenomic insights of enteric bacterial pathogens in Kolkata, India

Presenting Author – *Kei Kitahara, Okayama University, Japan*

Author/s – *Debmalya Mitra, Goutam Chowdhury, Kei Kitahara, Basilua Andre Muzembo, Suman Kanungo, Asish Mukhopadhyay, Hemanta Koley, Shanta Dutta, Shin-Ichi Miyoshi*

Abstract Content

A developing country like India is sensitively affected to infectious diseases due to its sanitary and socioeconomic conditions. *Vibrio cholerae* O1/ O139, which is endemic to India, is extremely contagious and has caused seven pandemics till date. We aimed to understand the transmission routes of the pathogens and elucidate the gut microbiome pattern of Indian patients. Stool samples of diarrhea patients were collected from a pediatric hospital in Kolkata and screened for bacterial enteric pathogens using rapid diagnostics tests and culture tests along with PCR. Stools of age-matched family controls and drinking water samples were also collected and screened similarly. Furthermore, DNA samples were extracted to carry out next-generation sequencing studies. A total of 933 stool samples were screened from patients with active diarrhoea, of which *V. cholerae* O1 Ogawa (n =18) and *V. cholerae* non-O1 non-O139 (n=15), *Salmonella* sp. (n=8), *Shigella* sp. (n=69), and *E. coli* (n=88) were isolated. From the 500 control samples collected, healthy carriers of *V. cholerae* O1 Ogawa (n =3) and V. fluvialis (n=21) were identified. *V. cholerae* non-O1 non-O139 was also isolated from the 79 drinking water samples. Next generation sequencing revealed typical microbiome patterns of the diarrhea patients which were characterized by the dominance of the causative bacteria along with Firmicutes, Bacteroidetes and Enterobacteriaceae. Healthy people's microbiome patterns were classified into four different groups. Age was found to be a significant factor in determining the group to which a person's microflora belongs.

M283 - Influence of intracellular Zn-starvation on hemagglutinin/protease (HA/protease) activity and its transcriptional regulation

Presenting Author – *Vera Fengler, Universität Graz, Austria*

Author/s – *Vera Fengler, Martina Woelflingseder, Ada Walter, Ines Bilban, Joachim Reidl*

Abstract Content

Vibrio cholerae represents a facultative pathogen and transits between two dissimilar habitats, the aquatic ecosystems and the human hosts. In the latter one, it colonizes intestinal epithelia cells. To reenter the aquatic ecosystems after successful colonization, *V. cholerae* has to detach from the intestinal mucus. One of the involved factors is the typ II secreted metalloprotease hemagglutinin/ protease (HA/protease), encoded by hapA. Expression of hapA is induced by the quorum sensing regulator HapR at high cell density and is further dependent on the alternative sigma factor RpoS, which is crucial under starvation conditions. Until now it is not known if RpoS acts on the hapR-promotor and is thus enhancing hapA expression or if the hapA-promotor itself is RpoS dependent [1, 2, 3].

In our study we define so far unknown conditions acting on HapA activity. Namely, by reducing intracellular zinc (Zn) concentration, monitored by proteolytical activity of secreted HapA on skim milk agar plates. This increased HapA activity is not caused by enhanced hapA transcription although rpoS transcription is increased during Zn-starvation, shown by chromosomal phoA-fusions to the respective genes. Further, we have evidence, that the hapR- as well as the hapA-promotors are dependent on RpoS, although influence of another transcriptional regulator on the hapA-promotor cannot be excluded. Taken all together, we add another aspect in the tight control of HapA activity, which is complex at the transcriptional as well as the post-translational level.

M284 - TraN key residues affect conjugative pIP501 transfer in multi-resistant *Enterococcus faecalis*

Presenting Author – *Claudia Michaelis, Berliner Hochschule Für Technik, Germany*

Author/s – *Claudia Michaelis, Tamara Berger, Rangina Ghulam, Kirill Kuhlmann, Walter Keller, Elisabeth Grohmann, Lukas Petrowitsch, Maria Besora, Tea Pavkov-K*

Abstract Content

Conjugative transfer enables rapid gene transfer of antibiotic resistance among bacteria, facilitated by a membrane-embedded multiprotein complex, called the Type IV Secretion System (T4SS). Conjugation plays an important role in the development of multi-resistant bacteria.

The conjugative plasmid pIP501 from *Enterococcus faecalis* serves as a model for studying conjugative transfer among Gram-positive bacteria. It encodes its own T4SS and is organized in a single transfer (tra) operon comprising of 15 tra genes. The cytosolic transfer protein TraN is a repressor of the pIP501 tra operon. It acts as a transcriptional regulator by binding specifically to a site upstream of the origin of transfer of pIP501. Specific amino acids enabling TraN to interact with pIP501 DNA were identified through co-crystallization of TraN with its cognate pIP501 DNA (1).

Here, we examined putative TraN key residues *in vivo* and *in vitro*. A markerless pIP501ΔtraN knockout resulted in significantly enhanced conjugative transfer among *E. faecalis*. In trans complementation with wild-type traN fully restored the wild-type transfer rate. Multiple alanine substitutions of putative TraN key residues were conducted by site-directed mutagenesis. The influence of the resulting TraN mutant proteins on pIP501 transfer rate was tested using biparental mating assays. The TraN variants were recombinantly expressed, purified, and applied to microscale thermophoresis (MST) to determine the binding affinities of mutant proteins to their cognate pIP501 target DNA in comparison to wildtype TraN.

We show that several key residues are essential for TraN binding on pIP501 DNA and, therefore repressor activity.

M285 - Morphology and life cycle of the human blood microbiota in health and disease assessed by light and electron microscopy

Presenting Author – *Stefan Panaiotov, National Center Of Infectious And Parasitic Diseases, Bulgaria*

Author/s – *Stefan Panaiotov, Borislava Tsafarova, Yordan Hodzhev, Georgi Yordanov, Reni Kalfin*

Abstract Content

The existence of blood microbiota in clinically healthy individuals was proven during the last 50 years. Indirect evidence from radiometric analysis suggested the existence of living microbial forms in erythrocytes. NGS techinique demonstrated rich microbial biodiversity in the blood of healthy and diseased individuals. The morphology and proliferation cycle of blood microbiota are obscure. To study the life cycle of blood microbiota we focused on light and electron microscopy analysis. We studied freshly drawn blood and stress-cultured lysed whole blood at 43 ○C in presence of vitamin K from healthy individuals and sarcoidosis patients. We demonstrated by microscopy that free circulating microbiota in the PMBC fraction possess a well-defined cell wall and proliferate by budding or through a mechanism similar to the extrusion of progeny bodies. Stress-cultured lysed whole blood microbiota proliferated as cell-wall deficient microbiota by forming electron-dense or electron-transparent bodies. The electron-dense bodies proliferated by fission or produce in chains Gram-negatively stained progeny cells or enlarged and burst to release progeny cells of 180 – 200 nm size. Electron-transparent bodies enlarged and emit progeny cells through the membrane. A novel proliferation mechanism of blood microbiota called by us “a cell within a cell” was observed. It combines proliferation of progeny cells within a progeny cell which is growing within the “mother” cell. The microscopy results suggested different proliferation mechanisms in whole and cultured blood. Our documented evidence and conclusions provide a more comprehensive view of the existence of normal blood microbiota in healthy and diseased individuals.

M286 - Prediction of protein toxins using protein language models

Presenting Author – *Tanja Krueger, LMU Munich - Walther Straub Institute of Pharmacology and Toxicology, Germany*

Author/s – *Tanja Krueger, Luisa F. Jimenez-Soto*

Abstract Content

Biological protein function is determined by its amino acid sequence, which can be represented in various ways for machine learning applications. Here protein sequences were translated to embeddings with the help of a pre-trained natural language model. Embeddings are a type of numerical representation that capture some aspect of proteins from their sequence. The aim of this study is to explore the use of embeddings to predict bacterial toxins by their protein sequence since embeddings could capture hidden function information.

We used a highly curated database of manually annotated bacterial toxins as the basis for our machine learning models and performed careful redundancy reduction to further refine the data. We established a baseline model using amino acid compositions. The baseline predictor results showed that the specialized dataset allowed for the prediction of bacterial toxins with an accuracy and precision of 85% and 87%. Preliminary data show embeddings successfully separating toxins from non-toxins, outperforming the baseline model in terms of accuracy (93%) and precision (92%). This suggests that embeddings can be a useful tool for predicting protein function from sequence data.

M289 - Back-to-Africa introductions of *Mycobacterium tuberculosis* as the main cause of tuberculosis in East Africa

Presenting Author – *Daniela Brites, Swiss Tropical and Public Health Institute, Switzerland*

Author/s – *Daniela Brites, Michaela Zwyer, Liliana K. Rutaihwa, Etthel Windels, Jerry Hella, Sebastien Gagneux*

Abstract Content

East-Africa has a high tuberculosis (TB) endemicity, with many distinct genotypes of the *Mycobacterium tuberculosis* complex (MTBC) causing TB. These genotypes often differ in prevalence yet, the factors leading to these differences remain poorly understood. We studied the MTBC population in Dar es Salaam, Tanzania over a six-year period, using 1,082 unique patient-derived MTBC whole-genome sequences (WGS) and associated clinical data. We show that the TB epidemic in Dar es Salaam is dominated by multiple MTBC genotypes introduced to Tanzania from different parts of the world during the last 300 years. The most common MTBC genotypes deriving from these introductions exhibited differences in transmission rates and in the duration of the infectious period, but little differences in overall fitness, as measured by the effective reproductive number. Moreover, measures of disease severity and bacterial load indicated no differences in virulence between these genotypes during active TB. Instead, the combination of an early introduction and a high transmission rate accounted for the high prevalence of L3.1.1, the most dominant MTBC genotype in this setting. Yet, a longer co-existence with the host population did not always result in a higher transmission rate, suggesting that distinct life-history traits have evolved in the different MTBC genotypes. Taken together, our results point to bacterial factors as important determinants of the TB epidemic in Dar es Salaam.

M290 - Uncovering epigenetic changes in early-stage MTBC-infected macrophages

Presenting Author – *Jana Schönfeld, Research Center Borstel, Germany*

Author/s – *Tobias Dallenga, Susanne Homolka, Stefan Niemann*

Abstract Content

Background: Tuberculosis, caused by strains of the *Mycobacterium tuberculosis* complex (MTBC), remains a major public health problem worldwide. Previous research indicates that MTBC infection can have deleterious effects on the epigenetic makeup of host cells, resulting in immune exhaustion. As macrophages play a critical role in host defense against MTBC, studying the epigenetic alterations in response to infection can offer valuable insights into host-pathogen interactions.

Objectives: In this study, we aim to investigate the changes in epigenetic profiles of human macrophages during early-stage MTBC infection. We focus on analyzing the impact on gene expression, DNA methylation, and histone modifications in macrophages upon infection with different MTBC strains.

Methods: One significant challenge in studying epigenetic changes in infected macrophages is the need to balance sterility with preserving RNA and DNA integrity. To address this, we developed a protocol that ensures biosafety while maintaining the quality of nucleic acids. To achieve this, we tested different sterilizing techniques to determine their capacity to effectively inactivate *Mycobacterium tuberculosis* and analyzed the resulting nucleic acid quality.

Results: Surprisingly, commercially available reagents did not completely inactivate our samples. The developed protocol allows for analyzing epigenetic alterations in infected macrophages while ensuring sterility and preserving the quality of RNA and DNA. Our protocol can be valuable for future studies aimed to understand the complex interplay between MTBC and host cells and may contribute to the development of novel therapeutic strategies for tuberculosis.

M292 - Negative hysteresis is a widespread envelope stress response in Gram-negative bacteria that can improve antibiotic therapy

Presenting Author – *Florian Buchholz, University of Kiel, Germany*

Author/s – *Lina Marie Upterworth, Leif Tueffers, Roderich Roemhild, Hinrich Schulenburg*

Abstract Content

Background: Antimicrobial resistance is a growing concern, and evolutionary-informed treatment approaches show promise in constraining resistance evolution. Sequential administration of antibiotics can enhance treatment efficacy through negative hysteresis, which is the change in cellular physiology induced by one antibiotic that increase susceptibility to later antibiotics. However, the distribution of this phenomenon and the underlying molecular mechanism were unknown.

Objectives: The aims of our study are to improve our understanding of the phenomenon of negative hysteresis by characterizing its molecular underpinnings and its distribution across Gram-negative bacteria, the latter as a necessary prerequisite for its use in therapy.

Methods: We conducted a systematic analysis of negative hysteresis across the genomic diversity of the high-risk human pathogen *Pseudomonas aeruginosanosa*, including highly-resistant strains and patient populations, as well as multiple strains of other Gram-negative pathogens, such as *Acinetobacter baumanii*, *Escherichia coli*, and *Klebsiella pneumoniae*. We characterized the molecular basis of hysteresis by combining transcriptomics, functional genetic analyses, and physiological assays.

Results: Our analysis revealed that negative hysteresis is widespread and common across the Gram-negative pathogens, particularly when a beta -lactam antibiotic is followed by an aminoglycoside drug. Our molecular characterization identified the Cpx envelope stress response system as central to the expression of this inducible physiological effect.

Conclusion: Our findings yield new insights into the mechanisms underlying negative hysteresis, which is widely expressed across bacterial taxa and may thus be a promising focus for evolution-informed, sustainable antibiotic therapy.

M293 - Antimicrobial activity of ceftazidime-avibactam and ceftolozane-tazobactam against carbapenem-resistant *Pseudomonas aeruginosanosa*

Presenting Author – *Ina Gajic, University of Belgrade, Serbia*

Author/s – *Ina Gajic, Jovana Kabic, Milos Jovicevic, Dusan Kekic, Lazar Ranin, Natasa Opavski, Sanja Zornic, Snezana Delic, Anita Sente Zigmanovic, Lidija Boskovic*

Abstract Content

Background: Carbapenem-resistant *Pseudomonas aeruginosanosa* (CRPA), is listed by the World Health Organization as a “priority pathogen” requiring urgent antibacterial drug research.

Objective: In light of limited treatment options for CRPA infections, this study compared the activity of ceftazidime–avibactam and ceftolozane–tazobactam and assessed the susceptibilities rates of various antimicrobials against clinical CRPA isolates.

Methods: A total of 129 CRPA isolates were collected from patients admitted to ten hospitals throughout Serbia from 2020 to 2023. Susceptibility testing was done using disk diffusion, gradient test, and broth microdilution, according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing, 2023. PCR was used to characterize the most common genes encoding carbapenemases.

Results: The vast majority of the isolates were obtained from the respiratory tract (N=62; 48.1%) and skin and soft tissue (N=31; 24%), followed by urine (N=19; 14.7%) and blood (N=17; 13.2%). The susceptibility rate of the CRPA isolates to ceftazidime-avibactam and ceftolozane-tazobactam was 63.6% (N=82/129) and 40.3% (N=52/129), respectively. The difference between the obtained susceptibility rates was statistically highly significant (p<0.01). Ceftazidime-avibactam was active against 30 (36.6%) of ceftolozane-tazobactam-resistant isolates and ceftolozane-tazobactam had no *in vitro* activity against ceftazidime/avibactam-resistant CRPA isolates (Figure 1). The overall susceptibilities rates to other antibacterial agents were as follows: colistin – 98.44%; piperacillin-tazobactam – 0%; ceftazidime– 0%; cefepime– 0%; aztreonam – 0%; amikacin – 31%; levofloxacin– 3.1%; ciprofloxacin– 7.75%. Only two isolates (1.6%) were pan-drug resistant. A total of 31 (24%) CRPA isolates harboured the blaNDM-1 gene. As expected, all 31 blaNDM-1-positive isolates were resistant to both novel antibiotics.

M294 – Horizontal gene transfer in predominantly clonal tuberculosis-causing mycobacteria

Presenting Author – *Roland Brosch, Institut Pasteur, France*

Author/s – *Jan Madacki, Mickael Orgeur, Guillem Mas Fiol, Wafa Frigui, Laurence Ma, Roland Brosch*

Abstract Content

Background: Current models of horizontal gene transfer (HGT) in mycobacteria are based on "distributive conjugal transfer" (DCT), an HGT type described in the fast-growing, saprophytic model organism *Mycobacterium smegmatis*, which creates genome mosaicism in resulting strains and depends on ESX-1 and ESX-4 type VII secretion systems. In contrast, only few data on inter-strain DNA transfer are available for tuberculosis-causing mycobacteria, represented by members of the *Mycobacterium tuberculosis* complex (MTBC) and closely related, early-branching *Mycobacterium canettii* strains.

Objectives: Here, the objectives were to study a wide range of human and animal-adapted MTBC members and *M. canettii* strains, to obtain new insights into HGT in pathogenic mycobacteria that might have contributed to the outstanding pathoevolution of *M. tuberculosis* to become one of the deadliest pathogens in the history of humankind.

Methods: We developed an optimized filter-based mating assay, and explored various combinations of strains and conditions. We obtained a high yield of thousands of recombinants containing transferred chromosomal DNA fragments from various MTBC donor strains and identified 4 *M. canettii* strains that were able to act as recipients. We confirmed several dozens of randomly selected clones by whole-genome sequence analysis.

Results: In contrast to previous results for *M. smegmatis*, we found that in tubercle bacilli, HGT is an ESX-1-independent process, whereas our data point to an involvement of the ESX-4 secretion system in the process.

Our findings provide new insights into the genetic events driving the pathoevolution of *M. tuberculosis* and radically change our perception of HGT in mycobacteria.

M295 - Genomic characterization of enteroaggregative *Escherichia coli* strains isolated from symptomatic urinary tract infections

Presenting Author – *Tânia Gomes, Universidade Federal De São Paulo, Brazil*

Author/s – *Rodrigo Hernandes, Waldir Elias, Ulrich Dobrindt, Ana Carolina Santos*

Abstract Content

Background: *Escherichia coli* is the most frequent urinary tract infection (UTI) agent worldwide. Recently, various reports implicated intestinal pathogenic enteroaggregative *E. coli* (EAEC) as UTI and bloodstream infection agents. The aggregative adherence plasmid (pAA), which characterizes typical EAEC strains, encodes various virulence factors (VFs) related to intestinal pathogenesis, including five variants of the aggregative adherence fimbriae (AAF). Despite the various reports of EAEC strains in UTIs, few evaluated their genomic content.

Objectives: To characterize and compare the genetic background and pAA content of EAEC strains isolated from UTI in three cities.

Methods: Thirteen strains were sequenced using the Illumina platform and characterized according to their phylogroups, sequence types (ST), and VFs. A comparative phylogenetic tree was built using *E. coli* genomes publicly available at the NCBI.

Results: The strains belonged to three phylogroups (A, B1, and D) and six STs. Phylogroups A and D were the most frequent (five strains each). Five strains belonged to ST10-A, four to ST69-D, and the remaining to ST501-D, ST2741-B1, ST1049-B1, and ST278-B1. The AAF/I, AAF/III, and AAF/IV variants were identified, with AAF/I prevailing (61.5 %). The phylogenetic tree built highlighted the close relationship of the ST10-A strains, which displayed AAF/I and a similar set of VFs. The B1 strains harbored AAF/I, AAF/III, or AAF/IV, whereas all ST69-D strains carried AAF/I or AAF/III. The presence of pAA in all ST10-A strains and their isolation source suggest that this EAEC cluster is mainly responsible for extraintestinal diseases. Further studies are required to confirm this hypothesis.

M296 - Nose microbiome composition as a novel biomarker for bronchiectasis

Presenting Author – *Julija Armalyte, Vilnius University Life Sciences Centre, Lithuania*

Author/s – *Julija Armalyte, Aleksandras Konovalovas, Laurita Klimkaite, Tomas Liveikis, Brigita Jonaityte, Edvardas Danila, Edvardas Bagdonas, Ruta Aldonyte*

Abstract Content

Background: Human body is a host for many distinct microbial ecosystems, both inside and outside. One of the organs that was considered mostly microorganism-free until recently is human lungs. More and more research reports distinct communities of microorganisms inhabiting the lungs, their roles in maintaining healthy states or causing disease are still not fully understood. Sampling human lung microbiota is an invasive procedure, thus upper respiratory tract could be used to gain information about lung health.

Objectives: Compare the composition of nose microbiota in patients with bronchiectasis disease and healthy individuals, and select biomarkers, which could reveal information about the progress and prognosis of the disease.

Methods: Samples were collected by anterior nasal swabbing and kept in Shield reagent (Zymo Research) until DNA purification by Quick-DNA Microprep Plus Kit (D4074, Zymo Research), 16S rRNA gene libraries were prepared for sequencing using Oxford Nanopore Technologies 16S Barcoding Kit (SQK-RAB204) and sequenced using Flongle Flow Cell (R9.4.1). Taxonomic classification was performed by using Emu and NanoCLUST pipelines.

Results: A total of 64 nose swab samples were collected, with 25 from healthy individuals and 39 from patients with bronchiectasis. Our analysis of the taxonomic diversity showed no significant differences in the microbiota richness and diversity between the groups. Environmental factors such as age, sex, workplace environment or pet ownership were found to have no impact on the differences observed in the nose microbiome. However, differences in microorganism composition were detected and several possible biomarkers were identified that may be associated with bronchiectasis.

M297 – The importance of HipA/B toxin-antitoxin system in extraintestinal *Escherichia coli* persistence

Presenting Author – *Roxane Piazza, Butantan Institute, Brazil*

Author/s – *Thais Mitsunari, Rosa Maria Silva, Camila Henrique, Waldir Elias, Roxane Maria Piazza*

Abstract Content

Background: Toxin-antitoxin (TA) systems comprise a set of genes that are widespread in prokaryotes. On the chromosome, the systems may be involved in the induction of cell death, persistence in response to stressful conditions, biofilm formation, colonization of new niches and maintenance of bacterial motility. In *Escherichia coli* K12, 36 TA systems have been described, of which type II is the most abundant, including hipA/B whose role in bacterial persistence is still unclear.

Objectives: To evaluate the role of hipA/B TA system in the pathogenesis of extraintestinal *E. coli* (ExPEC).

Methods: The prevalence of genes encoding type II TA systems was searched by PCR. hipA/B was deleted and complemented in two strains, and the effects of this mutation was evaluated by means of serum resistance, macrophage survival and persistence induced by antibiotics.

Results: Among the eight type II toxins searched in this work, hipA was present in 76 of the 100 ExPEC strains studied. The hipA/B system did not influence the phenotype of resistance to human serum or intracellular survival in macrophages. Among the hipA/B positive strains, only EC182 showed persistence after being exposed to ciprofloxacin for 24h. Accordingly, the mutated EC182 variant had this characteristic abolished. Our data highlights the importance of hipA/B in persistence phenotypes of ExPEC. Further studies are required to define if this phenotype is species, strain or antibiotic specific.

M298 - Temporal Hierarchy and Context-Dependence of Quorum Sensing Signal in *Pseudomonas aeruginosanosa*

Presenting Author – *Volker Behrends, University Of Roehampton, United Kingdom*

Author/s – *Volker Behrends*

Abstract Content

Background: The Gram-negative bacterium *Pseudomonas aeruginosanosa* can cause infections in a broad range of hosts including plants, invertebrates and mammals and is an important source of nosocomial infections in humans.

Objective: We were interested in how differences in the bacteria's nutritional environment impact bacterial communication and virulence factor production.

Methods: We grew *P. aeruginosa* in 96 different conditions in BIOLOG Gen III plates and assayed quorum sensing (QS) signalling over the course of growth. We also quantified pyocyanin and biofilm production and the impact of sub-inhibitory exposure to tobramycin.

Results: We found that 52 conditions supported metabolic activity, 43 supported growth, and 42 supported both. While 3-oxo-C12 homoserine lactone remained the dominant QS signal to be produced, timing of PQS production differed between media types. Further, whether cells grew predominantly as biofilms or planktonic cells was highly context dependent. Tobramycin exposure negatively affected production of butyryl homoserine, PQS and pyocyanin with increasing severity.

Conclusion: Our data suggest that understanding the impact of the nutritional environment on the bacterium can lead to valuable insights into the link between bacterial physiology and pathology.

M300 - More than just bacteria - Metagenomic analysis reveals a diverse, skin-like microbial community on worn spectacles

Presenting Author – *Birgit Fritz, Furtwangen University Of Applied Sciences, Germany*

Author/s – *Susanne Jacksch, Siegfried Wahl, Focke Ziemssen, Markus Egert*

Abstract Content

Spectacles are widespread devices aiding human vision. They represent a reservoir for (potentially) pathogenic microbes, which threaten eye health and might promote the spread of infectious diseases. Using cultivation and 16S rRNA gene amplicon sequencing, we obtained a comprehensive view of the bacterial community composition on spectacle and similar surfaces. Here, we report the establishment of metagenomic shotgun sequencing in order to obtain a more comprehensive view of the spectacle microbiota and its hygienic relevance.

Harvesting sufficient template DNA was difficult, due to the smooth and small sampling areas. Sequencing swab samples obtained from the total surfaces of 33 worn spectacles resulted in 737,109 raw sequences per sample, of which 249,021 non-human reads per sample remained after quality trimming. Read-based analysis assigned ~ 88 % of the sequences to skin and environmental bacteria, dominated by cutibacteria, staphylococci, corynebacteria and streptococci, corroborating previous studies. In addition, ~ 12 % of the reads were assigned to eukaryotes (mainly yeasts, such as *Malassezia* sp.), ~ 0.5 % to viruses and ~ 0.08 % to archaea. Viruses mostly comprised bacteriophages, but also human-associated types, such as HPV. Few sequences clustered with SARS-CoV-2, which, however, is a RNA-virus. Future work aims at optimizing the metagenomic workflow and the analysis of functional aspects of the spectacle microbiota, e.g. the presence of virulence factors, such as antibiotic resistance genes.

M301 - High variability in infection-relevant phenotypes was detected between and within *S.* Enteritidis lineages belonging to ST11.

Presenting Author – *Melissa Berni, Izsler, Risk Analysis And Genomic Epidemiology Unit, Italy*

Author/s – *Erika Scaltriti, Martina Tambassi, Ilaria Menozzi, Alessandra Dodi, Marina Morganti, Luca Bolzoni*

Abstract Content

Background: *Salmonella enterica* serovar Enteritidis (SE) is the first cause of foodborne outbreaks in Europe, due to its high diffusion in the poultry sector. After a period of reduction of human salmonellosis due to SE, an increase in human cases and isolation in poultry was observed in the last years. It was hypothesized that the emergence of more virulent SE lineages can represent one of the reasons behind this reversal trend.

Objectives: The aim of this study was to test if there exist differences in virulence in genetically related isolates of the SE population circulating in Europe.

Methods: The invasion and replication rates of SE isolates belonging to ST11 global epidemic and outlier clades was assessed in epithelial cells by gentamicin protection assay.

The virulence of SE isolates of the genomic cluster, belonging to the outlier clade and responsible for a large outbreak in Italy, was then analysed in detail transforming SE isolates with pCHAR-Duo fluorescence reporter plasmid and using automated image analysis to quantify invasion, vacuolar load and cytosolic replication with single cell resolution.

Results: We observed differences in virulence both between and within SE epidemic and outlier clades. The same results were obtained analyzing invasion, vacuolar load and cytosolic replication of different SE isolates belonging to genomic cluster. These results confirm that genetically related isolates with different virulence profiles may frequently emerge within the SE population. Work is ongoing to find the genetic features responsible for the observed phenotypes.

M302 - Genomic and phenomic analyses of *Acinetobacter baumannii* isolated from the community and co-located hospital in the tropics.

Presenting Author – *sadequr rahman, Monash University, Malaysia*

Author/s – *Nazmul Muzahid, Md Hamed Husain, Marie Huet, Jacky Dwiyanto, Kah Ern Ten, Tin Tin Su, Daniel Reidpath, Faizah Mustapha, Qasim Ayub, Hock Siew Tan, Sadequr Rahman*

Abstract Content

Acinetobacter baumannii is a common cause of multidrug resistant (MDR) nosocomial infections around the world and is an ESKAPE pathogen. However, little is known about the persistence and dynamics of *A. baumannii* in healthy individuals in a community. This study investigated the role of the community in a tropical country as a prospective reservoir for *A. baumannii* and explored genotypic and phenotypic associations between hospital and community isolates.

To investigate the *A. baumannii* isolates from the feces of healthy individuals in a community and examine genotypic and phenotypic associations with co-located hospital isolates.

Genome sequences of twelve community and fifteen h ospital isolates were compared using bioinformatic tools. Antibiotic resistance was determined by means of disc diffusion assays and micro-broth dilution. Pathogenicity of selected isolates was determined using a *Galleria mellonella* killing assay

SNP-based phylogenetic analysis and pangenome analysis of core genes showed clustering between four community and two hospital strains. A higher proportion of the community strains contained CRISPR arrays. One hospital strain had identical CRISPR spacer sequences to a co-clustered community isolate. Similar numbers of putative virulence genes and a slightly higher number of resistance genes were found in the hospital isolates compared to the community isolates. More hospital strains were multi-drug resistant than community isolates. The *Galleria* killing assay revealed that both community and hospital strains could be highly pathogenic. This study highlights the possible threat to public health by virulent *A. baumannii* present in the gut of asymptomatic individuals in the community.

M303 - Tracking the threat of *Acinetobacter baumannii*: A European Perspective

Presenting Author – *Jovana Kabic, University of Belgrade, Serbia*

Author/s – *Jovana Kabic, Ina Gajic, Dusan Kekic, Milos Jovicevic, Natasa Opavski, Lazar Ranin*

Abstract Content

Background: The increased rate of infections caused by multidrug-resistant isolates of *Acinetobacter baumannii* has become a global problem, especially the isolates carrying the carbapenemases encoding genes, necessitating the epidemiologic surveillance of such strains.

Objectives: This study aimed to investigate the diversity and geographic distribution of *A. baumannii* isolates from European countries and detect the most prevalent beta-lactamase and carbapenemase encoding genes among them.

Methods: This study included all *A. baumannii* genomes available in the NCBI Reference Sequence Database, isolated from European countries. A core-SNP-based phylogenetic tree was constructed using raxmlHPC-PTHREADS and was visualised using iTOL software. Sequence types (STs) based on the Pasteur scheme for all isolates were inferred from assembled genomes using mlst v2.19.0. The identification of beta-lactamase and carbapenemase encoding genes was performed using ABRicate set against the comprehensive antibiotic resistance database.

Results: The phylogenetic tree of 1296 *A. baumannii* isolates from 26 European countries indicated a high genetic diversity of circulating *A. baumannii* isolates, with 115 different STs identified. The ST2 was most prevalent (54.08%), followed by ST1 (9.79%), ST636 (4.70%), ST78(3.39%), ST25(3.16%) and ST492 (2.93%) (Figure 1). Overall, 328 (25.30%) isolates carried the blaTEM (blaTEM-1, blaTEM-191) genes, while 54 (4.16%), 43 (3.31%), 23 (1.77%), and one (0.07%) isolate harboured the blaPER (blaPER-7, blaPER-8, blaPER-10, blaPER-13), blaNDM (blaNDM-1, blaNDM-9), blaGES (blaGES-5, blaGES-11, blaGES-12, blaGES-22, blaGES-35) and blaVEB-1 genes, respectively (Figure 1). The blaNDM gene was detected among the isolates from eight countries (France, Czech, Germany, Spain, Belgium, Serbia, United Kingdom, and Greece).

M304 - Regulatory mechanism of host cell contact-dependent T3SS gene expression in *Vibrio parahaemolyticus*

Presenting Author – *Toshio Kodama, Nagasaki University, Japan*

Author/s – *Sarunporn Tandhavanant, Hiroyuki Terashima, Nopadol Precha Precha, Hirotaka Hiyoshi, Tetsuya Iida, Shigeaki Matsuda, Narisara Chantratita*

Abstract Content

Some pathogenic bacteria with Type III secretion system (T3SS) have a strict regulated mechanism in which gene expression and protein secretion are coordinated to efficiently inject effectors into host cells and establish infection. One of the mechanisms is the regulation of gene expression in response to contact with host cells. Here, we show that *Vibrio parahaemolyticus*, an enteropathogenic bacteria, has a host cell contact-dependent regulatory mechanism for virulence gene expression. T3SS2, an essential virulence determinant for acute gastroenteritis encoded by *V. parahaemolyticus* pathogenicity island (Vp-PAI), recognizes host cell contact by sensing high intracellular K+ levels and switches secretory substrates. This secretory substrate switching is regulated by proteins called gatekeepers. Mutant deficient in the gatekeeper gene loses the ability to switch secretory substrates and lock the secretory state into contact with the host cell. Transcriptome analysis of T3SS2 gatekeeper gene-deficient strains showed the genes encoded in Vp-PAI was specifically upregulated in a T3SS2 secretory activity-dependent manner, implying the presence of a T3SS2 secreted negative regulator. Comparative proteomic analysis identified a VtrN whose secretion is enhanced under conditions that mimic host cell contact as well as other T3SS2 effectors. The vtrN gene deletion specifically upregulated Vp-PAI genes expression, but unlike the gatekeeper gene deletion, it was independent of T3SS2 secretory activity. Furthermore, VtrN interacted with VtrB, a transcription factor essential for Vp-PAI-encoded genes expression, and repressed its transcriptional activity. Thus, *V. parahaemolyticus* has a mechanism to upregulate virulence gene expression in a host cell contact-dependent manner using VrtN, a secreted transcriptional repressor.

M306 - Virulence related traits in clinical and environmental isolates of opportunistic pathogen *Stenotrophomonas maltophilia*

Presenting Author – *Laurita Klimkaitė, University, Lithuania*

Author/s – *Radvilė Drevinskaitė, Karolis Krinickis, Edita Sužiedėlienė, Julija Armalytė*

Abstract Content

Background: *Stenotrophomonas maltophilia* is gram-negative multidrug resistant biofilm forming bacterium widely distributed in natural environment and recently more known as opportunistic human pathogen causing severe infections in immunocompromised patients. Environmental and clinical *S. maltophilia* isolates are known to exhibit similar phenotypic and genotypic characteristics and it is not clear which traits are the most important for this bacterium to become pathogen.

Objectives: To analyse virulence associated phenotypic and genotypic traits in clinical and environmental isolates of *Stenotrophomonas maltophilia*.

Methods: 33 clinical and 42 environmental isolates were analysed in this study. In order to evaluate virulence associated phenotypic traits at different temperatures, biofilm formation, swarming and twitching motility were analysed at 28°C and 37°C. Isolates' susceptibility to trimethoprim-sulfamethoxazole, chloramphenicol, ciprofloxacin, gentamicin, ceftazidime and tigecycline was determined, virulence and antibiotic resistance genes detection was performed.

Results: Majority of environmental isolates expressed virulence related phenotypic traits only at lower temperature (28°C), while at host body temperature (37°C) these characteristics were lost. Only some environmental isolates retained virulence associated traits at 37°C and showed similarity to clinical isolates. Despite different phenotypic characteristics, genes associated with virulence and antibiotic resistance have been detected in both clinical and environmental isolates, although some genes were more abundant in clinical isolates. Antibiotic susceptibility analysis revealed that chloramphenicol, trimethoprim-sulfamethoxazole, tigecycline and ciprofloxacin were effective against majority of isolates. Small fraction of environmental *S. maltophilia* isolates showed similar phenotypic and genotypic traits to clinical isolates and could be considered as source of *S. maltophilia* infections.

M307 - Uncovering a global stress response in the early-branching species *Fusobacterium nucleatum*

Presenting Author – *Falk Ponath, Helmholtz Institute for RNA-based Infection Research, Germany*

Author/s – *Falk Ponath, Yan Zhu, Valentina Cosi, Jörg Vogel*

Abstract Content

The oral commensal *Fusobacterium nucleatum* (*F. nucleatum*) has recently garnered attention for its ability to colonize tissues and tumors elsewhere in the human body. However, the growing interest in this emerging cancer-associated bacterium contrasts with a lack of knowledge about its basic gene expression features and physiological responses. This includes an understanding of global stress response pathways that typically ensure the survival of bacteria outside their primary niche.

Tackling this, we have generated high-resolution global RNA maps for five clinically relevant fusobacterial strains. We have used these data sets to uncover fundamental aspects of fusobacterial gene expression architecture and a suite of noncoding RNAs, which includes a conserved fusobacterial oxygen-induced small RNA, FoxI. Advancing the poor genetics in this phylum, we have developed gene deletion and overexpression tools to study the activity of fusobacterial genes and the targetome of FoxI. Collectively, our results uncover the fusobacterial homolog of the envelope stress sigma factor, σE, and reveal FoxI as its noncoding RNA arm that represses mRNAs of several abundant outer membrane proteins as well as proteins with cytosolic function. Interestingly, given that *F. nucleatum* is an early-branching species, our findings with σE and the FoxI sRNA suggest that σE regulons with a coding arm and a noncoding arm might have independently evolved multiple times in bacterial evolution. In addition to the characterization of a global stress response in *F. nucleatum*, the genetic tools developed here will enable further discoveries and dissection of regulatory networks in this early-branching bacterium.

M308 - Exploring the potential of drug repositioning for inhibiting intracellular infection caused by *Staphylococcus aureus*

Presenting Author – *Blanca Lorente Torres, University of León, Spain*

Author/s – *Blanca Lorente Torres, Helena Álvarez Ferrero, Farzaneh Javadimarand, Álvaro López García, Pablo Castañera Estrada, Alberto Antolín Lerma, Jesús Llano Verdeja, Álvaro Mourenza, Luis M. Mateos, Michal Letek*

Abstract Content

Staphylococcus aureus has been traditionally considered an extracellular pathogen. However, increasing evidence suggests that it is an intracellular facultative superbug. The ability to survive within host cells contributes to the emergence of antimicrobial resistance, since many antibiotics fail to reach intracellular pathogens, and the ones that do, are often at sub-minimum inhibitory concentrations. Furthermore, the intracellular niche also allows the pathogen to evade the host's immune response. *S. aureus* is able to infect professional phagocytes and is capable of disrupting the phagosomal membrane, allowing replication within the host cell's cytoplasm.

Given the aforementioned considerations, drug repurposing may be a promising strategy for identifying new treatments for *S. aureus* infections. It would allow to identify new drugs with shorter development time frame, as the preclinical testing, safety assessment and formulation development may already have been completed for the drugs considered for repositioning.

In this study, we aimed to investigate the potential of alternative drugs to block the intracellular survival of *S. aureus*. We infected A-549 lung epithelial cells with *S. aureus* USA300 and subsequently treated them with 3,744 drugs with repurposing potential. The drug library consisted of a wide variety of compounds, including antibiotics, antifungal, antiviral, anticarcinogenic, cardiovascular and neuroprotective drugs, which were in different stages of development. From our data 16 compounds are very promising, with most of them being host-directed drugs. Novel combinatorial strategies utilizing these drugs may prove valuable in controlling *S. aureus* infections, an increasingly valued approach to combat the emergence of multidrug resistance.

M309 - Understanding *Escherichia coli* persister diversity to improve treatment strategies

Presenting Author – *Carolin Kobras, University of Oxford, United Kingdom*

Author/s – *Chinenye Akpulu, Mathew Stracy*

Abstract Content

Background: Many bacterial infections cannot be cured, even when caused by a pathogen that is not resistant to antibiotics. Antibiotic tolerance and persistence enable bacterial cells to survive transient exposure to antibiotics at concentrations that would otherwise be lethal, often by entering a slow-growing or dormant state. After treatment ends these cells can revive and regrow, leading to recurrent and chronic infections.

Objectives: Developing new strategies to eradicate persisters and reduce antibiotic treatment failures requires better understanding of how the physiology of diverse persister mutants affect their susceptibility to different antibiotic classes. To this end, we test how different molecular triggers of a high-persister phenotype affect *Escherichia coli*'s ability to survive antibiotic treatment, describing their antibiotic tolerance profile across clinically relevant drugs and aiming to identify any chinks in the bacteria's armour.

Methods and Results: We screened a collection of *E. coli* persister mutants against a large panel of different antibiotics and drug combinations, using high-throughput phenotyping. While persister cells are typically tolerant to multiple classes of antibiotics, our results suggest that this tolerance is not uniform and depends on the specific cell physiology underlying the persister state.

Ultimately, the knowledge gained from this study will reveal the most promising treatment for infections caused by tolerant and persistent bacteria, opening the door to developing strategies to target specific persister types.

M311 - A humanized mouse model to dissect the interplay between *Campylobacter jejuni*, the gut microbiota and host immunity

Presenting Author – *Nizar Shayya, Charité – Universitätsmedizin Berlin, Germany*

Author/s – *Soraya Mousavi, Markus Heimesaat, Stefan Bereswill*

Abstract Content

Background: *Campylobacter jejuni* is a leading causative agent of bacterial foodborne disease worldwide. While the host immune response and gut microbiota play a critical role in the outcome of *C. jejuni* infections, a comprehensive understanding of the complex interactions between the pathogen, host immunity, and gut microbiota remains elusive.

Objectives: The objective of this study is to investigate the interactions between *C. jejuni*, host immunity and gut microbiota using human-microbiota-associated mice as a model system.

Methods: Human-microbiota-associated and secondary abiotic mice were infected with *C. jejuni* and the dynamics of bacterial colonization, host immune response, and gut microbiota were analyzed using culture-dependent analysis, PCR-based microbial community analysis and flow cytometry.

Results: The study revealed that *C. jejuni* colonizes the gut of human-microbiota-associated mice in comparable densities to that of secondary abiotic mice. Additionally, changes in the gut microbial communities were explored. The host innate and adaptive immune responses to *C. jejuni* in both types of mice were comparable, yet differential pro-inflammatory cytokine secretion was observed. Our findings provide new insights into the complex interactions between *C. jejuni*, host immunity, and gut microbiota and this has important implications for the development of intervention strategies to prevent and treat *C. jejuni* infections. The use of human-microbiota-associated mice as a model system is valuable in understanding the interplay between host immunity, gut microbiota, and the pathogen, and highlights its importance in dissecting the pathogenesis of *C. jejuni* infections.

M312 - Selective effects of teat disinfectants on composition and susceptibility of bovine udder microbiomes

Presenting Author – *MD SHAHINUR ISLAM, Christian-albrechts-universität Zu Kiel, Germany*

Author/s – *Evelyn Lass, Ole Lamp, Julia Anna Schwenker, Christina Hölzel*

Abstract Content

Antimicrobial resistance (AMR) is broadly studied all over the world. In recent years, awareness of the impact of using disinfectants on developing AMR also increased. Teat disinfection in the lactation period is widely practiced for teat hygiene and preventing mastitis, possibly changing the susceptibility pattern of udder microbes towards applied substances.

In the ongoing study presented here, we aim to assess the co-selection effect of teat disinfectants on the distribution and susceptibility of bacterial isolates from the bovine udder towards chlorhexidine, lactic acid and antibiotics.

Milk samples from 26 cows were collected before and after a week-long intervention following a split-udder design with chlorhexidine and lactic acid. Isolates identified by MALDI-TOF MS (Autoflex 3 Smartbeam, Bruker Daltonics GmbH) and currently await susceptibility testing by micro- and macrodilution. Results indicate that a selective effect of lactic acid towards the increased occurrence of *Corynebacterium* spp., as seen immediately after intervention, lasted for at least five weeks.

M313 - Thiostrepton inhibits the stringent response and the expression of virulence determinants in *Neisseria gonorrhoeae*

Presenting Author – *Silvia Caterina Resta, Università del Salento, Italy*

Author/s – *Adelfia Talà, Matteo Calcagnile, Antonio Pennetta, Giuseppe Egidio De Benedetto, Pietro Alifano*

Abstract Content – *Neisseria gonorrhoeae* (the gonococcus) is a Gram-negative bacterium causing gonorrhoea. It's raising its genetic resistance to available antibiotics. Moreover, failure of gonorrhoea therapy may result from non-mutational resistance. This response includes persistence, mediated by a fraction of the population poorly killed by the antibiotic because of a reprogramming of gene expression due to the stringent response (SR), triggered by the alarmone guanosine pentaphosphate/tetraphosphate [(p)ppGpp]. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) showed gonococci sensitivity to ampicillin, tetracycline, gentamicin, nalidixic acid, rifampicin and thiostrepton. Nevertheless, time-kill curves showed survivors in all treatments except thiostrepton. This, interacting with L11 protein of 23S rRNA, inhibits translation and reduces pppGpp synthesis. We then analysed the transcriptome of gonococci treated with serine hydroxamate (SH) to trigger the SR, and/or thiostrepton. SH-treated gonococci showed a transcriptional profile coherent with the SR. Conversely, thiostrepton treatment downregulated genes for amino acid biosynthesis and central carbon metabolism. Notably, unlike SH, double drug treatment didn't downregulated genes for ribosomal proteins and translational factors. Deeper analysis showed changes in toxin-antitoxin modules and genes for host-pathogen interaction. For instance, mafA/mafB/mafI system, involved in interbacterial competition, adhesion and transcytosis, was upregulated by SH and downregulated by thiostrepton, while genes for lipooligosaccharide (LOS) biosynthesis were downregulated by all treatments. Remarkably, gonococcus sialylates its LOS to hide from the complement system. In conclusion, our data revealed the transcriptional reprogramming occurring during SR and how thiostrepton counteracts it, indicating it as an effective drug against gonococci.

M314 - Functional characterization of the VgrG4 protein from *Klebsiella pneumoniae*

Presenting Author – *Talyta Soares do Nascimento, Fundação Oswaldo Cruz, Brazil*

Author/s – *Talyta Soares do Nascimento, Amanda da Silva Sarmento, Leticia Miranda Santos Lery*

Abstract Content – *Klebsiella pneumoniae* (KP) is an opportunistic pathogen that is of concern to public health systems around the world, as multi-resistant isolates are frequently identified. One of its virulence factors is the Type VI Secretion System (T6SS), a macromolecular complex that may translocate effector proteins. VgrG proteins are structural components of the tip of T6SS, but may also contain a variable C-terminal extension with an effector role. In a previous study, we identified that at least 100 KP isolates present a VgrG containing a conserved C-terminal extension of 138 amino acids, although its function is not yet known. Among them, there is the VgrG4 protein from Kp52145 strain. We showed that VgrG4-CTD interacts with cytoskeletal proteins and induces the remodeling of actin filaments in macrophages. The aim of this project is to characterize the role of VgrG4 and VgrG4-CTD in the modulation of host-pathogen interaction. The recombinant proteins VgrG4-CTD and VgrG4 were delivered to macrophages. Infection assays and fluorescence microscopy were performed to analyze the cell cytoskeleton, reactive oxygen species (ROS) production. It was also verified whether the proteins were able to alter KP adhesion and internalization in macrophages. Preliminary results suggest that both proteins appear to have the ability to induce alterations in the actin cytoskeleton, and appear to induce ROS production. Preliminary results suggest that both proteins appear to have an effect on modulating the actin cytoskeleton and ROS production in macrophages. Moreover, initial data suggest that both proteins appear to have no role in KP internalization into macrophages.

M315 - Impact of Western diet on enterohaemorrhagic *Escherichia coli* (EHEC) colonisation in the human *in vitro* Mucosal Artificial Colon

Presenting Author – *Deborah O'Sullivan, Université Clermont, France*

Author/s – *Deborah O'Sullivan, Claude Durif, Ophélie Uriot, Cormac Gahan, Lucie Etienne-Mesmin, Stéphanie Blanquet-Diot, Morgane Brun*

Abstract Content

Enterohemorrhagic *Escherichia coli* (EHEC) is a major food-borne pathogen causing human disease ranging from diarrhea to life-threatening complications. Relatively little data is available on interactions between EHEC and the human gut microbiota. Accumulating evidence demonstrates the involvement of Western diet in gut microbiota shifts that enhance susceptibility to enteric infection, but the effect of diet on EHEC pathogenesis remains unknown. Our research aimed to investigate the effects of healthy versus Western diet on gut microbiota composition and activities and EHEC colonisation in an *in vitro* human colon model M-ARCOL (Mucosal ARtificial COLon). This model reproduces the main nutritional, physicochemical and microbial (luminal and mucus-associated microbiota) parameters of the colonic environment. Two bioreactors were inoculated with human fecal samples (n=4) and ran in parallel, one receiving a healthy diet, the other a Western diet and infected with EHEC strain EDL933. EHEC survival was determined by qPCR, gut microbiota composition was assessed by 16S metabarcoding and microbial activities were evaluated through gas and short chain fatty acid analysis. Diet, donor and EHEC infection impacted beta-diversity in luminal and mucosal samples. EHEC survival was dependant on both donor and diet in luminal samples. EHEC was more rapidly depleted when treated with a healthy diet compared to a Western diet and eliminated sooner in some donors. EHEC was maintained in mucosal samples without elimination, suggesting a possible niche environment for colonisation and survival. The prolonged EHEC colonisation sustained by a Western diet *in vitro* could suggest an increased susceptibility to infection in humans.

M316 - Heterogeneity of capsular polysaccharide synthesis in *Staphylococcus aureus*: promotor activity, native mRNA transcript levels an

Presenting Author – *Naisa Vetter, Interfaculty Institute for Microbiology and Infection-medicine Tübingen (IMIT), Germany*

Author/s – *Shilpa George, Christiane Wolz*

Abstract Content

Intra-population diversity has been reported across bacteria of different genera, giving them a selective advantage under changing environmental conditions. This includes phenotypic heterogeneity, where different phenotypes are present in a genetically homogeneous population.

In *Staphylococcus aureus*, the capsular polysaccharide (CP) protects against phagocytosis but also has the drawback of impeding adherence to host cells. CP synthesis was shown to be highly heterogeneous and growth phase dependent. The biosynthetic enzymes responsible for CP synthesis are encoded by the capA-P operon with the principal promotor (Pcap) located upstream capA. This was achieved using single-cell assays such as promotor-fluorescent protein fusion (Pcap-cfp/yfp) and CP immunofluorescence. We also established an in situ hybridization method to detect individual mRNA molecules within single *S. aureus* cells (mRNA-FISH). All methods verified the growth phase dependency and high cell variability of cap/CP expression.

Extrinsic regulation of Pcap activity was verified by double promoter constructs (Pcap-yfp-Pcap-cfp). However, by combining the different methods on the single cell level we found that Pcap driven cfp/yfp expression (fluorescence) does not correlate with the native cap mRNA level (mRNA-FISH) or CP synthesis (immunofluorescence) within the same bacterial cells. The same was evident when cap and cfp/yfp mRNA species, both resulting from Pcap activity, were correlated using dual mRNA-FISH. Thus, mRNA structure/stability and other post-transcriptional mechanisms are likely detrimental for the timely CP synthesis. Therefore, on the single-cell level, analyses of promoter activity using promoter-fusion constructs may not necessarily reflect native promoter activity.

M317 - *Origanum vulgare* extracts as a promising source of compounds with anti-pathogenic activity against dental plaque bacteria

Presenting Author – *Sybren Van Ginneken, KU Leuven, Belgium*

Author/s – *Fouzia Idir, Guglielmo Coppola, Daniel Grenier, Farida Bendali, Hans Steenackers*

Abstract Content

Despite improvements in oral hygiene over the past decades, dental caries still plays a major role in tooth decay. To identify new bioactive compounds to combat caries, we systematically screened the antimicrobial and anti-biofilm activity of extracts obtained from nine medicinal plants against an extensive collection of dental plaque isolates.

Broad-spectrum antimicrobial and anti-biofilm properties were observed, especially among ethanolic extracts, which marks them as a promising source for bioactive compounds to control oral biofilms. The ethanolic extract of *Origanum vulgare*, which showed the most promising effects in the initial screening, was further characterized. We first verified the biocompatibility of this extract using human oral keratinocytes and selected a range of non-cytotoxic concentrations to further validate its anti-biofilm and anti-virulence potential. At these concentrations, the extract not only prevented biofilm formation of most dental plaque isolates, but also showed curative potential against mature biofilms grown under conditions mimicking the oral niche. In addition to these anti-biofilm properties, we also observed an inhibition of multiple virulence-associated genes and traits.

Thymol was identified as an important active compound of the extract using GC–MS analysis, but synergy with other compounds was also detected, suggesting a potential advantage of using the whole extract over purified thymol. Further research into the bioactive compounds of the *O. vulgare* ethanolic extract could yield novel products to fight dental caries.

M318 - Organic acids produced by *Lactobacillus* strains isolated from urine reduce *Proteus mirabilis* adhesion and biofilm formation

Presenting Author – *Dominika Szczerbiec, University of Lodz, Poland*

Author/s – *Agnieszka Torzewska*

Abstract Content

Lactobacillus spp. secrete molecules such as H2O2, organic acids or bacteriocins, which have an antimicrobial effect and impact on the pathogenicity of bacteria.

The aim of the study was to determine the influence of extracellular substances secreted by *Lactobacillus* (*L. gasseri*, *L. jensenii*) on adhesion of *P. mirabilis* strains to bladder epithelium and antibiofilm activity.

The anti-adhesion effect of *Lactobacillus* against *P. mirabilis* was evaluated *in vitro* on cultured HCV-29 cells. The assay was performed in a mixture of culture medium and synthetic urine, using membrane inserts, which allowed the diffusion of metabolic products without mixing the cells of both strains. The degree of adhesion was assessed after 1h incubation by microscopic observation of the stained cells and determination of the number of adhered bacteria (CFU/mL) by plating method. Moreover, the main organic acids secreted by tested *Lactobacillus* were quantified colorimetrically. Antibiofilm effect of these acids against 24-hour biofilm of *P. mirabilis* strains formed on polystyrene surface was investigated using MTT assay.

Lactobacillus strains inhibited the adhesion of *P. mirabilis* to the bladder epithelium. *L. gasseri* showed higher anti-adhesion properties reaching even 60%. It was determined that those strains produce lactic and succinic acids. Both acids showed antibiofilm effect against *P. mirabilis* and their highest concentrations inhibited the growth by up to 100%.

The results indicate that extracellular substances produced by *Lactobacillus* inhibit the *P. mirabilis* adhesion to urothelium as well as biofilm formation. Obtained results could contribute to a better treatment or support of UTI and comorbidities.

M319 - RNA biology of KH domain proteins in the emerging cancer-associated microbe *Fusobacterium nucleatum*

Presenting Author – *Yan Zhu, Helmholtz Institute for RNA-based Infection Research, Germany*

Author/s – *Falk Ponath, Jörg Vogel*

Abstract Content

Fusobacterium nucleatum (Fn), long known as a common oral microbe, has recently garnered much attention when found to colonize tumors throughout the human body. Our recent work generated global fusobacterial RNA maps which enabled us to discover a suite of small noncoding RNAs (sRNAs). Further, we showed that the sRNA FoxI acts as posttranscriptional regulator of several envelope. Intriguingly, fusobacteria do not seem to encode any of the three common RNA-binding proteins (RBPs), i.e., CsrA, Hfq and ProQ, suggesting that Fn could harbor new bacterial sRNA-associated RBPs.

Using RNA aptamer tagged sRNA pull-downs, we have now identified the predicated fusobacterial homologs of KhpA and KhpB - KH domain proteins as potential major sRNA-associated RBPs in *F. nucleatum*. KhpA and KhpB show the amino acid sequence conservation within fusobacteria species and are constitutively expressed during growth phase in Fn. Deletion of khpA or khpB leads to reduced growth and cell length in Fn. RIP-seq showed that KhpA and KhpB bind RNAs, including mRNAs and sRNAs. We further revealed that KhpA and KhpB affect the steady-state levels of sRNAs positively or negatively by changing the stability of several sRNAs.

Transcriptome assays showed khpB deletion changes the levels of > 50 mRNA transcripts in stationary growth phase. The highly overrepresented transcripts are ethanolamine-utilization genes (eut). Growth assays found that deletion of khpA or khpB delayed the growth of Fn in a minimum medium supplemented with ethanolamine. We are currently investigating potential molecular mechanism of the newly identified RBPs in regulation of eut operon.

M320 - Experimental evolution of *Stenotrophomonas maltophilia* in an *in vitro* lung epithelium biofilm model

Presenting Author – *Claire Taylor, Research Center Borstel, Germany*

Author/s – *Claire Taylor, Ulrich Schaible, Sascha Brunke, Uwe Mamat*

Abstract Content

Background: Up to 80% of all human infections are caused by biofilms, with colonisation of the cystic fibrosis lung being a prime example. It is known that resistance of bacteria to antibiotics within a biofilm is increased, however most studies of antibiotic resistance evolution are carried out on planktonic bacteria. Therefore, there is a great interest in understanding evolution in biofilms.

Objectives: To study evolution of *Stenotrophomonas maltophilia* in biofilms formed on lung epithelial cells at the air-liquid interface (ALI), including investigations of the impact of co-infections with *Candida albicans* on the evolution of *S. maltophilia* in the biofilm.

Methods: We investigated *S. maltophilia* in single and mixed biofilms with *C. albicans*, using an *in vitro* infection model with human lung epithelial Calu-3 cells at the ALI, over seven days. *S. maltophilia* clones which developed resistance to rifampicin were isolated on selective agar and assessed for rifampicin resistance stability to identify potential mutants.

Results: These data indicated no significant differences between the number of rifampicin resistant clones isolated from the single-species biofilms and the mixed-species biofilms with *C. albicans* at each time point. There is an indication that ALI culture conditions on Calu-3 cells may select for rifampicin cross resistance.

A selection of rifampicin resistant clones demonstrated an increased minimum inhibitory concentration to rifampicin and retained their resistance after passage over five days without exposure to rifampicin, demonstrating heritability. Genome sequencing is being used to identify possible mutations in rifampicin resistant isolates.

M321 - Investigating peptide nucleic acids (PNAs) as antimicrobial agent against *Fusobacterium nucleatum*

Presenting Author – *Valentina Cosi, Helmholtz Institute for RNA-based Infection Research, Germany*

Author/s – *Valentina Cosi, Falk Ponath, Chandradhish Ghosh, Jörg Vogel*

Abstract Content

The oral microbe *Fusobacterium nucleatum* has recently gained attention for its ability to colonize tissues as well as tumors distal from its original niche. There fusobacteria can enhance tumor growth, metastasis and resistance to chemotherapy. Removal of the bacteria has been shown to reduce the tumor burden. The aim of this project is to explore the potential of programmable antibiotics in the form of antisense oligomers such as peptide nucleic acid (PNA) to eliminate or modulate fusobacteria.

The antisense PNAs are usually coupled to cell-penetrating peptides (CPPs) for delivery across the bacterial cell wall, but the efficiency of theses CPPs varies between bacterial species. In order to determine the most efficient CPP for fusobacteria we examined the uptake of fluorescently labeled CPPs using confocal laser scanning microscopy. Of the tested CPPs (KFF)3K and RXR peptides resulted as the most promising candidates with the highest cytosolic uptake efficiency. The selection of the mRNA target, choice of PNA binding position, oligonucleotide length and base composition can also influence the killing capacity of the PNA. We designed PNAs targeting essential genes of *F. nucleatum* such as acpP, gyrA, and ftsZ. These PNAs showed concentration-depended growth-delay *in vitro*. To understand the induced translational inhibition and potential downstream effects of PNAs, we plan to establish translational reporter systems as well as using RNA-seq for monitoring global transcriptomic changes. Our final goal is to eradicate fusobacteria at the tumor site using PNAs specifically targeting the oncomicrobe.

M322 - Comparison of the antimicrobial spectrum of fidaxomicin, thuricin CD, vancomycin and nisin

Presenting Author – *Lauren Walsh, University College Cork, Ireland*

Author/s – *Lauren Walsh, Paul Ross, Colin Hill*

Abstract Content

Vancomycin is a traditional treatment for *Clostridium difficile* infection (CDI). However, recurrence of infection can be high. The gold standard treatment for CDI, fidaxomicin, is considered a narrow-spectrum antibiotic which has been shown to reduce the likelihood of recurrence with *C. difficile* infection. This antibiotic is reported to have minimal effects on the commensal gut bacteria. Each antimicrobial was tested against 50 strains by well diffusion assay. MIC's were performed against a select number of those strains. The micro-Matrix™ fermentation system (ex vivo model of the distal colon) was treated with 30µM of each antimicrobial, spiked with *C. difficile* and incubated for 24 hours. A no-treatment control was included and T0 samples were taken. Metagenomic sequencing was carried out on extracted DNA, from which absolute abundance was determined. Following incubation *C. difficile* present in samples was quantified. All antimicrobials were active against most gram-positive bacteria tested by well diffusion assay. Except for thuricin CD which was specific for *C. difficile* and some Bacillus and Listeria species. The MIC's demonstrated that thuricin CD elicited high levels of activity towards *C. difficile* and B. firmus. Fidaxomicin, vancomycin and nisin exhibited lower MIC's against all strains tested when compared to thuricin CD, with the exception of *C. difficile*. These results were mirrored in the micro-Matrix™ system. The activity of fidaxomicin is comparable to that of the broad-spectrum antibiotic vancomycin. While active against *C. difficile*, fidaxomicin does show activity against gut commensal bacteria, unlike thuricin CD which has a very narrow spectrum.

M323 - Evolutionary trajectories of antibiotic susceptibility in longitudinal bacterial isolates

Presenting Author – *Lieze Agten, KU Leuven, Belgium*

Author/s – *Laura Schillebeeckx, Noémie Luyts, Natalie Verstraeten, Wouter Everaerts, Jan Michiels*

Abstract Content

Bacteria can evade the detrimental effects of antibiotics using an array of strategies and the resulting therapy failure poses a major threat to public health. Most notorious among these strategies is antibiotic resistance yet persistence, too, can complicate treatment. Persistence refers to a subset of transiently multi-drug tolerant cells within an otherwise susceptible population. In recent years, these persister cells have been the subject of thorough investigation and several mechanisms underlying persistence have been elucidated. Moreover, persisters are now known to contribute to the development of resistance and persister levels have been shown to correlate with the frequency of antibiotic exposure *in vitro*.

To gain mechanistic insights in the *in vivo* evolution of persistence, we collected bacterial isolates from longitudinal urine samples of patients with suprapubic catheters. Isolates were identified by culturing on CHROMagar™ Orientation medium and subsequent 16S rRNA sequencing. In a next step, the antibiotic susceptibility of the isolates was determined by *in vitro* minimum inhibitory concentration assays and persistence assays. Additionally, possible correlations between antibiotic treatments and alterations of antibiotic susceptibility were analyzed. In ongoing research, we are searching for genetic alterations underlying antibiotic susceptibility and variants are being subjected to physiological or biochemical analyses to gain mechanistic insights. Finally, results will be validated in models mimicking the *in vivo* situation, such as biofilm models, human bladder cell models and mouse models. Combined, our results will paint a comprehensive picture of persistence evolution in a clinical context.

M324 – Pathogenicity island-mediated prophage induction

Presenting Author – *Yin Ning Chiang, National University of Singapore, Singapore*

Author/s – *John Chen*

Abstract Content

The *Staphylococcus aureus* pathogenicity islands (SaPIs) are mobile genetic elements that carry genes for superantigens, toxins, and virulence factors. They are molecular parasites that have evolved to exploit bacteriophages as helpers for their own propagation and high frequency dissemination. To achieve this, the SaPIs are known to utilize several mechanisms that interfere with distinct aspects of phage life cycles. Here, we observed a reduction of SaPI transfer into *S. aureus* strains lysogenic for certain prophages. We first determined that this reduction was due to SaPI-mediated induction of lysogenic phages to enter their lytic life cycle. We found that this form of lysogenic induction bypasses the host SOS response. The SaPI-encoded determinant responsible for this phenomenon was identified through a genetic screen and its expression was shown to be sufficient for prophage induction. Next, we carried out a screen to identify the prophage target of the SaPI-encoded determinant. Currently, work is being done to demonstrate the interaction between the SaPI-encoded determinant and its prophage target. Our findings highlight the complexities of the co-evolution of genetic elements and its impact on the bacterial host. A better understanding of mobile genetic element transfer and factors affecting their stability would help us in predicting their role in the emergence and selection of pathogenic *S. aureus* strains.

M325 - Investigation of the wavelength dependence of fungal inactivation by standardized irradiation device using UV-LEDs

Presenting Author – *Yushi Onoda, Tokushima University Graduate School, Japan*

Author/s – *Kai Ishida, Yasuko Kadomura-Ishikawa, Miharu Nagahashi, Michiyo Yamashita, Shiho Fukushima, Toshihiko Aizawa, Shigeharu Yamauchi, Yasuo Fujikawa, Tomotake Tanaka, Takashi Uebanso*

Abstract Content

Background: With the development of UV-LEDs (light emitting diodes), it has become important to examine the biological effect of the UV light using standardized evaluation method and uniform irradiation methods suitable for LED optical characteristics. In this study, a light source with high uniformity of irradiance and collimated UV light was developed to analyze the photo-inactivation effect on fungi and compared the inactivation effect with bacteria and viruses.

Methods: An irradiation device suitable for LED optical characteristics was developed and quantified optical characteristics by optical simulation. Thirteen different peak wavelength UV-LEDs (250, 253, 257, 260, 263, 267, 270, 275, 280, 290, 300, 308, and 365 nm; Nichia, Tokushima, Japan) were used. *Rhodotorula mucilaginosa*, *Cladosporium sphaerospermum*, *Aspergillus brasiliensis*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, influenza A virus, herpes simplex virus were used for these experiments. The effects of each of UV irradiation were evaluated using colony forming units for fungi and bacteria, and plaque-forming units for viruses.

Results and Discussion: Fungi were more photo-resistant than bacterias and viruses. Interestingly, the same A. brasiliensis spores with longer growth time were found to be more photo-resistant. There was a strong wavelength dependence of the inactivation effect in the range of 250 to 300 nm wavelength. Interestingly, UV irradiation in the range of 263 to 270 nm wavelength were found to be highly inactivation effect, but with little wavelength dependence in this wavelength range. These results are important knowledge for the development of sanitation management method by photosterilization, and applied products using UV-LEDs.

M326 - Adaptations of *Listeria monocytogenes* in *Drosophila melanogaster* an invertebrate host

Presenting Author – *Natalie Munroe, Imperial College London, United Kingdom*

Author/s – *Marc Dionne*

Abstract Content

Background: *Listeria monocytogenes* is a ubiquitous broad-range-host intracellular pathogen associated with food-borne illness from contaminated Ready to Eat and dairy products. *Listeria* infection can have serious consequences, with a mortality rate of approximately 29.3% in immunocompromised and 17.2% in healthy populations.

Drosophila melanogaster is a well characterised model host for bacterial infection. The experimental tractability of *Drosophila*—in particular, the ease of infection and genetic manipulation—enables the rapid identification of immune mechanisms and host and pathogen effectors.

Objectives and Methods: We aim to characterise *Listeria* infection in *Drosophila melanogaster* by monitoring survival time and bacterial load of wild-type and mutant *Drosophila* infected with *Listeria*. We will then use in-host experimental evolution to derive host-adapted Listeria strains by serial passage through *Drosophila*. We will identify selected mutations by whole-genome sequencing of evolved strains. Additionally, we will assay the influence of any mutations in mammalian infection by comparing the interaction between cultured mammalian cells and ancestral and *Drosophila*-selected strains.

Results: Screening is ongoing. So far, we have performed basic characterisation of the infection in order to identify appropriate conditions for selection experiments. The results of these experiments have been in agreement with the literature, showing dose dependent lethality of infection. By analogy with previous analyses of *in vivo* experimental evolution, we expect that selected strains will exhibit evasion of specific immune killing mechanisms, but this remains to be seen.

M327 - Cell death during nutrient starvation in *Staphylococcus aureus* cells lacking (p)ppGpp is linked to disturbed GTP homeostasis

Presenting Author – *Andrea Salzer, Interfaculty Institute for Microbiology and Infection-medicine Tübingen (IMIT), Germany*

Author/s – *Andrea Salzer, Sophia Ingrassia, Lisa Sauer, Johanna Rapp, Hannes Link, Christiane Wolz*

Abstract Content

In *S. aureus*, the alarmones ppGpp and pppGpp are synthesized upon amino acid limitation or in response to cell-wall stress by the alarmone synthetases RelSau, or RelP and RelQ, respectively. (p)ppGpp is important for bacterial survival, virulence and persistence (1-3). Upon synthesis of (p)ppGpp, GTP levels decrease sharply via consumption of GTP and inhibition of enzymes involved in GTP synthesis (1, 5). *S. aureus* wildtype and isogenic (p)ppGpp0 mutants show similar growth rates and final yields throughout growth. However, in the stationary phase (p)ppGpp0 mutants show a significantly decreased ability to form colonies indicating that stringent response induction either prevents cell death and/or supports escape from a “viable but non-culturable” state. Accordingly, we observed a stringent response-like transcription profile (4) or rsaD expression. rsaD is indirectly regulated by GTP levels through derepression by the GTP-responsive transcriptional factor CodY. However, (p)ppGpp dependent survival is independent of codY. Metabolome analysis further confirm dysregulation of GTP metabolism in the (p)ppGpp0 strain during starvation. When we compared the growth of guanine-auxotrophic mutants (guaAB) in wildtype and (p)ppGpp0 strains, no difference in survival in late stationary phase was observed. This indicates that the uncontrolled increase of GTP in the (p)ppGpp0 strain is sufficient to promote cell death under starving conditions. Cell membrane staining and analysis of membrane potential with the voltage-sensitive probe DiOC2(3) reveals alterations in membrane architecture and function in the (p)ppGpp0 strain. Further, we employed RNAseq to reveal global metabolic changes upon starvation allowing survival by regulating GTP levels.

M328 - Interaction analysis of *Chlamydia* glycogen synthase and human caspase-9 interaction analysis

Presenting Author – *Takuma Otani, Kindai University, Japan*

Author/s – *Saki Motoyoshi, Yoshinao Azuma*

Abstract Content

Chlamydia pneumoniae is an obligate intracellular bacterial pathogen responsible for acute and chronic respiratory diseases, such as pneumonia and asthma, and is widely considered to be a cause of atherosclerosis. As strategies to facilitate its survival in the host cells, *Chlamydia* modulates host-cell pathways including apoptosis regulation. We previously showed that the host apoptotic factor caspase-9 plays a crucial role in chlamydial multiplication and host apoptosis inhibition amid chlamydial infection. Based on the yeast two-hybrid screening, five chlamydial genes interacting with human caspase-9 were isolated, Cpj0444 (PmpG), Cpj0838 (tRNA modification GTPase, MnmE), Cpj0056 (phosphoglucomutase, PgcA), Cpj0948 (glycogen synthase, GlgA), and Cpj0512 (hypothetical). Interactions of MnmE and PmpG with the caspase-9 protein were physically presented. Attempting to clarify the relationship between the chlamydial glycogen synthetic proteins, PgcA and GlgA, and the host caspase-9, we prepared specific antibodies against the PgcA and GlgA, and immunocytochemical and pulldown analyses were performed. PgcA protein was located mainly in chlamydial inclusion but partially in host cytoplasm, while GlgA was found in chlamydial inclusion, especially in the chlamydial cells. Caspase-9 protein was observed in the host cytoplasm while activated caspase-9 was observed in the inclusion. But physical interactions between the PgcA/GlgA and caspase-9 were not detected by the pulldown assay.

M329 - Elucidation of the impact of age-related changes in the host response on the severity of pneumococcal infections

Presenting Author – *Masaya Yamaguchi, Osaka University, Japan*

Author/s – *Momoko Kobayashi, Kunio Kawanishi, Masayuki Ono, Daisuke Motooka, Daisuike Okuzaki, Shigetada Kawabata*

Abstract Content

Background: *Streptococcus pneumoniae* is one of the main causative organisms of pneumonia. Pneumococcal pneumonia is associated with high mortality and morbidity in the elderly.

Objectives: In this study, the effect of ageing on the pathogenesis of pneumococcal infection was analysed using an animal model.

Methods: Intranasal infection with *S. pneumoniae* strain TIGR4 was performed in young and old mice. The bacterial burden and several cytokines were measured in alveolar lavage fluid at 24 hours after infection, in addition to comparing viability after infection. Lung tissue was stained for histopathological analysis. In addition, neutrophils were isolated from the mice and their bactericidal capacity against *S. pneumoniae* was compared.

Results: Infection experiments showed significantly reduced survival in old mice compared with young mice. In addition, significantly higher bacterial counts were detected in alveolar lavage fluid after infection in old mice compared to young mice. Bacterial single-cell genomic analysis of the alveolar lavage fluid suggested that different mutations occurred in each single organism detected. In lung tissue at 24 hours after infection, the percentage of neutrophil elastase-positive cells was significantly higher in the old mice compared to the young mice. On the other hand, MMP-8 was highly expressed in the alveolar lavage fluid of the young mice. Furthermore, neutrophils from old mice were less capable of killing *S. pneumoniae* than neutrophils from young mice.

These results indicate that neutrophils respond differently in the lungs of old mice than in young mice, leading to increased host lethality.

M330 - Common mechanisms control resistance to antimicrobials and insect immune responses in *Enterococcus faecalis*

Presenting Author – *Ashima Wadhawan, Imperial College London, United Kingdom*

Author/s – *Ashima Wadhawan, Carolina J Simoes da Silva Pereira, Catarina Nunes, Andrew Edwards, Marc Dionne*

Abstract Content

Enterococcus faecalis is an opportunistic Gram-positive bacterium found in the gut microbiota of diverse species, including vertebrates and invertebrates, and is common in the environment. One of the hosts *E. faecalis* can infect is the fruit fly, *Drosophila melanogaster*. The *Drosophila* immune response is distinct from that of humans and interacts with *E. faecalis* differently. To study this interaction we carried out experimental evolution of *E. faecalis* in *Drosophila*. We generated *E. faecalis* strains with much-enhanced ability to survive and proliferate within this host. Strains selected in this way are specifically resistant to the Toll-induced Bomanin family of effector peptides, resulting not only in higher *E. faecalis* numbers but also in a significant increase in pathogenicity. Many of these *Drosophila*-selected strains also show exhibit marked increases or decreases in antimicrobial resistance. Whole genome sequencing showed that most selected strains carried single mutations and that many of these mutations were in genes encoding proteins known to be involved in bacterial surface characteristics and antimicrobial resistance (mprF_2, liaF, yxdM, croS, bgsA). To test if *Drosophila* antimicrobial peptides kill *E. faecalis* using mechanisms similar to antibiotics we generated *E. faecalis* strains that were resistant to daptomycin. Some of these daptomycin-selected strains also acquired resistance to the Drosophila immune response. Daptomycin-selected *E. faecalis* strains have mutations in the same genes or the same regulatory systems as were observed in *Drosophila*-adapted strains. Taken together our results indicate common genetic mechanisms underlie killing of *E. faecalis* by daptomycin and the *Drosophila* immune response.

M331 - Using transposon directed site insertion sequencing to identify genes involved in interspecies competition of *P. aeruginosa*

Presenting Author – *Valentin Egle, Interfaculty Institute for Microbiology and Infection-medicine Tübingen (IMIT), Germany*

Author/s – *Theresa Tanios, Monika Schütz, Erwin Bohn*

Abstract Content

Background and Objectives: *Pseudomonas aeruginosanosa* (Pa) and species of the *Burkholderia* cepacia complex (Bcc) are opportunistic lung pathogens often found in cystic fibrosis (CF) patients. While Pa can initiate long-term infections in younger CF patients, Bcc infections only arise in teenagers and adults1. Both Pa and Bcc use type VI secretion systems (T6SS) to mediate interbacterial competition2. Previously Perault et al (2020) demonstrated that adaptations of Pa to the lung might lead to mutations which may abrogate T6SS activity of Pa and make the host susceptible to fatal Bcc superinfections3. In the present study we wanted to address which factors besides the already defined ones are critical that Pa can outcompete Bcc.

Methods and Results: We established an interspecies competition assay based on FACS analysis of a fluorescently labelled clinical Pa isolate and the Bc ATCC 25416 strain before and after co-incubation on solid media. So far, we employed genetic knock-out mutants of Pa to demonstrate that the H1-T6SS, but not the H2-T6SS of Pa is crucial to outcompete Bc. Different amounts of NaCl in the media seems to impact competition as well.

We are currently using a Transposon-Directed Insertion Site Sequencing (TraDIS) approach to address the prinicipal armament of Pa required to withstand the attacks of Bc and will discuss first results.

M332 - Characterization of adhesion factors from the microbial fish pathogen *Yersinia ruckeri* and their association with host receptors

Presenting Author – *Anna Lislerud, University of Oslo, Norway*

Author/s – *Hajime Nakatani, Maria Spence, Athanasios Saragliadis, Katsutoshi Hori, Dirk Linke*

Abstract Content

Background: Infectious diseases caused by bacteria lead to vast economic losses in international aquaculture. The Gram-negative bacterium *Yersinia ruckeri* is the causative agent of enteric redmouth disease (ERM) – an infectious disease that mostly affects salmonoids (1). ERM from infection by *Y. ruckeri* can have a mortality rate of up to 70% in infected fish farms.

Objectives: For the purpose of this project, we are studying the *Y. ruckeri* type V secretion systems (T5SSs). More specifically two invasin adhesion molecules belonging to the Ve inverse autotransporter (IAT) subtype (2). We want to investigate the role of the adhesion proteins, *Y. ruckeri* Invasin (YrInv) and *Y. ruckeri* Invasin-like molecules (YrIlm) in the pathogenesis of *Y. ruckeri*.

Methods: We have used single and double-gene knockouts of *Y. ruckeri* in *in vivo* bath infection experiments with live zebrafish to investigate the importance of YrInv and YrIlm as virulence factors in *Y. ruckeri*. Salmon-cell culture experiments and a gentamycin assay are used to investigate this further. Expression and purification of YrInv and YrIlm protein constructs have enabled us to perform pull-down essays to determine host-cell receptor interactions with the adhesins.

Results: The results of the experiments will be presented. The data indicate a decrease in the virulence of the knock-out strains compared to the wildtype strain. Quite dramatic differences in survival rates of the different strains have also been shown following the gentamycin assay.

M333 - Electrochemical impedance biosensor for the detection of *Vibrio vulnificus* zoonotic pathogen

Presenting Author – *Arnau Perez Roig, University Of Valencia, Spain*

Author/s – *Arnau Pérez Roig, Bergoi Ibarlucea, Carmen Amaro, Gianaurelio Cuniberti*

Abstract Content

Vibrio vulnificus (Vv) is a zoonotic pathogen linked to fish farms able to infect aquatic animal species and humans by contact and ingestion. Recently, Vv is spreading due to climate change. Current gold standard for detection consists on PCR. However, this method is time consuming and requires expensive equipment and trained personnel. New alternatives such as electrical biosensors are emerging due to their advantages. These devices allow designing low-cost platforms with a high sensitivity and great miniaturization. Among them, impedance sensors have proven great detection limits without sacrificing size. Additionally, functionalization of the surface with biological elements increase the specificity of the sensor, turning it into a biosensor. For these reasons, biosensors constitute a flexible, highly sensitive and portable approach for the detection of Vv, helping to reduce the spread and mortality of the disease. The objective of this work consists on the development of an impedance biosensor, functionalized with single stranded DNA sequences complementary to the vvha gene. This gene encodes a species-specific hemolysin widely used as a species marker. The specificity and sensitivity of this biosensor was tested in buffer samples containing from 1 nM to 1 pM of synthetic DNA. Finally, the biosensor was tested with DNA extracted from pure cultures of the bacteria. Along the conducted experiments, the biosensor was able to detect the presence of DNA from the bacteria and successfully differentiate it from other Vibrio species.

M334 - Virulence properties and antimicrobial resistance among canine uropathogenic *Escherichia coli* strains isolated in a French veter

Presenting Author – *Patrick Di Martino, Cergy Paris University, France*

Author/s – *Gilles Mayot, Cassandre Langreau, Aurélie Bogey*

Abstract Content

All *Escherichia coli* strains isolated from dogs with urinary tract infections in the Vet'Analys veterinary laboratory in Hyères, France, during a period of two consecutive months were studied. The twenty isolates were screened for haemolytic properties on blood agar, biofilm formation in microtiter plates after crystal violet staining, antibiotic resistance by disc diffusion method, and the presence of virulence-associated genes fimA, papC and hlyA by PCR. Ten strains expressed haemolysis on blood agar, five of them harboured the hlyA gene. fimA was found in all strains, fimA and papC were detected together in fourteen isolates, and fimA, papC and hlyA were observed together in five isolates. Five of the strains studied efficiently formed a biofilm *in vitro*. Resistance to penicillin was observed in all isolates, twelve of them did not show any other resistance. Of the eight strains with additional resistance, two were multidrug resistant to at least three classes of antibiotics, one of which expressed an extended spectrum beta-lactamase. There was no correlation between biofilm formation, the presence of virulence genes and antibiotic resistance. In conclusion, among the uropathogenic *Escherichia coli* strains isolated over a period of two months in the Vet'Analys laboratory, the frequency of the presence of the fimA and papC genes was very high, the frequency of haemolytic power was high, the ability to efficiently form a biofilm *in vitro* and multi-resistance to antibiotics were low.

M335 - Development of multi-species biofilm models for evaluating medical approaches in dentistry

Presenting Author – *Jan-Ole Reese, University of Oslo, Norway*

Author/s – *Jan-Ole Reese, Athanasios Saragliadis, Håvard Jostein Haugen, Dirk Linke*

Abstract Content

Background: Biofilm formation occurs on 40% of all dental implants inserted, potentially leading to devastating infections known as peri-implantitis. The consequences include tissue damage, bone atrophy, and eventual loss of the implant. Unfortunately, at present, tremendously needed new solutions for biofilm control have to be tested in animal experiments, which are not only far too costly but also ethically inappropriate.

Objectives: To assess novel treatment and prevention strategies *in vitro*, we develop a reproducible multispecies biofilm model, intended to simulate key aspects of biomaterial-derived infections in a laboratory setting. A dynamic approach was chosen by culturing highly relevant oral bacterial species under flowing conditions to achieve optimal replication of the specific environmental conditions in the oral cavity.

Methods: *In vivo* growth conditions are recreated by cultivation within a bioreactor. Coupling the latter to a modified "Robbins device" flow chamber allows growing biofilms directly on implant surfaces under constant flowing conditions.

Biofilms are examined by combining biomass quantification with crystal violet assay and structural analysis with scanning electron microscopy (SEM), as well as species-specific bacterial quantification by quantitative polymerase chain reaction (qPCR) and fluorescence in situ hybridization (FISH).

Results: We present data showing how many species can be included in a multispecies biofilm model without losing a high degree of reproducibility. Furthermore, the composition of various species within the biofilm is demonstrated. Once established, the model is used to evaluate the potential of medical applications to remove biofilms from the implant.

M336 - Antibiotic-tolerant persisters are pervasive in clinical *Streptococcus pneumoniae* isolates

Presenting Author – *Nele Geerts, University of Antwerp, Belgium*

Author/s – *Nele Geerts, Linda De Vooght, Ioannis Passaris, Peter Delputte, Bram Van den Bergh, Paul Cos*

Abstract Content

Background and Objectives: *Streptococcus pneumoniae* is considered a serious threat by the Centers for Disease Control and Prevention because of rising antibiotic resistance. Next to resistance, bacteria can also survive lethal antibiotic treatment by developing antibiotic tolerance through persistence. This phenotypic variation seems omnipresent among bacterial life, is linked to therapy failure, and acts as a catalyst for resistance development. The aim of this study was to make a broad characterization of persistence in *S. pneumoniae*.

Methods: To proof the presence of persister cells, time-kill curves were obtained by treating pneumococci with high concentrations of antibiotics (amoxicillin, cefuroxime, moxifloxacin and vancomycin) in different growth phases. To exclude resistance, persisters were re-grown and time-kill curves as well as antibiotic susceptibility were determined. Finally, a set of clinical *S. pneumoniae* isolates was screened for survival after antibiotic treatment at 100-fold the MIC.

Results: Pneumococci treated with 100-fold the MIC of the antibiotics resulted in time-kill curves showing a biphasic killing pattern, which is proof of the presence of persister cells. Resistance was excluded as similar time-kill curves were obtained from regrown persisters compared to the original strain and they remained susceptible to the antibiotics. We also detected a high variety in antibiotic survival levels across a diverse collection of *S. pneumoniae* clinical isolates, which assumes that a high natural diversity in persistence is widely present in *S. pneumoniae*. Currently, we are screening a large set of clinical *S. pneumoniae* isolates for persistence to confirm our findings.

M337 - HmuS protein from *Porphyromonas gingivalis* - a novel dechelatase involved in anaerobic heme metabolism

Presenting Author – *Patryk Cierpisz, University of Wroclaw, Poland*

Author/s – *Patryk Cierpisz, Michal Smiga, Teresa Olczak*

Abstract Content

Periodontal diseases belong to a group of infectious inflammatory diseases, resulting in the destruction of tooth-supporting tissues, gum bleeding, and very often tooth loss. One of the main etiological agents and keystone pathogens of chronic periodontitis is *Porphyromonas gingivalis*. It is an anaerobic, Gram-negative bacterium that lacks the functional pathway of protoporphyrin IX (PPIX) biosynthesis. Moreover, it does not produce a classical system used for iron uptake. Therefore, for survival and effective virulence, it requires heme as a source of iron and PPIX. The main system used for heme uptake by *P. gingivalis* is the Hmu heme acquisition system, containing hemophore-like protein (HmuY), outer membrane TonB-dependent receptor (HmuR), and four additional proteins with unknown function. A HmuS protein, which is a part of the Hmu system. It is a putative chelatase, with homology to CobN/Mg-chelatases. Using transcriptomic methods, we showed that HmuS is produced in an iron/heme-dependent manner. We overexpressed and purified recombinant HmuS protein and using spectrophotometric methods we showed that HmuS binds heme and other metalloporphyrins. Moreover, we showed that HmuS exhibits reverse chelatase activity. We suspect that HmuS could be involved in extracting iron from heme under anaerobic conditions. Our results also show that HmuS activity may be important in iron acquisition, enabling *P. gingivalis* survival and virulence potential.

M338 - Cyanidin chloride can reduce the expression of virulence factors in *Acinetobacter baumannii*

Presenting Author – *Jaeu Won, Chungnam National University, Republic of Korea*

Author/s – *Ho-Sung Park, Kyungho Woo, Dong Ho Kim, Chul Hee Choi*

Abstract Content

Acinetobacter baumannii (*A. baumannii*) is a representative opportunistic pathogen among gram-negative bacteria that can damage patients with compromised immune systems. Among flavonoids, cyanidin chloride, anthocyanidin family, is reported to have cytoprotective and anticancer effects. However, the antibacterial mechanism of cyanidin chloride against *A. baumannii* has not been reported. To confirm the effect of cyanidin chloride on *A. baumannii*, bacterial growth was measured with CFU. Surface motility, biofilm formation and acyl-homoserine lactone (AHL) inhibition was measured to confirm pathogenic inhibition. Inhibition at gene level was measured through qRT-PCR. To determine the minimum inhibitory concentration (MIC) of *A. baumannii*, an antimicrobial susceptibility test (AST) was performed based on the CLSI guideline, and a checkerboard assay was performed to test the synergistic effect. Results showed that cyanidin chloride did not affect the growth of ATCC 17978, the standard strain *A. baumannii*, but significantly inhibited the biofilm formation, motility and AHL production. Cyanidin chloride inhibited the expression of biofilm-related and motility-related genes (bap, bfmR, csuAB) and QS system-related genes (AbaR, AbaI) at the genetic level, and also down-regulated ompA, a major virulence gene of *A. baumannii*. In the synergy test, it was confirmed that cyanidin chloride exhibited additive effects with the antibiotics; tetracycline, amikacin and ciprofloxacin. In addition, it was confirmed that there is a synergistic effect when ceftazidime and cyanidin chloride are treated together, and that already formed biofilms can also be effectively lowered. These results indicate the possibility that cyanidin chloride can be used as a pathogenic inhibitor against *A. baumannii*.

M339 - Outer membrane protein A of *Acinetobacter baumannii* suppresses Xenophagy Mechanism via CAMKK2/AMPK pathway.

Presenting Author – *Kyungho Woo, Chungnam National University, Republic of Korea*

Author/s – *Dong Ho Kim, Ho Sung Park, Jau Won, Chul Hee Choi*

Abstract Content

Acinetobacter baumannii has been designated by the World Health Organization as an important pathogen urgently in need of research. Outer membrane protein A (OmpA) plays important roles including bacterial adhesion and evasion of host defenses. Xenophagy is an autophagic phenomenon that specifically involves pathogens. *A. baumannii* triggers xenophagy, yet little is known about the evasion pathway. Here we tried to evaluate the induction of xenophagy in *A. baumannii* infection as well as explore the related evasion mechanisms by OmpA.

To determine whether autophagy activity and inhibition were associated with the AMPK pathway, Analysis was performed using both the *A. baumannii* ATCC 17978 Strain, the isogenic OmpA deletion mutant, and Exgeonus OmpA. Xenophagy activity was determined by microscopic analysis, flow cytometry, and confocal imaging systems by staining cells with Acridine Orange (AO), Monodansylcadaverine (MDC), and antibodies. The Co-IP was performed to confirm the physiological interaction protein of camkk2 with the OmpA. In this study, we demonstrated that the isogenic OmpA deletion mutant has significantly increased AMPK phosphorylation, as well as autophagosome-Lysosome fusion in Raw 264.7 cells, compared to the WT strain. OmpA inhibited phosphorylation by binding to Camkk2, thereby inhibiting the AMPK pathway. Inhibition of the Xenophagy pathway by each inhibitor and exogenous OmpA increased intracellular *A. baumannii*. Our study has revealed an important role of the evasion mechanism by OmpA in the *A. baumannii*-induced autophagic process. These findings provide basic data for a promising therapeutic target against *A. baumannii* infection.

M340 - The application of plasma functionalised liquids in the treatment of orthopaedic implant infections

Presenting Author – *Orla Nic Shiurdain, Instituto Ramón Y Cajal De Investigación Sanitaria (irycis), Ireland*

Author/s – *Orla Nic Shiurdain, Sean Kelly, Peter Dobbyn, Daniela Boehm, Paula Bourke*

Abstract Content

Orthopaedic implant infections cause a significant burden on healthcare systems globally. Orthopaedic implants are commonly used to help heal broken bones or to replace joints such as hip replacements. If the implantation site becomes infected the infection can be difficult to treat. This is because of the formation of bacterial biofilms on the surface of the implant and antimicrobial resistant bacteria. Current treatments for these infections include antibiotic treatment and wound debridement, however in some cases this is not adequate, and the implant must be removed via revision surgery. Cold atmospheric plasma (CAP) and plasma functionalised liquids (PFLs) have antimicrobial properties which could be used a treatment for orthopaedic implant infections. The aim of this research is to optimise a PFL treatment to treat methicillin resistant *Staphylococcus aureus* and *Pseudomonas aeruginosanosa* biofilm infections which could be used in combination with direct CAP therapy.

PFLs generated from several plasma systems including an in-house reactive species specificity (RSS) system in Spark and Glow discharges, a microwave discharge – Midiplex were investigated. The efficacy of the liquids was assessed against planktonic and biofilm forms of *S. aureus* and *P. aeruginosa*. The most effective liquids will be investigated further regarding combination with direct plasma treatments and biocompatibility. This study has found that PFLs generated from different plasma systems had distinct chemistry as well as different efficacies. This study aims to assess the efficacy and safety of the optimised PFLs against mature biofilms, used both alone and in combination with direct CAP treatments.

M341 - Post-transcriptional regulation of the PQS quorum sensing system in *Pseudomonas aeruginosanosa*

Presenting Author – *Dimitra Panagiotopoulou, University of Nottingham, United Kingdom*

Author/s – *Natalia Romo Catalan, Miguel Cámara, Stephan Heeb*

Abstract Content

Pseudomonas aeruginosanosa is an opportunistic pathogen, highly resistant to antibiotics and a major cause of nosocomial infections. The ability to survive in diverse environments, allows *P. aeruginosa* to colonise the lungs of cystic fibrosis patients and establish chronic infections.

The presence of three quorum sensing (QS) systems (rhl/las/pqs) and a wide range of small non-coding RNAs (sRNAs) have been shown to play an important role in the adaptation of this organism to different environments. sRNAs regulate gene expression at the post-transcriptional level aiding the adaptation of this organism to rapidly changing environments. In this study, we have identified a region encoding a sRNA that overlaps with the promoter of the pqsABCDE operon from the pqs QS system and named it pqsX. After validating the presence of the PqsX transcript by Northern blot in the *P. aeruginosa* model sublines PAO1 and PA14, the secondary structure of the sRNA was predicted *in silico*. The relationship of PqsX with all three QS systems of *P. aeruginosa* was investigated using a lux-based bioreporter system. In addition, the impact of pqsX overexpression on the production of QS molecules from the three QS systems was determined. This study highlights the importance of sRNAs in the post-transcriptional regulation of the QS systems adding another layer of complexity to the regulation of virulence in *P. aeruginosa*.

M342 - RNA Seq analysis of multispecies biofilms provides information about interaction and competition among lung pathogens

Presenting Author – *Raphael Moll, Universität Hamburg, Germany*

Abstract Content

Background: Microbial biofilms harboring several pathogens coexisting in close proximity are related to lung infections. The bacteria *Pseudomonas aeruginosanosa*, *Staphylococcus aureus*, *Stenotrophomonas maltophilia* and *Candida albicans* are widely known for their substantial role in pathogenicity in infected lungs.

Objectives: By RNA seq analysis and the usage of promotor fusion constructs we want to investigate the interaction and competition among lung pathogens in multispecies biofilms. Furthermore, we want to examine the distribution and organization of each species in these biofilms by CLSM imaging.

Methods: For this study chromosomally labeled fluorescent strains of *P. aeruginosa* PA01, *S. aureus* SH1000, *S. maltophilia* K279a and *C. albicans* SC5314 were used and multispecies biofilms were formed successfully in flow and static settings. Multispecies biofilms were processed to RNA seq analysis and promotor fusion constructs were generated.

Results: LSM imaging showed that species interactions affect the structural composition of multispecies biofilms. Layer formation was often observed. In multispecies biofilms, PA01 dominated, while SH1000 was often reduced. The bottom layer was initially colonized by K279a. In a dual species biofilm of K279a with SC5314, K279a cells were attached to the hyphae of SC5314. In coculture with SH1000, lactate metabolism was elevated in K279a. On the other hand, propionate degradation in K279a was upregulated in the presence of PA01. K279a and PA01 metabolism in multispecies biofilms was primarily fermentative with cytochromes used for anaerobic respiration. The expression of virulence factors, QS signalling and cyclic diGMP was decreased in PA01 in coculture with K279a.

M343 - Characterization of gene-microbiome interactions in the mammalian lung

Presenting Author – *Abdulgawaad Saboukh, Max Planck Institute for Evolutionary Biology, Germany*

Author/s – *Cecilia Juryung Chung, John Baines*

Abstract Content

Background: Complex communities of microbes inhabit every surface of the human body. The lungs were traditionally viewed as sterile in healthy individuals, but it is increasingly clear that microbes inhabit the lower airway tract and contribute to important traits such as lung development and immunity. While many external factors have been studied and shown to influence lung microbiota composition, the degree to which host genetics play a role in structuring lung microbiota is less understood. In our working group, we identified a strong association between *Lactobacillus* and interesting candidate genes such as interleukin-10 (Il-10) and mitogen-activated protein kinase–activated kinase-2 (Mk2), which are known to play a role in lung disease.

Objectives: Further explore the identified *Lactobacillus*-Mk2 association *in vitro* and *in vivo*. 2. In an asthmatic mouse model, we aim to Investigate whether *Lactobacillus* modulates the allergic airway inflammation by regulating the expression levels of Mk2.

Methods: We are using cell culturing techniques to co-culture human and mouse lung resident *Lactobacillus* strains with human and mouse lung epithelial cells, respectively. Additionally, the 16S rRNA sequencing and the ddPCR are used to determine the lung microbial composition in Mk2 knockout and wildtype mice.

Results: Our preliminary results from the *in vitro* cell culture experiments indicate strain-specific effects on the transcription levels of the Mk2 and the downstream pro-inflammatory cytokines, Il-6 and Il-1B. Additionally, 16S rRNA sequencing revealed differences in the abundance levels of the top genera when comparing the different the lung bacterial composition between the different Mk2 mouse genotypes.

M344 - Involvement of the positively charged residues located near acylated lysins in the membrane insertion of *Bordetella* CyaA toxin

Presenting Author – *Anna Lepesheva, Institute of Microbiology of the Czech Academy of Sciences, Czech Republic*

Author/s – *Adriana Osickova, Michaela Grobarcikova, David Jurnecka, Sarka Knoblochova, Peter Sebo, Jiri Masin*

Abstract Content

The adenylate cyclase toxin-hemolysin (CyaA) belongs to the Repeats in ToXin (RTX) family of leukotoxins and plays a key role in virulence of the whooping cough agent *Bordetella pertussis*. CyaA translocates an adenylyl cyclase enzyme subunit into phagocytes expressing the complement receptor 3 (CR3, also known as the CD11b/CD18) and subverts the bactericidal functions of phagocytes by unregulated conversion of cytosolic ATP to cAMP. In parallel, CyaA permeabilizes cellular membrane by forming small cation-selective pores. With a reduced efficacy, CyaA can also interact with a variety of other host cell types that lack CR3, or even with naked lipid bilayer membranes. Both cytotoxic activities depend on the activation of proCyaA by fatty acyl modification of two lysine residues, Lys860 and Lys983 by acyltransferase CyaC. Based on recently solved structure of the C-terminal fragment of CyaA in complex with CR3, we mutagenized two blocks of positively charged arginine and lysine residues located in the vicinity of Lys860, as well as the Arg984 located near the acylated Lys983 residue. Our results show that replacement of Arg984 and Lys857 reduces the ability of CyaA to enter and penetrate the membrane of CR3-positive THP-1 human monocytes, CR3-negative sheep erythrocytes, or artificial lipid membranes. We hypothesize that the positively charged Arg984 and Lys857 play a key role in anchoring of the acylated segment to the plasma membrane of target cells and may be directly involved in interaction with the negatively charged head groups of lipids, or with the negatively charged glycans on cell surface.

M345 - *Tenacibaculum*: toxins and potential bacteriophage isolation

Presenting Author – *Patricija Petrikonyte, University of Oslo, Norway*

Author/s – *Helga Pernille Bergvol, Sophanit Mekasha, Dirk Linke*

Abstract Content

Background: *Tenacibaculum* sp. strains are associated with causing tenacibaculosis in both wild and farmed fish – a disease that causes skin lesions, fin- and tail rot. The bacteria pose a major threat having an impact on fish welfare in addition to being responsible for high financial losses for the fish industry. Only recently virulence factors and pathogenicity mechanisms in *Tenacibaculum* have garnered more attention, however, the lack of knowledge persists and is an obstacle dealing with the ulcerate disease caused by the bacteria.

Objectives: The research project aims to identify and characterize *Tenacibaculum maritimum* virulence factors and its potential novel bacteriophage in a goal to develop effective preventive mechanism of tenacibaculosis.

Methods: Combinations of LC-MS proteomics and RT qPCR methods were used to identify, quantify and compare virulence factors among different strains of *T. maritimum*. Potential phage isolation and characterization is performed using a double-layer plaque assay and imaging techniques.

Results: Complete genome sequence and secretome proteomics of the Gram-negative fish pathogen T. maritimum provides information about virulence mechanisms and relevant secretion systems. Comparative overview over secretome profiles obtained from five *T. maritimum* species indicate the presence of protein toxin candidates belonging to diverse enzyme families that could potentially play a role in pathogenecity. Four of the five T. maritimum strains have indications to contain prophages which infection might be induced while grown in the laboratory. These findings increase and broaden our knowledge on *Tenacibaculum* sp. infection and potential ways to fight tenacibaculosis

M346 - Characterization of multicellular aggregates formed by *Burkholderia multivorans*

Presenting Author – *Mirela R. Ferreira, iBB - Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Portugal*

Author/s – *Sara C. Gomes, William H. DePas, Vaughn S. Cooper, Leonilde M. Moreira*

Abstract Content

The formation of biofilms, including attached biofilm and planktonic cellular aggregates, is of relevance in natural environments, but also during interaction with host cells resulting in pathogenicity. In patients with cystic fibrosis (CF), microorganisms from the *Burkholderia cepacia* complex, namely *Burkholderia multivorans*, often establish a chronic infection. Several *Burkholderia* clinical isolates share the ability to form cellular aggregates, possible mediators of the transition to chronic infection due to their ability to better resist the harsh conditions existing in CF lungs[1]. Due to their relevance, it is crucial to better understand these structures and characterize them. Therefore, to study the dynamics of aggregation, several carbon/nitrogen ratios were tested, revealing that low-nitrogen concentration leads to higher aggregation. Other stressful conditions found within the CF lungs that could affect aggregate formation were also tested. Results showed that in the presence of antimicrobials, hypoxia and low pH, the formation of aggregates is favored. To better visualize the structure of aggregates, a tissue-clearing technique – MiPACT - was performed, allowing three-dimensional imagining of aggregates and identification of some extracellular matrix components[2]. The labeling of aggregates with lectins identified some matrix's carbohydrates such as α-Mannose, D-Galactose, D-Galactose-β3-N-acetylgalactosamine, and N-acetylglucosamine, leaving some clues of possible polysaccharides present in these structures. Deletion of bep genes by CRISPR/Cas revealed that Bep is the main polysaccharide present in these aggregates and no significant contribution of cepacian and cellulose to aggregates formation was observed. Together, our results highlight the complexity of the multicellular aggregate structure and the conditions favoring its formation.

M348 - Characterisation of *B. thailandensis* virulence factors in biochemical studies and cellular infection assays

Presenting Author – *Mirko Himmel, Universität Hamburg, Germany*

Author/s – *Daria Dretvic, Samantha Klein, Stefan Linder, Wolfgang Streit*

Abstract Content

The genus *Burkholderia* contains several human-pathogenic bacteria species. Among them, highly virulent *Burkholderia pseudomallei* and *B. mallei* are closely related to low virulent *B. thailandensis*. The intracellular lifestyle of these *Burkholderia* species relies on different virulence factors, which enable active escape from the phagosome, actin-based intracellular motility, and fusion of host cell membranes into multi-nucleated giant cells. *B. thailandensis* (BTH) shows a much lower virulence than B. mallei and B. pseudomallei. The bacterial protein BimA is essential for actin tail formation but seems to require activation by a yet unknown mechanism. At present, it is not fully understood how actin-dependent motility and cell-to-cell spread are regulated on the molecular level (Fig. 1). Potentially involved are the *Burkholderia* proteins BimC, BimD, and BipC. Here, we present results of cellular infection assays using different *B. thailandensis* gene knock-down strains combined with biochemical analyses of BimC to further elucidate critical bacterial factors determining *Burkholderia* virulence.

M349 - Study of the emerging European/Russian multidrug-resistant 100-32 clone of *Mycobacterium tuberculosis*

Presenting Author – *Isabelle Bonnet, Université De Paris, France*

Author/s – *Isabelle Bonnet, Florence Brossier, Fadel Sayes, Wafa Frigui, Yan Madacki, Wladimir Sougakoff, Roland Brosch, Régis Tournebize*

Abstract Content

Background: Tuberculosis (TB) remains a leading worldwide infectious disease as demonstrated by an increase of people newly diagnosed with TB post-COVID-19 pandemic in 2021. The multidrug-resistance (MDR) in *Mycobacterium tuberculosis* (Mtb) represents a serious hurdle to global TB control with cure rates around 50%. Within the lineage 2 Mtb strains of the socalled “Beijing” family, the 100-32 clonal complex (CC) (or B0/W148 clone) shows a rapid and recent expansion across Eurasia and has quickly acquired drug resistance mutations.

Objectives: Understand whether specific mutations might explain the higher transmissibility of this MDR 100-32 clone.

Methods: We investigated by whole genome sequencing 50 MDR 100-32 CC isolates. Among the specific genetic variants, we focused on a frequently-found mutation in whiB6 (T51P), a regulator of the ESX-1 system, and a two-nucleotide deletion in kdpD creating a KdpDE fusion protein. Mutants and complemented strains of both genes have been constructed in the Mtb reference strain H37Rv and will be characterized by Western Blot, cytokine production in THP-1-derived macrophages, RNA-seq and virulence studies in macrophage and/or mouse models.

Results: As initial results, we found that the whiB6 T51P mutant i) produces lesser amounts of ESAT-6 protein than clinical strains harboring a WT whiB6 gene, indicating reduced ESX-1 functions, and also ii) produces less proinflammatory cytokines than WT whiB6-strains. These preliminary results suggest that lower inflammatory responses might explain a higher transmissibility due to a delay in diagnosis and treatment and therefore favor the emergence of this clone.

M350 - Iron overload induces the inflammatory responses in *Mycobacterium abscessus* infection

Presenting Author – *Dong Ho Kim, Chungnam National University, Republic of Korea*

Author/s – *Kyungho Woo, Ho-Sung Park, Jaeu Won, Chul Hee Choi*

Abstract Content

Mycobacterium abscessus (M.abs) is a rapidly growing non-tuberculous mycobacterial species that infects macrophages of the lung in human. Iron is essential for organisms, including M.abs. However, this metal is not freely available in the mammalian host. Due to its poor solubility and tendency to catalyze the production of reactive oxygen species, host iron is kept in solution bound to specialized iron binding proteins. The success of mycobacterial infection appears to be inherently related to the bacteria's ability to regulate intracellular iron levels, primarily using iron storage proteins. In this study, we are investigating the role of iron in M.abs infection. BMDMs were infected with M.abs UC22 or ATCC 19977 and then, extracellular were removed. Infected BMDMs were incubated in presence of Fe3+ (ammonium iron (Ⅲ) citrate) or DFO (deferoxamine). The number of intracellular bacteria were significantly decreased in presence of Fe 3+ compared to BMDMs were incubated with DFO. ELISA analysis showed that Fe 3+ enhanced production of TNF in BMDMs infected with M.abs UC22, but production of IL-10 was decreased as the concentration of Fe 3+ increases. Next, we monitored the autophagic flux by measuring level of autophagic markers. The expression of RUBCN and iNOS were decreased, but the expression of LC3, p62 and FTH1 was increased in presence of Fe 3+. Taken together, these results suggest that Fe 3+ could restrict M.abs infection by increasing autophagy and inflammatory response.

M351 - Modulation of inflammatory response by *Tannerella forsythia* OMVs in the presence of quorum sensing inhibitors

Presenting Author – *Bong-Kyu Choi, Seoul National University, Republic of Korea*

Author/s – *Sun-Jin An, Kyung-Won Ha, Hye-Kyoung Jun, Hyun Young Kim*

Abstract Content

Background and Objectives: Outer membrane vesicles (OMVs) of bacteria harbor physiologically active molecules, and quorum sensing inhibitors (QSIs) are expected to regulate bacterial virulence. The purpose of this study was to compare the proinflammatory activity of OMVs of a periodontal pathogen grown in the absence or presence of QSIs.

Methods: The periodontal pathogen *Tannerella forsythia* was grown to late exponential phase in the absence or presence of D-arabinose (100 mM) or D-galactose (100 mM) as QSIs. OMVs released from *T. forsythia* were isolated using density gradient ultracentrifugation and characterized by nanoparticle tracking analysis and transmission electron microscopy. THP-1 monocytes were treated with OMVs for 6 and 24 h, and the expression of proinflammatory cytokines including TNF-α, IL-1β, IL-6, and IL-8 was analyzed by qPCR and ELISA. The effect of OMVs on intracellular signaling was evaluated. In addition, *T. forsythia* OMVs were tested for their activation of TLR2 and TLR4 using a human NF-κB reporter cell line and bone marrow-derived macrophages from TLR2-/- mice.

Results: Compared to OMVs of nontreated *T. forsythia* (TF OMVs), OMVs released from QSI-treated *T. forsythia*, designated as TF ara-OMVs and TF gal-OMVs, showed reduced production of TNF-α, IL-1β, IL-6, and IL-8 in THP-1 monocytes through decreased activation of NF-κB/MAPKs. TF ara-OMVs and TF gal-OMVs showed less activation of TLR2 than TF OMVs.

These results demonstrate that QSIs provide a dual advantage against bacterial infection by inhibiting bacterial biofilm formation and generating OMVs with reduced proinflammatory activity.

M352 - Macrolide resistance in *Pseudomonas aeruginosanosa* through uL4 and uL22 ribosomal protein mutations

Presenting Author – *Lise Goltermann, Rigshospitalet, Denmark*

Author/s – *Ivan Pogrebnyakov, Helle Krogh Johansen, Søren Molin, Ruggero La Rosa*

Abstract Content

Background: *Pseudomonas aeruginosanosa* is an opportunistic Gram-negative pathogen often found in the lungs of patients suffering from cystic fibrosis (CF). Previously, *P. aeruginosa* has been deemed insusceptible to macrolide antibiotics. Yet, CF patients infected with *P. aeruginosa* are often prescribed prolonged courses of macrolide treatment because of a supposed immunomodulatory effect without regarding resistance development.

Objectives: We aimed to show that macrolides are active antibacterial compounds against *P. aeruginosa* and that macrolide resistance can occur via ribosomal protein mutations.

Methods: *P. aeruginosa* isolates from various sources were screened for mutations in ribosomal proteins. Most of the identified mutations mapped to the ribosomal proteins uL4 and uL22. A selection of mutations was transferred into the reference strain PAO1 and all resulting strains were characterized with respect to antimicrobial tolerance, growth characteristics, proteome composition and an array of phenotypes.

Results: Adjusting the traditional susceptibility testing protocol revealed that wild-type *P. aeruginosa* is indeed susceptible towards macrolides. Importantly, all three uL4 and four uL22 mutants exhibited significantly increased tolerance towards this drug class. Greater concentrations of macrolide antibiotic were needed to inhibit the growth, reduce motility, and induce redox sensitivity of each uL4 and uL22 mutant. Also, proteome composition changed for the uL4 and uL22 mutants compared with the PAO1 wild-type.

Macrolide antibiotics should, therefore, be considered as active antimicrobial agents against *P. aeruginosa* and resistance development should be contemplated when patients are treated with prolonged courses of macrolides. Importantly, improved macrolide susceptibility testing is necessary for the detection of resistant bacteria.

M353 - The morphological change of nontuberculous mycobacteria caused by cell surface glycopeptidolipid

Presenting Author – *Nagatoshi Fujiwara, Tezukayama University, Japan*

Author/s – *Minoru Ayata, Hiroyuki Yamada, Takehiko Kobayashi, Shiomi Yoshida, Shinji Maeda*

Abstract Content

Background: *Mycobacterium avium*, *Mycobacterium intracellulare* and *Mycobacterium abscessus* are the most common isolates of nontuberculous mycobacteria (NTM), which cause pulmonary diseases. These species express glycopeptidolipid (GPL) antigen on the cell surface. The colony morphology of NTM is diverse, and the rough-typed strain is considered high virulence.

Objectives: *M. intracellulare* Ku11 strain was isolated from a patient in Japan. This strain produced a novel GPL, which showed a different Rf value on thin-layer chromatography (TLC) from those of other standard GPLs. *M. abscessus* smooth- and rough-typed strains were isolated in the same patient. The structure and biosynthesis gene of GPL were clarified, and discuss the relationship between the colony morphology and GPL expression.

Methods: The oligosaccharide of GPL was analyzed by using TLC and mass spectrometry (MALDI/TOF-MS/MS). The gene cluster involved in GPL biosynthesis was isolated and sequenced. We checked the open reading frames (orfs). The function of orfs were identified by the CRISPR-Cas9 technique. We tried to introduce mps1-2 gene into the *M. abscessus* rough-typed strain and checked the existence of GPL.

Results: The sugar moiety of a novel Ku11-GPL was defined as α-Rha-(1→3)-2-O-Me-α-Rha-(1→3)-α-Rha-(1→3)-α-Rha-(1→3)-α-Rha-(1→2)-6-d-α-Tal. We identified the orfs that are functionally responsible for the elongation of oligosaccharide, glycosyltransferase. Moreover, the intact GPLs in nature were acetylated at some positions of oligosaccharide, and recognized via toll-like receptor 2. As for *M. abscessus*, it is considered that smooth-typed strain changed to rough-type during chemotherapy in a patient. We clarified that the change to rough-type from smooth-type was caused by deletion of GPL.

M354 - The Interplay between Intrinsic Invader Factors and Environmental Factors in the invasion of the human gut microbiome

Presenting Author – *Ricardo Leon Sampedro, , Switzerland*

Author/s – *Mathilde Boumasmoud, Katja Pfrunder, Markus Reichlin, Adrian Egli, Alex Hall*

Abstract Content

Background: To address the global crisis of antibiotic resistance, it is critical to understand the spread of antibiotic resistance in complex microbial communities. Despite its importance, we lack direct quantitative observations on how resistance emerges in human-associated communities.

Objective: To identify the factors determining the success of antibiotic-resistant *E. coli* clinical isolates at invading human gut microbiome communities.

Methods: Combining experimental and metagenomic approaches, we tracked the invasion of four clinical *E. coli* strains carrying ESBL and carbapenem- resistance plasmids (IncI-blaCTXM-1, IncF-blaCTXM-14, IncU-blaKPC-2, IncL-blaOXA-48) into anaerobic gut microcosms. We sampled three anonymous, healthy adults to prepare faecal slurry and set-up different microcosms. We then inoculated each microcosm with one of the *E. coli* strains propagating them, in the presence and absence of ampicillin, to measure invasion success. In addition, we carried out supernatant experiments to test the effects of the presence of the community vs the microcosms environment. Finally, we analysed the community composition to identify the interactions between the resident gut microbiome and the invader strain.

Results: Our results revealed that, in the absence of antibiotics, invasion success varied among both invading antibiotic-resistant *E. coli* strains and among microbial communities isolated from different humans. Crucially, we show that one invading strain was successful at invading all microbial communities even without antibiotics. In further experiments, we found invading strains also varied in their intrinsic growth profiles and their direct interactions with other strains. These results provide new information into drivers of invasion success in human-associated microbial communities.

M355 - Rifapentine can be a viable alternative to RIF in the recent treatment of tuberculosis

Presenting Author – *bora shin, National University of Singapore, Korea, Republic of*

Author/s – *Barry Boon Liang Choo1, Pablo Bifani*

Abstract Content

Tuberculosis (TB) is a major global health issue, with millions of new cases diagnosed annually. Rifampin (RIF) is a crucial drug for treating TB and is used in multidrug regimens to prevent drug resistance and improve cure rates. Rifapentine (RPT), a semisynthetic derivative of RIF, has rarely been used. A recent clinical trial showed that RPT-based therapy in patients with drug-susceptible pulmonary TB could reduce treatment from 6 to 4 months. However, limited microbiological data is available on RPT for TB treatment. This study compared the frequency of mutagenesis between RIF and RPT at various concentrations (1, 2, 5, 10 ug/ml) in three TB strains (W4, H37Rv, and CDC1551). 720 RIF and RPT-resistant isolates were also collected and evaluated. Results showed that RPT had similar mutational frequency to RIF, confirming its similar effectiveness against TB. Additionally, the distribution of mutation types in rpoB was found to be strain-specific. These findings provide valuable insights into the continued evaluation of RPT as a potential alternative to RIF in TB treatment and suggest directions for future research.

M357 - Redundant DksA paralogs control virulence and oxidative stress response in *Pseudomonas aeruginosanosa*

Presenting Author – *Alessandra Fortuna, University Roma Tre, Italy*

Author/s – *Diletta Collalto, Veronica Schiaffi, Paolo Visca, Fiorentina Ascenzioni, Giordano Rampioni, Livia Leoni, Valentina Pastore*

Abstract Content

Background: The stringent response regulator DksA plays a major role in the virulence of Gram-negative pathogens. *Pseudomonas aeruginosanosa* is unique as it has two functional DksA paralogs: DksA1 is constitutively expressed and contains a zinc-finger motif; DksA2 is expressed only under zinc starvation conditions and does not contain zinc. In zinc-containing media, the two DksA paralogs are interchangeable in the regulation of hundreds of *P. aeruginosa* genes, including virulence genes and genes involved in tolerance to reactive oxygen species (ROS). However, the role played by DksA1 and DksA2 in *P. aeruginosa* oxidative stress response has not been investigated so far.

Objectives: Studying the role of DksA1 and DksA2 in *P. aeruginosa* hydrogen peroxide (H2O2) tolerance.

Methods: ROS tolerance of the following *P. aeruginosa* strains was compared: wild type, its derivative dksA1 dksA2 double mutant, the double mutant complemented with either dksA1 or dksA2 genes. In particular, H2O2 tolerance of *P. aeruginosa* planktonic and biofilm-growing cultures was determined through CFU counts and biofilm assays. Furthermore, intra-macrophage survival of *P. aeruginosa* strains was investigated, paralleled by catalase activity measurement and catalase-encoding genes expression assessment.

Results: DksA1 is required for *P. aeruginosa* H2O2 tolerance in biofilm and planktonic cultures and survival within macrophages. This activity is at least partially due to the DksA1-dependent positive control of catalase genes expression. Moreover, DksA2 can replace DksA1 functions related to *P. aeruginosa* H2O2-tolerance. Hence, our results strengthen the hypothesis that DksA2 could replace DksA1 under zinc-starvation conditions, a situation common at the infection site.

M358 - Profiling the cell wall proteome of Mexican *Staphylococcus aureus* isolates associated with bovine mastitis

Presenting Author – *Girbe Buist, University of Groningen, University Medical Center Groningen, Netherlands*

Author/s – *Yaremit Mora-Hernández, Jasper Stinenbosch, Elias Vera Murguia, Sandra Maaß, Stefano Grasso, Dörte Becher, Jan Maarten van Dijl*

Abstract Content

Mastitis is a common problem in dairy farms that leads to large economic losses. This infection of the bovine mammary gland is often caused by the Gram-positive bacterial pathogen *Staphylococcus aureus*. Mastitis is usually treated with antibiotics, but a vaccine to prevent this infection would be preferable, because *S. aureus* rapidly acquires antibiotic resistances. However, no anti-staphylococcal vaccine is currently available. This relates to huge genomic plasticity of the different *S. aureus* lineages, and an even higher variability in the presentation of potential antigens. Importantly, the best vaccine targets are exposed on the bacterial cell surface, where they are directly accessible to complement and immunoglobulins. Therefore, the present study was aimed at profiling the cell wall proteome of six different *S. aureus* isolates associated with bovine mastitis. To this end, we performed *in silico* target predictions, and identified cell surface-exposed protein domains by surface shaving with trypsin and subsequent mass spectrometry (MS). In parallel, non-covalently cell wall-bound proteins were extracted with potassium thiocyanide and identified by MS. To mimic conditions in the bovine mammary gland, the bacteria were grown in whey permeate. Altogether, 258 different cell wall-associated proteins were identified, including 42 bovine proteins that form a 'corona' on the bacterial surface. Notably, merely 47 proteins were shared by all six investigated *S. aureus* isolates of which 39 were exposed on the cell surface. Altogether, our observations argue in favor of proteomic profiling to identify the best possible candidate proteins for development of future vaccines that protect against bovine mastitis.

M359 – First report Fluoroquinolones resistance in Group B Streptococci in Serbia

Presenting Author – *Udruzenje mikrobiologa Srbije, University of Belgrade, Serbia*

Author/s – *Dušan Kekić, Miloš Jovićević, Jovana Kabić, Nataša Opavski, Lazar Ranin, Ina Gajić*

Abstract Content

Background: Group B *Streptococcus*, GBS is among the leading causes of invasive neonatal infections and a potential invasive pathogen in pregnant women, the elderly, and immunocompromised individuals. Fluoroquinolones are increasingly being used due to the increase in macrolide resistance in GBS.

Objective: The research aimed to examine the frequency of resistance to fluoroquinolones, the prevalence of GBS capsular serotypes, and the association of capsular types with resistance to the tested antibiotics.

Methods: The study included the collection of GBS isolates from the period from 2015 to 2022. Antibiotic resistance testing was performed using the disk diffusion method and the E test, according to EUCAST standards. Serotyping was performed using the multiplex PCR method.

Results: Overall from 2360 tested GBS isolates, 22 (0.93%) were resistant to fluoroquinolones. The majority of the samples were from women (90.91%), while two were from men (9.09%). All isolates were non-invasive, except for one invasive. The frequency of antibiotic resistance was: moxifloxacin (45.45%), levofloxacin (72.73%), tetracycline (90.91%), erythromycin and clindamycin (63.64%), chloramphenicol (22.73%) and gentamicin high doses (9.09%). The minimum inhibitory concentration values range for moxifloxacin and levofloxacin from 3 mg/L to >32 mg/L and 2 mg/L to >32 mg/L, respectively. Five capsular types were identified, Ib (13.6%), II (18.2%), III (13.6%), IV (4.6%) and V (50.0 %). All isolates were susceptible to penicillin and vancomycin. Serotype V was the most frequent in terms of multi-resistance to macrolides (66.6%), tetracyclines (100%), chloramphenicol (33.0%) and high-dose gentamicin (6.6%).

M360 - Carbapenemases (NDM-1) in outer membrane vesicles from clinical *Acinetobacter baumannii* isolates, a way to bacterial persistence

Presenting Author – *Rodrigo Monteiro, University of Minho, Portugal*

Author/s – *Beatriz Santamarina, Antoni P.A. Hendrickx, Erik Bathoorn, Joana Azeredo, Jan Maarten van Dijl*

Abstract Content

Background: Carbapenem-resistant *Acinetobacter baumannii* is a priority target of WHO to develop new antimicrobial drugs. Outer membrane vesicles (OMVs) are small vesicles released by Gram-negative bacteria that carry virulence factors (VF), protect against natural predators, and help in the colonization of mammalian cells.

Objectives: Our main goal is to disclose how OMVs are impacting virulence and persistence of *A. baumannii* by characterizing OMVs proteome of clinical carbapenem-resistance isolates.

Methods: Three recently isolated *A. baumannii* isolates (WT1/CR1/CR2) genomes were sequenced by NGS and nanopore. OMVs were isolated from the three clinical isolates upon 24-hour culturing through ultracentrifugation of growth medium fractions. For carbapenemase activity detection the carbapenem inhibition method (CIM) and CarbaNP test were used. To assess protection conferred by OMVs, growth curves were performed by mixing a susceptible strain (ATCC 17978) and a pre-incubated solution of OMVs with imipenem. OMVs proteome was analyzed by liquid chromatography coupled with mass-spectrometry.

Results: Clinical *A. baumannii* isolates CR1/CR2 were found to produce carbapenemases, encoded by a 260-kb plasmid with mobility potential. OMVs proteome analysis showed a high diversity of proteins on CR1/CR2 isolates when compared with WT1, which might be correlated with the plasmid presence. Among the different VF detected, two carbapenemases (OXA-97/NDM-1) were present in CR1/CR2 OMVs. Purified CR1/CR2 OMVs were positive by CIM and conferred susceptible bacteria protection against imipenem. Our study reinforces that *A. baumannii* OMVs are extracellular reservoirs of VF with active carbapenemases. Therefore, we propose that OMVs depositing carbapenemases in the extracellular milieu support bacterial resistance to carbapenemases.

M361 - Mutations increasing the catalytic activity of TEM-1 increase the hetero-resistance of *E. coli*. at single cell level

Presenting Author – *Shakeel Ahmad, Institute Of Physical Chemistry PAS, Poland*

Author/s – *Shahab Shahryari, Paweł Jankowski, Adam Samborski, Ilona Foik, Shreyas Vasantham, Piotr Garstecki*

Abstract Content

Bacterial cells may present different phenotypes even within monoclonal populations and some confer them selective advantages during stress. On antibiotic exposure, if individual cells show differential minimum inhibitory concentration (MIC), they are called hetero-resistant. Droplet microfluidics offers a promising method to study these single cell responses, as it allows separation of individual cells via stochastic confinement.

Hetero-resistant is believed to play a major role in selection, evolution and resistance in bacteria. One strategy of resistance used by bacteria is production of β-lactamase enzymes that degrade the β-lactam antibiotics. Frequent mutations and quick evolution of β-lactamases is making new drugs obsolete rapidly.

We studied hetero-resistance in *Escherichia coli* carrying different clinically relevant variants of TEM-1 β-lactamase having different catalytic activity for cefotaxime hydrolysis. Three variants were chosen-wild type and two mutants generated by one amino-acid substitutions. Individual cells were encapsulated inside a “media in oil” nanolitre droplets with appropriate antibiotic concentrations and incubated. Imaging enumerated the negative/positive droplets based on signal due to proliferation. Further, the single cell-MIC (scMIC) was calculated and its probability distribution defines heterogeneity.

Results showed that mutations increasing catalytic activity of TEM-1 against cefotaxime significantly increase the scMIC and hetero-resistance. This altogether may implicate that hetero-resistance as well as scMIC of a strain, against a particular antibiotic, might increase due to specific small mutations in the wild. The data from the study can be very crucial for better understanding of the resistance mechanism, thereby helping in its efficient management and new drug development.

M362 - The prevalence of fungal pathogens in alternative sources of contamination in carrot fields in Lithuania

Presenting Author – *Simona Chrapačienė, Lithuanian Research Centre for Agriculture and Forestry, Institute of Horticulture, Lithuania*

Author/s – *Neringa Rasiukevičiūtė*

Abstract Content

Background: Cultivating high-quality carrots becomes challenging because many economically important pathogenic fungi are actively spreading during the vegetation of vegetables. In this case, seed and planting material is the most important input for the success of the development of horticulture. However, even when using the finest plant material, an infestation of carrot crops may appear due to external sources of infection, such as soil or weeds. Soil and weeds can host pathogens that later infect carrots under favorable conditions. Therefore, the knowledge of the prevalence of fungi infecting carrots would help to manage fungal disease risks and achieve more effective plant protection.

Objectives and Methods: This study aimed to investigate the prevalence of fungal pathogens causing carrot diseases in soil and weeds. To accomplish the research objective, seven farms in Lithuania were selected based on achieved annual carrot yield for collecting soil and weeds samples. Soil and weeds were chosen as alternative sources of crop contamination in this study as they can act as additional hosts for pathogens. Targeted metagenomic sequencing was performed for soil samples. From weed samples, microscopic fungi were isolated and described according to cultural and morphological characteristics typical to the colonies.

Results: Fungi of the Ascomycota phylum were dominant in all soil samples. 69% of all detected fungi were identified as ascomycetes. When looking deeper, *Fusarium*, *Cladosporium*, *Penicillium*, *Alternaria* and other genera were found most often. A similar tendency was observed when examining the prevalence of fungal pathogens causing carrot diseases in weeds.

M363 - Serological monitoring of *Mycoplasma gallisepticum* used enzyme-linked immunosorbent assay from chicken farm in South Korea

Presenting Author – *Sung Il Kang, Animal And Plant Quarantine Agency, Republic of Korea*

Author/s – *Sung-Il Kang, O-Mi Lee, So-Hee Lee, Chung-Hyun Kim, Myeong Ju Chae, Ji-Yeon Jeong, Yong-Kuk Kwon, Min-Su Kang*

Abstract Content

Mycoplasma gallisepticum (MG) causes chronic respiratory disease in chickens. Especially, this disease results in severe economic losses due to reduced feed efficiency and drop in egg production in broiler breeders and layers. Therefore, live vaccines (ts-11, 6/85, F strain) have commonly been used to prevent the disease in the chicken flocks. In the present study, we conducted serological monitoring of the chicken flocks vaccinated with the live vaccines.

A total of 2,298 blood sample were collected from chickens rearing in 11 flocks. The serological test for detection antibodies against MG was performed using commercially available ELISA kit (IDEXX, USA).

Antigen detection out of 11 flocks was confirmed in 4(36.4%) vaccinated flocks and 1(9.1%) non-vaccinated, respectively. In the vaccinated flocks, the mean antibody titer of the antigen-negative flocks was 915±734 at 16-35 weeks of age. Titers of some ts-11 vaccinated flocks ranged from 1,100 to 2,800 at 20-30 weeks of age and were antibody negative thereafter.

The flocks vaccinated with 6/85 and F-strains showed the level of below 400 until 56 weeks of age. Whereas, the mean antibody titers of antigen positive flock among vaccinate flocks were 386±543 (1-15 weeks of age), 1,404±886 (16-35 weeks of age), 1,393±821 (36-55 weeks of age), and 6,545±5,298 (over 56 weeks of age). When the antigen was detected, the antibody titer was maintained above 1500 until 56 weeks of age.

Consequently, the routine serological monitoring provides a due to the presence of MG field infection even in vaccinated chicken flocks.

M364 - Reducing public health risks via understanding of high-risk poultry enterotypes and resistomes

Presenting Author – *Melanie Hay, Royal Veterinary College, United Kingdom*

Author/s – *Melanie Hay, Ankit Hinsu, Prakash Koringa, Madhvi Joshi, Fiona Tomley, Damer Blake, Ramesh Pandit, Haidaruliman Paleja, Chaitanya Joshi, Dong Xia*

Abstract Content

Background: The poultry industry in Asia is at high risk for the emergence of antimicrobial resistance (AMR) because of the widespread use of antibiotics. Bacteria acquire and spread AMR via horizontal transfer of antimicrobial resistance genes (ARGs) on mobile genetic elements. Thus, zoonotic pathogens such as *Campylobacter*, *Salmonella* and *Escherichia coli* from poultry could become enriched in ARGs, resulting in increased antibiotic-resistant infections in humans.

Objectives: Identifying poultry farming practices that decrease the abundance of zoonotic pathogens and ARGs is valuable for public health. Enterotypes represent distinct microbial community phenotypes and resistomes represent population AMR phenotypes. Variation in both can have significant effects on chicken health and public health risk. Our first objective is to determine whether enterotypes and resistomes can be stratified in terms of public health risk. Our second objective is to identify farm, market and environmental factors that contribute to high-risk phenotypes.

Methods: We use dimensionality reduction and clustering to identify enterotypes (from 16S rRNA data) and resistomes (from AMR AmpliSeq data) in chickens from farms and markets in India. Thereafter, we use Random Forest Models to identify (various combinations of) factors that associate with specific enterotypes and resistomes on farms.

Results: New results will be presented. A pilot study of this approach showed that enterotypes could differ in *Campylobacter* abundance. Furthermore, farm characteristics could predict enterotype, suggesting that enterotypes are influenced by farming practices and that modification of farming practices could potentially be used to reduce *Campylobacter* burden.

M365 - Cooperative use of copper in fungal - bacterial biofilms

Presenting Author – *Seána Duggan, MRC Centre for Medical Mycology, United Kingdom*

Abstract Content

Co-infections caused by the fungus *Candida albicans* and the bacterium *Staphylococcus aureus* result in prolonged treatment and poorer patient outcome due to a phenomenon termed “lethal synergy” where the fungi and bacteria interact to enhance virulence resulting in worse disease. This phenomenon is echoed in dual species biofilms of both organisms, which result in greater biomass and anti-microbial resistance compared to single species biofilms. To understand processes involved in this synergy, a proteomics approach was taken, where single and dual species biofilms were subject to total protein extraction and Tandem Mass Tagging coupled to Mass Spectroscopy. Within the dual species biofilm *C. albicans* differentially regulates 115 proteins and *S. aureus* differentially regulates 146 proteins. Amongst these are proteins relating to metabolism, stress response and protein turnover. Interestingly, while *C. albicans* up-regulates a copper binding transcriptional regulator Mac1, *S. aureus* down regulates proteins for copper resistance, chaperone activity and transport (CsoR, Crt1, CopZ). These data indicate *C. albicans* and *S. aureus* engage in copper interplay during dual species biofilm growth. Dual species biofilm biomass, metabolic activity and structure were altered to a greater extent than their single species counterpart when cultured in low and high copper media, implicating copper as a definitive resource for *C. albicans* and *S. aureus* dual species biofilms. Future work will employ mutants of copper sensing and transport to delineate the role of copper in *C. albicans* and *S. aureus* biofilm synergy. This knowledge has the potential to unveil avenues to rationally interfere with synergism in co-infections.

M366 – Antimicrobial resistance is associated with increased Biofilm formaion and altered motility in *Acinetobacter baumannii*

Presenting Author – *Sérgio Mendes, University of Coimbra, Portugal*

Author/s – *Sofia Combo, Thibault Allain, Sara Domingues, Andre G. Buret, Gabriela Da Silva*

Abstract Content

Background: *Acinetobacter baumannii* is a non-motile opportunistic pathogen associated with mechanical ventilation pneumonia. Multidrug resistance (MDR) and biofilm formation emergence in *A. baumannii* represent a major clinical challenge. Biofilm formation can lead to motile biofilm-dispersed microbes release, which may further infect other mucosal surfaces and/or enter the circulation. In this context, we investigated co-regulatory mechanisms of antibiotic resistance, biofilm formation and increased motility as a means of increased bacterial virulence.

Objectives: The objective of this study was to evaluate whether antimicrobial resistance acquisition impacts *A. baumannii* virulence.

Methods: We used 1) ciprofloxacin susceptible *A. baumannii* ATCC 19606, 2) isogenic-resistant mutant (CipR) of *A. baumannii* ATCC 19606 obtained by ciprofloxacin exposure (0,01 to 30 mg/L) in serial passages in LB broth, 3) two MDR clinical isolates. Microdilution method was used to determine MIC. Biofilms were produced in the Calgary Biofilm Device (24 hours incubation). Biofilms biomass was evaluated by Crystal Violet staining and CFU/mL counting. Motility assays were performed in 0.3% agar media. CipR genomes were analyzed by Whole-Genome Sequencing (WGS).

Results: CipR (MIC of 512 mg/L) produced more biofilms than *A. baumannii* ATCC 19606. Clinical isolates demonstrated lower biofilm formation compared to CipR. CipR biofilm phenotype showed characteristic air-liquid interface and immersed mode of growth. CipR and MDR strains showed ditching/branching motility phenotypes. Whole-genome single-nucleotide-polymorphism analysis showed differences between CipR and *A. baumannii* ATCC 19606. Ciprofloxacin resistance is associated with altered biofilm phenotype and increased virulence.

M367 - Effect of the human amniotic membrane homogenate on biofilm formation of selected uropathogenic *Escherichia coli* strains

Presenting Author – *Marija Najdovska, University of Ljubljana, Slovenia*

Author/s – *Aleksandar Janev, Marjanca Starčič Erjavec, Mateja Erdani Kreft*

Abstract Content

The emergence of multiple drug-resistant uropathogenic *Escherichia coli* (UPEC) strains remains a major public health concern. A large proportion of these microbes are capable of biofilm formation, which poses an additional problem for their effective treatment. Alternative antimicrobial agents are needed(1).

The aim of this study was to test the effect of a new potential antimicrobial agent - the homogenate of human amniotic membrane (hAM) on biofilm formation of selected UPEC strains.

Five different UPEC strains were employed. hAM homogenate was prepared in phosphate-buffered saline (PBS)(1). Overnight cultures grown in Luria broth (LB) were diluted 1:100 in fresh LB, PBS or hAM homogenate, inoculated into a 96-well microtiter plate and incubated at 37°C for 24 h to test the ability to form biofilms(2).

Mean OD580 values reflecting the biofilm growth of the employed UPEC strains are shown in Figure 1. For 4 out of 5 UPEC strains tested (DL9, DL13, DL14 and DL88), it was found that the highest capacity for biofilm formation was in LB, an intermediate capacity in PBS, and the lowest capacity for biofilm formation was in the hAM homogenate. One UPEC strain (DL8) was also found to have the highest capacity for biofilm formation in LB, but an almost identical capacity for biofilm formation in PBS and hAM homogenate. The hAM homogenate was found to negatively affect the ability to form biofilms in the UPEC strains used, and therefore has potential as an alternative treatment to prevent the formation of biofilms by UPEC.

M368 - The role of cosmetic products in the fight against dermatophytes

Presenting Author – *Cemre Özkanca, Istanbul University, Turkey*

Author/s – *Cemre Özkanca, Sibel Döşler*

Abstract Content

Backgrounds: The skin, which is the physical barrier of the immune system with its low pH and the presence of sweat glands, creates a suitable environment for colonization of microorganisms. Skin infections caused by fungi, which are eukaryotic microorganisms, are called dermatophytes. It is known that fungi can develop resistance due to environmental stress such as biofilm formation, target mismatch, drug transport systems and cell permeability. Cosmetic products which are applied externally to the skin, also likely to create environmental stress. There are many types of cosmetic products on the market, for moisturizing, protection and care-giving purposes.

Objectives: In this study, we determined the antifungal and anti-biofilm effectiveness of some raw materials that are frequently encountered in formulation of cosmetics against clinical dermatophyte isolates.

Methods: The biofilm forming capacities of the isolates were determined by crystal violet staining method. The minimum inhibitory concentrations (MIC) and minimum biofilm eradication concentrations (MBEC) of raw materials were determined by microbroth dilution technique.

Results: While three *Microsporum canis* and two *Trichophyton rubrum* isolates were strong in regards to forming biofilm, *Trichophyton tonsurans* was weak. Among the cosmetic raw materials glycerin, UV filter octocrylene, tea tree oil, vitamin A, panthenol, Centella asiatica extract, UV filter benzophenone-4, and UV filter diethylamino hydroxybenzoyl hexyl benzoate have antifungal activities between 0.002-0.25 µg/ml concentrations. Also these materials inhibit the mature biofilms up to 3-log10 cfu/ml. According to these results, cosmetic raw materials used in daily life have the antifungal and antibiofilm potential for treatment of dermatophytes.

M369 - *In silico* identification of bacteriocin gene clusters in the rumen based on the Hungate1000 culture collection.

Presenting Author – *David Hourigan, APC Microbiome Ireland, Ireland*

Abstract Content

The Hungate1000 collection is a catalogue of rumen isolates. Bacteriocins are secondary metabolites with narrow and broad range antibacterial activity. They have gathered much interest as a natural alternative to antibiotics due to their reduced collateral damage to the microbiome. Bacteriocins within the microbiome have roles in niche clearing, colonisation resistance and spatial segregation. Bacteria do not live in monotypic isolation and are considered a social life form within polymicrobial communities. Bacteriocins act as signaling molecules between gram-positive species within these communities. Genome mining gives insights into the mechanisms of production and ecology of bacteriocin production. This study aimed to identify putative novel bacteriocin biosynthetic gene clusters (BGCs) from the rumen microbiome by screening whole genome sequences (WGS) of the Hungate 1000 culture collection. To gain deeper insight into the mechanisms of production of bacteriocin gene clusters using synteny and homology to existing BGCs, and characterize their abundance and diversity. BGCs were predicted using Antismash and genes were functionally annotated using InterPro. The distribution of BGCs was overlayed on a phylogenetic tree constructed using PhyloPhlan. Propeptides were predicted using homology to existing peptides and key motifs within short open reading frames (sORF). Regions of synteny were analysed using progressiveMauve and gggenomes. A total of 1072 BGCs were predicted across 349 genomes (85.1%); of these, 525 are putative RiPP/bacteriocin BGCs. The most abundant BGC determined were putative ranthipeptide BGCs, mainly among Clostridia. Lanthipeptides were predicted across multiple genera, with class II lanthipeptides the most diverse and large subset predicted.

M370 - RNA-Seq based transcriptomic profiling to understand off-target effects of antisense antibiotics

Presenting Author – *Jakob Jung, University of Wurzburg, Germany*

Author/s – *Linda Popella, Phuong Thao Do, Patrick Pfau, Jörg Vogel, Lars Barquist*

Abstract Content

Conventional antibiotics generally work against a broad spectrum of bacterial pathogens. This promotes the development of antibiotic resistance and damages our protective microbiota, which can have unwanted effects on our health. New antibiotics are therefore needed to directly target individual pathogens, leaving beneficial bacteria unharmed.

Antisense oligomers (ASOs) such as peptide nucleic acids (PNAs), designed to inhibit the translation of essential bacterial genes, have emerged as attractive sequence- and species-specific programmable RNA antibiotics. Yet, potential drawbacks include unwanted side effects caused by their binding to transcripts other than the intended target.

To facilitate the design of PNAs with minimal off-target effects, we developed MASON (Make AntiSense Oligomers Now, https://mason.helmholtz-hiri.de/), a webserver for the design of PNAs that target bacterial mRNAs. MASON generates PNA sequences complementary to the translational start site of a bacterial gene of interest and reports critical sequence attributes and potential off-target sites.

We based MASON's off-target predictions on experiments in which we treated *Salmonella enterica* serovar Typhimurium with a series of 10mer PNAs derived from a PNA targeting the essential gene acpP but carrying two serial mismatches. Growth inhibition and RNA-sequencing (RNA-seq) data revealed that PNAs with terminal mismatches are still able to target acpP, suggesting wider off-target effects than anticipated. Comparison of these results to an RNA-seq dataset from uropathogenic *Escherichia coli* (UPEC) treated with eleven different PNAs confirmed our findings are not unique to *Salmonella*.

We believe that MASON's off-target assessment will improve the design of specific PNAs and other ASOs.

M371 - A microfluidic based platform for studying clinically relevant bacterial biofilms

Presenting Author – *Mahbuba Akter Lubna, Technical University of Denmark, Denmark*

Author/s – *Laura Serioli, Hau Van Nguyen, Mikkel Anbo, Lars Jelsbak, Kinga Zór, Anja Boisen*

Abstract Content

Biofilm-related persistent infections with epidemic 'high-risk clones' (HiRiCs) of *Pseudomonas aeruginosanosa* have raised serious concerns due to their extreme multidrug resistance capability. Previous studies showed that enhanced biofilm forming potential due to specific O-antigen structure might be a contributing factor for their global clonal success. The major challenges with the study of biofilm often involve huge setup, high reagent volume, and commonly provide a single data point. Microfluidic platforms are promising tools and have been shown to facilitate experiments that better reflect the natural growth environment of bacterial biofilms.

Here we present the application of a centrifugal microfluidic system, Bacterial Culture on Disc (BCoD), earlier shown to enable bacterial biofilm growth and monitoring using confocal microscope. This device contains three cell culture chambers enabling simultaneous biological replicates and reduces the overall experimental variation, time, and cost. We use BCoD to monitor the growth of two genetically engineered HiRiCs of *P. aeruginosa* ST111 each with alterations to its O-antigen (ΔOSA and O12) to understand overall cell growth and biofilm formation.

Bacteria expressing O-antigen O12 (ST111-O12) was found to have better biofilm formation abilities compared to the O-antigen deleted strain, ST111-ΔOSA. Moreover, ST111-ΔOSA has less viable cells than ST111-O12 after 24hrs of growth. Overall, these results point towards an important role of the specific chemistry of the lipopolysaccharide O-antigen in relation to clinically relevant phenotypes. As a next step, we are carrying out studies to evaluate the effect of various antibiotics on the studied clones employing the BCoD device.

M372 - Rational design of a live attenuated vaccine for *Brucella* melitensis using Tn-seq

Presenting Author – *Emeline Barbieux, University of Namur, Belgium*

Author/s – *Emeline Barbieux, Georges Potemberg, Audrey Fraikin, Xavier De Bolle, Eric Muraille*

Abstract Content

Because of the importance for public health and damages induced by *Brucella* (B.) infection in livestock farming, scientists try to control and eradicate the disease in animals. However, existing vaccines do not meet all the safety criteria. They are still virulent for humans, interfere with diagnostic tests and importantly, they can induce abortions in animals. Developing safe vaccines would help to reduce the impact of brucellosis. First generation of live attenuated vaccines (LAVs) relied on empirical and unpredictable attenuation. Recent advances in genetics make it possible to envisage the rational construction of LAVs. Our objective is to achieve by Transposon Sequencing (Tn-seq) approach a functional map of the B. melitensis genome in order to construct a candidate LAV capable of persisting long enough to induce protective immunity but incapable of persisting in tissues or invading the placenta in pregnant animals. For this purpose, Tn-seq analyses were performed under different selection conditions in the mouse model. This allow us to identify genes required for optimal persistence of B. melitensis in the lung and in the spleen. Clustering analysis showed that essential genes for the persistence of *Brucella* vary according to the analyzed tissues. Many of these genes are involved in bacterial metabolism, suggesting that *Brucella* faces different nutritional requirements depending on the nature of colonized tissues. Our Tn-seq predictions were validated by constructing deletion mutants and testing them in mice. On this basis, several candidate vaccines have been selected and are in the validation phase in mice.

M373 - The microbiome of healthy and acne-affected human skin and interferences of staphylococci and *Cutibacterium acnes*

Presenting Author – *Holger Brüggemann, Department of Biological and Chemical Engineering, Denmark*

Author/s – *Cecilie Feidenhansl, Michael Lund, Charlotte M. Ahle, Hans B. Lomholt*

Abstract Content

Background: Human skin is populated by trillions of microbes collectively called the skin microbiome. *Cutibacterium acnes* and coagulase-negative staphylococci are among the most abundant members of this ecosystem, with described roles in skin health and disease. However, knowledge regarding health-beneficial and disease-associated effects of these ubiquitous skin residents is still limited.

Objectives: Aims of this study were the characterization of the skin microbiome and its dysbiosis in acne vulgaris patients as well as the interrogation of the skin microbiome for health-beneficial functionality.

Methods: Culture-dependent and -independent methods were used. *C. acnes* and staphylococcal landscapes across two different skin sites of 36 healthy individuals and 36 acne patients were profiled with amplicon-based next-generation sequencing, and microbial interferences were assessed by antagonistic plate assays.

Results: Healthy skin sites were colonized with multiple staphylococcal species, dominated by S. epidermidis, followed by *S. capitis* and *S. saccharolyticus*. Distinct *C. acnes* phylotypes were identified, spanning the 10 known single-locus sequence typing (SLST) classes. Acne-affected skin was characterized by a loss of *C. acnes* phylotype diversity, an overrepresentation of A-type *C. acnes* as well as a shift of the staphylococcal population.

Relative abundance profiles indicated the existence of phylotype-specific co-existence and exclusion scenarios. Staphylococcal strains were identified on healthy skin that exhibited anti-*C. acnes* activities. Overall, these findings highlight the importance of skin-resident staphylococci and suggest that selective microbial interference is a contributor to healthy skin homeostasis.

M375 - Spread of multidrug resistant *Escherichia coli* and *Klebsiella pneumoniae* in long-term healthcare units' environment

Presenting Author – *Catarina Santos-Marques, Faculty of Pharmacy, Portugal*

Author/s – *Camila Teixeira, Catarina Santos-Marques, Josman Dantas Palmeira, Wolfram Manuel Brück, Douglas Teodoro, Sónia Gonçalves Pereira*

Abstract Content

Multidrug-resistant (MDR) bacteria are a major challenge for public-health. Carbapenem-Resistant and extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae*, are of critical priority1. Its prevalence in long-term healthcare-units (LTHU) is fairly unknown.

Clinical (hands/mouth/anal) and environmental (WC/bed rail/bedside table/handbell) samples were collected and culture-based screened for lactose-fermenting Gram-negatives. Antimicrobial susceptibility-testing (EUCAST2) was performed for 6 antimicrobial-classes. 16S rDNA/MALDI-TOF identification and ESBL/carbapenemase genes PCR-screening are currently ongoing.

From the isolates already identified, 23 are *E. coli* (6 environmental, 17 clinical) and 10 *K. pneumoniae* (1 environmental, 9 clinical). Regarding environmental-isolates, 3 *E. coli* presented MDR-phenotype, all of them harbouring bla CTX-M group 1. Resistance rates were: amoxicillin+clavulanic acid(n=6), amikacin(n=5), cefotaxime(n=3), ceftazidime(n=3). Regarding the environmental *K. pneumoniae* isolates they were resistant to amoxicillin/clavulanic acid and amikacin and didn't present the tested resistance-genes. Regarding clinical-isolates, 10 *E. coli* and 9 *K. pneumoniae* had an MDR profile. With 5 *E. coli* and 2 *K. pneumoniae* harbouring blaCTX-M G1, and KPC detected in 1 *E. coli* and 4 *K. pneumoniae*. Phenotypic-resistance of clinical *E. coli* was: amoxicillin+clavulanic acid(n=16), cefotaxime(n=9), cefoxitin(n=9), amikacin(n=9), ceftazidime(n=8), ciprofloxacin(n=8), meropenem(n=6), imipenem(n=1). For clinical *K. pneumoniae*: cefotaxime(n=9), ceftazidime(n=9), meropenem(n=9), amoxicillin+clavulanic acid(n=8), amikacin(n=6), cefoxitin(n=5), ciprofloxacin(n=5), imipenem(n=4) and aztreonam(n=4).

These preliminary-results demonstrate the spread of ESBL- and KPC-harbouring MDR-*E. coli* and -*K. pneumoniae* in LTHU-environment. This poses a menace to patients, healthcare-workers and visitors, and highlights the mandatory need of proper infection-prevention and control-programs in these settings. Results will be used to construct an artificial intelligence-based infection risk predictor, using deep-graph neural-networks approach.

M376 - Antibiotic resilience in the *Mycobacterium tuberculosis* complex

Presenting Author – *Annemarie Hintz-Rüter, Research Center Borstel, Germany*

Author/s – *Lindsay Sonnenkalb, Stefan Niemann*

Abstract Content

Drug resistant tuberculosis continues to challenge the existing treatment regimens. Recent studies indicate that resistance acquisition of *Mycobacterium tuberculosis* complex (MTBC) bacteria, is fueled by its capability to develop tolerance and persistence, i.e. antibiotic resilience. Resilient bacteria can survive antibiotic treatment that kills the susceptible population without gaining genomic variants or an increase in minimum inhibitory concentration.

Bacteria of the MTBC have a 99% conserved genome, yet even few single nucleotide polymorphisms (SNPs) can have major implications on their phenotype. Therefore, MTBC is divided into different lineages and sublineages. In this study, we investigated resilience mechanisms of strains belonging to lineages 2 and 4, as these lineages are responsible for most of the drug resistance observed world-wide.

Our aim is to determine whether resilience contributes to the association of lineage 2 with higher drug resistance rates, by increase the time bacteria survive in the presence of antibiotics.

We determined by growth curves that all used strains grow in a similar rate when not stressed. Then we investigated by Time-Kill Assays how these strains respond to stress by exposure to varying concentration of Rifampicin, a first line antibiotic against Mtb. Future prospects include next generation sequencing to understand the genomic and transcriptomic differences between susceptible, tolerant, and persistent cells.

This study will advance our understanding of the success and adaptability between the different MTBC lineages and give insights to help circumvent resistance evolution and prevent resilience.

M377 - Target identification of cell penetrating *Shigella* effector protein IpaH7.8

Presenting Author – *Theresa Meyer, University of Münster, Germany*

Author/s – *Theresa Meyer, Ivan Ngueya Yango, Yannick Teschke, Christian Rüter*

Abstract Content

Modulation of host cell processes by bacterial effector proteins is often referred to as a key virulence factor upon infection. By injecting these effector proteins directly into the cytoplasm of the host cell via the type 3 secretion (T3SS) Gram-negative bacteria can alter induced immue responses to their benefit.

Interestingly, previous reports could identify some, usually T3SS delivered proteins, to have the ability to translocate autonomously into eukaryotic cells such as the *Yersinia* outer protein M (YopM), being able to inhibit caspase 1 and thus promote *Yersinia* infection. Moreover, sequence analysis revealed a species-spanning family of effector proteins bearing strong homologies in protein structure and function including candidates from *Yersinia*, *Salmonella* and *Shigella*.

Another LPX-effector-family member is the *Shigella* effector protein IpaH7.8. By recombinantly expressing and purifying the protein we could show a T3SS-independent, endosomal uptake of IpaH7.8, mediated by two highly-conserved N-terminal α-helices. The latter could also be shown to facilitate an endosomal escape and thus, access to the cytoplasm. To eventually modulate the host's immune response, the well-conserved C-terminal domain of IpaH7.8, comprising an E3 ubiquitin ligase activity, allows hijacking the ubiquitination system of the host. To asses affected target proteins in human macrophages, we conducted a comparative signalotome and ubiquitome analysis upon treatment of THP-1 derived macrophages, revealing potential interaction partners of IpaH7.8. With regard to the anti-inflammatory properties of other LPX effector proteins, the impact of the ubiquitination of target proteins is currently investigated and may allow a therapeutic application of IpaH7.8 to combat inflammatory diseases.

M379 - Random peptide mixtures as safe and effective antimicrobials in mouse models of bacteremia and pneumonia

Presenting Author – *Einav Malach, Faculty of Dental Medicine, Hebrew University of Jerusalem, Israel*

Author/s – *Yael Belo, Hannah E. Caraway, Jonathan Z. Lau, Gee W. Lau, Zvi Hayouka*

Abstract Content

Antibiotic resistance is a daunting challenge in modern medicine, and novel approaches that minimize the emergence of resistant pathogens are urgently needed. Antimicrobial peptides are newer therapeutics that attempt to do this; however, they fall short because of low-moderate antimicrobial activity, low protease stability, susceptibility to resistance development and high cost of production. The recently developed random peptide mixtures (RPMs) are promising alternatives. RPMs are synthesized by incorporating a defined proportion of two amino acids at each coupling step rather than just one, making them highly variable but still defined in their overall composition, chain length and stereochemistry. Because of RPMs' extreme diversity, it is unlikely that bacteria would be capable of rapidly evolving resistance. However, their efficacy against pathogens in animal models of human infectious diseases remained uncharacterized. Recently, we have demonstrated that RPMs have strong safety and pharmacokinetic profiles. RPMs rapidly killed both *Pseudomonas aeruginosanosa* and *Staphylococcus aureus* efficiently, and disrupted preformed biofilms by both pathogens. Importantly, RPMs were efficacious against both pathogens in mouse models of bacteremia and acute pneumonia. Our results demonstrate RPMs are potent broad-spectrum therapeutics against antibiotic-resistant pathogens.

M381 - Analysis of the type II toxin-antitoxin system in the bacterial physiology of an *E. coli* hybrid strain

Presenting Author – *Jessika Cristina Silva, Butantan Institute, Brazil*

Author/s – *Jessika Cristina Silva, Lazáro Marques-Neto, Luciana Leite, Eneas Carvalho, Danielle Munhoz, Thais MITSUNARI, Roxane Maria Piazza*

Abstract Content

Background: The toxin-antitoxin (TA) systems are genetic modules associated with some bacterial processes, such as cell formation persistence, biofilm formation, and responses to stressful environments. In these systems, the toxin is related to the inhibition of physiological processes and the antitoxin protects the cell against the toxin. The hybrid strain BA1250 (atypical enteropathogenic *E. coli* and extraintestinal *E. coli* strain) can colonize multiple host niches, therefore facing different stress environments.

Objectives: Analyses the role of genes the TA type II, CcdB-CcdA, YhaV-PrlF, MazF-MazE, YoeB-YefM, and PasT-PasI systems in the hybrid strain BA1250 under different stress conditions by transcription analysis.

Methods: Genome analysis of the BA1250 strain was performed to predict type II TA genes. Transcription analysis of TA genes type II under stress conditions such as nutritional, oxidative, osmotic, and acid were evaluated both in the log and stationary phases of the BA1250 strain.

Results: Genome analysis revealed the presence of 21 TA loci in the genome of the strain BA1250 and five TA type II systems present in the chromosome were analyzed. We observed significant differences in the expression levels of the CcdB-CcdA, YhaV-PrlF, YoeB-YefM, and PasT-PasI systems in the presence of nutritional stress in the stationary phase. In acid stress, an increase in the gene expression of YhaV, YefM, and PasT toxins in the stationary phase was observed. These data indicate that these four TA systems seem to be involved in the stress response of the BA1250 strain.

M382 - Role of hydrogen sulfide in *Pseudomonas aeruginosanosa* physiology and virulence

Presenting Author – *Lorenzo Caruso, University Roma Tre, Italy*

Author/s – *Marta Mellini, Ortensia Catalano, Martina Nastasi, Elena Forte, Francesca Giordano, Alessandro Giuffrè, Livia Leoni, Giordano Rampioni*

Abstract Content

Background: Recent studies revealed that endogenous production of hydrogen sulfide (H2S) has a protective effect against antibiotics in several bacteria, including the multidrug resistant pathogen *Pseudomonas aeruginosanosa* [1]. Hence, enzymes involved in *P. aeruginosa* H2S synthesis could be considered promising targets for the development of new antibiotic adjuvants [2].

Objectives: Investigating the role of H2S in i) antibiotic resistance in the model strain PAO1 and in *P. aeruginosa* cystic fibrosis (CF) isolates; ii) the mechanism by which *P. aeruginosa* resists to endogenously produced H2S.

Methods: A set of *P. aeruginosa* PAO1 mutant strains with single and multiple deletions in genes possibly involved in H2S synthesis/oxidation was generated. An optimized protocol based on paper strips soaked with lead acetate allowed determining H2S levels in our set of mutants and clinical isolates. MIC assays performed on wild type, selected isogenic mutants showing the highest and lowest H2S-production, and CF isolates, allowed investigating H2S involvement in antibiotic resistance. O2 consumption assays were then used to study H2S sensitivity of *P. aeruginosa* terminal oxidases.

Results: In this work, we i) clarified the role played by distinct *P. aeruginosa* H2S-synthesizing and -consuming enzymes on H2S homeostasis, ii) showed that production of H2S in *P. aeruginosa* is not involved in antibiotic resistance, iii) demonstrated a protective role of the terminal oxidase CIO in growth conditions that exacerbate H2S production. Additional experiments aimed at characterizing the impact of H2S on the transcriptome and the expression of virulence traits in *P. aeruginosa* PAO1 are currently in course.

M383 - Experimental and bio-informatic determination of mutation rates in highly multi-drug resistant *M. tuberculosis* lineages

Presenting Author – *Emilie Rousseau, Research Center Borstel, Germany*

Author/s – *Thierry Wirth, Matthias Merker, Stefan Niemann*

Abstract Content

Mycobacterium tuberculosis (MTB) has been evolving with humans for millennia and has a complex genetic structure composed of several lineages. Certain strains of MTB belonging to one specific lineage, lineage 2 (L2), have become a major threat since they are present worldwide and are associated with specific multi-drug resistance (MDR) status (1), lower probability of therapeutic success, and higher potential for dissemination. Interestingly, L2 phylogenetic structure correlates with MDR phenotypes, as most recently emerged clones ("modern") display more drug resistances than ancestral ones, to a point where the two most recent clones are practically entirely MDR, leading to severe human and financial costs (2).

However, underlying mechanisms for L2 epidemically successful evolution toward MDR remain unclear. Diverse preliminary results tend to indicate a role of metabolic pathways into the evolution of L2 toward epidemic success (3-6).

This study uses a combined approach of experimental and bio-informatic estimation of mutation rates. The first part consists in *in vitro* estimations of differential mutation rates of isolates provided by the German National Reference Center for MTB, between lineage 4 (L4), the other most prevalent MTB lineage, and L2 ancestral and modern strains. The second part consists in bio-informatic estimation of several lineages mutation rates from on-field sequences, during epidemic phase.

Our results indicate that L2 modern strains spontaneously acquire rifampicin resistance twice faster than ancestral and L4 strains. Phylogenetic analysis also show that L2 modern clone W148 present a moderately but significantly higher mutation rate than L4 Haarlem strain during epidemic phase.

M384 - Uropathogenic *Escherichia coli* strains impact the lifespan of the *Caenorhabditis elegans* PX627 mutant

Presenting Author – *Gilles Mayot, Cy Cergy Paris University, France*

Author/s – *Hela Harbeouia, Patrick Di Martino, Gilles Mayot*

Abstract Content

The nematode *Caenorhabditis elegans* (*C. elegans*) is used in many areas of biological research, and in particular to study microbial pathogenesis. Uropathogenic *Escherichia coli* (UPEC) strains exhibit several virulence factors associated with urinary tract infections in humans and pets. The *C. elegans* model can be used to assess the virulence of UPEC. The PX627 mutant of *C. elegans* is an auxin inducible infertile strain i.e. exposure to auxin induces infertility.

The aim of this work was to develop a new *C. elegans* killing assay based on feeding bacteria by the nematode throughout its life from the egg and to compare in this model the lifespan of two *C. elegans* mutants, the temperature sterile rrf-3 mutant and the PX627 mutant fed with UPEC strains.

The behavior of three clinical UPEC strains and the nonpathogenic OP50 strain was compared. Survival curves were generated by the Kaplan–Meier method and compared by the log-rank test over 10 days of follow-up.

The lifespan of the *C. elegans* PX627 mutant fed with each of two UPEC was significantly different that of the mutant fed with OP50.

The early exposure of this PX627 mutant to pathogenic strains is an advantage over the *C. elegans* mortality tests described in the literature. This new mortality test using the *C. elegans* PX627 mutant can be used on a large scale to screen the virulence of many UPEC studies.

M385 - The Role of Bacteria derived Outer-Membrane-Vesicles in the Induction of Systemic Inflammation

Presenting Author – *Yannick Teschke, University of Münster, Germany*

Author/s – *Yannick Teschke, Theresa Meyer, Christian Rüter, Petra Dersch*

Abstract Content

Klebsiella pneumoniae and *Escherichia coli* are the leading cause of Gram-negative bacterial infections worldwide. Moreover, the spread of multi-resistant and hypervirulent strains has drawn attention to the increasing community acquired infections with these species.

Recently, outer-membrane-vesicles (OMVs) have been shown to disseminate virulence factors in host blood and tissues even without the presence of their parent bacteria, causing strong immune responses. Since OMVs are shed from the bacterial surface, they harbor all their surface proteins, polysaccharides (e. g. LPS) and even capsular components, that can be sensed by host immune cells. Furthermore, bacteria are able to selectively pack toxins and nucleic acids in and onto the vesicles, where they are mostly protected from the degradation through host lytic enzymes.

By purifying OMVs from different *K. pneumoniae* und *E. coli* strains using ultracentrifugation, we were able to analyze their morphology with nano-tracking analysis and electron microscopy. Our data show, that the OMVs differ in size distribution, membrane structure and interestingly also in protein content, which was further confirmed by proteome analysis. Fluorescently labeled OMVs have been shown to be rapidly taken up by eukaryotic cells, activating intracellular immune receptors. Indeed, further investigation by flow cytometry revealed, that OMVs trigger inflammasome formation and inflammatory cell death pathways in human THP-1 monocytes.

Although further investigation on the inflammatory pathways and factors that influence cargo selection into OMVs is required, OMV analysis holds potential for rapid clinical diagnostics and enables improved clinical decision making as well as identification of novel therapeutic agents.

M386 - Elucidating the dynamics and transmission potential of the aquatic pathogen in Rainbow trout

Presenting Author – *Alejandra Villamil Alonso, Technical University of Denmark, Denmark*

Author/s – *Argelia Cuenca Navarro, Giulia Zarantonello, Niccolò Vendramin, Lone Madsen*

Abstract Content

Renibacterium salmoninarum (Rs) is a facultative intracellular bacterium and the aetiologic agent of bacterial kidney disease (BKD), a fatal systemic infection and major cause of mortality in salmonids worldwide. Rainbow trout (Oncorhynchus mykiss) is susceptible to Rs although the mechanisms of transmission and the chronic state of Rs are not yet fully understood in the species. We aimed to characterize the bacterial dynamics and host survival through established infections at 6°C and 12°C. Groups of Rainbow trout were intraperitoneally injected with Rs (1x109, 1x108 cells dose-1) or saline water (control) in recirculating aquaculture systems (RAS), and subjected to survival analysis during 12 weeks post-infection (wpi). Simultaneously, a cohabitation trial was established to assess transmission potential and bacterial kinetics through qPCR of bacterial DNA and environmental DNA (eDNA) extracted from fish kidney and water samples, respectively. Survival rate was similar for the challenged groups regardless temperature, although mortality began at an earlier stage at 12°C. Abundance of bacterial transcripts was significantly higher at 4 wpi in the kidney. Interestingly, water eDNA analyses reveal bacterial shedding was at its highest at 3 wpi, and persistent at 12 wpi. Moreover, Rs was reisolated from the host by classical culturing at 12 wpi. Our results describe the chronic state of BKD and the possibility to detect Rs in water systems even at late infection stages, defining an infection model essential to further investigate the fate of Rs in Rainbow trout and the molecular mechanisms behind infection.

M387 - Rlo related aspartic proteases modulate biofilm formation, antibiotic resistance and cell wall homeostasis in *Pseudomonas aeruginosa*

Presenting Author – *Maria Jesus Garcia Garcia, CSSB-DESY, Germany*

Author/s – *Maria Jesus Garcia Garcia, Justin Lormand, Charles Savelle, Eva Lopez Lumbreras, Holger Sondermann*

Abstract Content

Background: *Pseudomonas aeruginosanosa* is a gram-negative opportunistic pathogen prevalent in the airways of cystic fibrosis patients, where it commonly establishes life-threatening chronic infections. Therapeutic treatments against these infections are complicated by *Pseudomonas* intrinsic and acquired mechanisms of antibiotic resistance, as well as by its propensity to form biofilm communities within the cystic fibrosis lung. Consequently, novel therapeutic approaches are needed to effectively treat cystic fibrosis patients.

Objectives: Here we describe the discovery and phenotypic characterization of three novel and highly conserved aspartic proteases, that we have named rloA, rrp1 and rrp2.

Methods and Results: The simultaneous disruption of rloA, rrp1 and rrp2 causes increased sensitivity to β-lactam antibodies and decreases the ability of Pseudomonas to form biofilms, indicating a potential for these proteins as future drug targets for the treatment of pseudomonal infections. Because triple protease mutants are sensitive to hypo-osmotic stress, we explored a possible role of these proteins in peptidoglycan homeostasis. Evidence from cell wall HPLC analysis and results from a suppressor mutagenesis screen support that peptidoglycan crosslinking is disrupted in rloA, rrp1, rrp2 triple mutants.

Results from our experiments increase our current understanding of the factors that control peptidoglycan homeostasis and reveal links between cell wall crosslinking, antibiotic resistance, and biofilm formation.

M388 - *In silico* analysis of *Klebsiella pneumoniae* lipoproteins and elaboration of a molecular toolbox to study host-pathogen interacti

Presenting Author – *Lisa Zierke, University Greifswald, Germany*

Abstract Content

Background: *Klebsiella pneumoniae* causes 5% of all nosocomial infections like urinary tract infections, bloodstream infections, pneumonia, liver abscesses and meningitis. High antimicrobial resistance of *K. pneumoniae* strains complicates treatment and currently, vaccination is not available. Lipoproteins represent promising candidates for protein-based vaccination strategies due to their surface-exposure, conservation and potential immunogenicity. A structure-function analysis of lipoproteins is required to understand their role in host-pathogen interactions and elucidate their immunogenic potential.

Methods: The genome of *K. pneumoniae* ATCC BAA2146 was analyzed *in silico* to identify genes encoding for lipoproteins, which were heterologously expressed in *E. coli*. To generate isogenic *K. pneumoniae* mutants, an insertion-deletion mutagenesis strategy was established and mutants with and without antibiotic resistance genes were generated.

Results: We included 35 out of 93 predicted lipoproteins of *K. pneumoniae* ATCC BAA2146 and heterologously expressed and purified 15 out of these 35 lipoproteins. The selection is based on their conservation, their molecular weight and outer membrane localization. Functional and structural characterization is currently analyzed. The mutagenesis of wza in *K. pneumoniae* ATCC BAA2146 resulted in a non-encapsulated phenotype and the lack of capsule expression was verified by electron microscopy.

Conclusion: We started to elaborate a molecular toolbox for *K. pneumoniae* aiming to investigate the role of *K. pneumoniae* lipoproteins in bacterial fitness and virulence in order to define new target molecules for antimicrobials. In addition, we generated isogenic mutants and obtained e.g., a capsule knockout strain, which can be used for *in vitro* cell-culture based infection experiments and proteome analysis.

M389 - Development of self-assembling antimicrobial coatings for medical devices

Presenting Author – *Cynthia Calligaro, Spartha Medical, France*

Author/s – *Philippe Lavalle, Nihal Engin Vrana*

Abstract Content

Background & Objectives: The development of infections is a major problem accompanying implant surgeries. Many of these implants have complex geometry and are made of various materials. Our group develops multimodal bioactive coatings made up of polypeptides and polysaccharides. Poly(arginine), PAR and hyaluronic acid, HA (PAR/HA) layer-by-layer films are thin films, easy to build, with promising antimicrobial properties (1-4). Here, we investigate the film behavior on various substrates and its activity on relevant microorganisms to nosocomial infections.

Methods: To construct (PAR/HA) multilayer films, bare substrates were alternatively dipped in PAR and HA solutions with intermittent rinsing steps until the desired number of layers, 24 or 48, is reached. For the antimicrobial assays, the coated substrates were incubated with microorganism suspensions (5-6 log CFU/mL) for 24h. The killing activity was evaluated at 0 and 24 h by microorganism enumeration.

Results: (PAR/HA)24 films, made with 24 layers, have a strong bactericidal activity (≥ 5 log reduction in CFU/mL) against all Gram-positive bacteria tested, and a static activity (≥ 1 log reduction in CFU/mL) against Gram-negative bacteria tested or against *Candida albicans*. With an increased number of layers, the film shows bactericidal activity against all the tested microorganisms (Table 1). This experiment was successfully repeated on various medical-grade substrates, such as titanium, silicone, polyurethane, or polypropylene, and after sterilization and storage.

These results show the capacity of (PAR/HA) films to ensure antimicrobial activity when coated on different substrates. (PAR/HA) coating appears to be a versatile and powerful system, suitable on all surfaces.

M391 - *Zonula occludens* toxin from PMC 53.7 binds to PAR2 via a peptide motif found in the C-terminal region

Presenting Author – *Cristian Iribarren, University Autonoma of Chile, Chile*

Author/s – *Gino Corsini, Katherine Garcia, Sinisa Bjelic*

Abstract Content

Non-toxigenic strain of *Vibrio parahaemolyticus* are responsible for about 10% of acute gastroenteritis associated with the consumption of raw or undercooked shellfish, suggesting that they have acquired unknown virulence factors. A *Zonula occludens* toxin (Zot) identified in a non-toxigenic clinical strain of *Vibrio parahaemolyticus*, called PMC53.7. Zot is an enterotoxin produced by *Vibrio cholerae* that alters intestinal permeability, through the disruption of intercellular tight junctions and the rearrangement of actin filaments in the cytoskeleton of intestinal cells by activation of the protein kinase C alpha (PKC-α) signaling pathway. The sequence "FCIGIRL" in the C-terminal region of *Vibrio cholerae* Zot was described to binds to the protease-activated receptor 2 (PAR2) of intestinal cells but is absent in PMC53.7-Zot. However, our results show a rearrangement of the actin filaments of the Caco-2 intestinal cell cytoskeleton when we treat them with PMC53.7-Zot. In addition, transcriptome analysis of cultured intestinal Caco2 cells infected with PMC53.7 identified repressed genes, biological processes, and pathways associated with rearrangement of the actin filaments and tight junctions. Also, an overexpressed mediator of the PKC-α signaling pathway was observed. The objective of this work is to demonstrate that a peptide motif in the C-terminal region of PMC53.7-Zot alters intestinal permeability by binding to PAR2 in a Caco2 intestinal cell model. The Rosseta computer software was used to obtain structures of the Zot-PMC53.7 protein and to generate the molecular coupling with PAR2. Then, we will identify candidate peptide motifs to synthesize and test for PAR2 binding and cellular effect.

M392 - Evaluation of volatile sulfur compounds- producing abilities in a collection of lactic acid bacteria

Presenting Author – *Giovanna Felis, University Of Verona, Italy*

Author/s – *Elisa Salvetti, Veronica Gatto, Giacomo Dalla Torre, Carolina Conter, Alessandra Astegno, Paola Dominici*

Abstract Content

Volatile sulfur compounds (VSCs) are crucial aromatic molecules deriving from the microbial enzymatic catabolism of sulfur-containing amino acids methionine and cysteine that define the aroma of several fermented foods, particularly dairy products.

Lactic acid bacteria (LAB), the most common starters in cheesemaking, have reduced VSC-producing abilities, which have been shown to correlate with C-S lyase activities.

Since understanding LAB potential in VSCs formation is pivotal in rationally modulating peculiar flavors in fermented products, the present study aims at (i) screening a collection of lactobacilli for the enzymatic potential to produce VSCs, thus exploring the diversity of C-S lyase natural activities, and (ii) investigating the presence of C-S lyases in the genome sequences of strains with different phenotypic behavior.

A collection of 280 strains belonging to *Lactobacillus delbrueckii* species, and *Lacticaseibacillus* and *Lactiplantibacillus* genera was screened for the qualitative production of VSCs, allowing the selection of 28 strains (13 *Lacticaseibacillus* and 15 *Lactiplantibacillus*) as best candidates for quantitative assays development of C–S lyase activities.

Further, the presence of genes with high sequence similarity with C-S lyases was explored in the genomes of 3 Lcb. paracasei and 3 Lpb. plantarum strains displaying different phenotypes, unraveling little correlation between copy number and phenotype.

The analysis of a broad collection of strains revealed the diversity of VSCs – related phenotypic and genotypic traits among different species and within the same species. In addition, comparative genomics shed light on the enzymes putatively associated with C-S lyase activities to be selected for future biochemical characterization.

M393 - First report of staphylococcal food poisoning due to staphylo-coccal enterotoxin type B in döner kebab (Italy)

Presenting Author – *Fabio Zuccon, IZS PLVA, Italy*

Author/s – *Angelo Romano*

Abstract Content

Staphylococcal food poisoning results from the consumption of food contaminated by staphy-lococcal enterotoxins. In July 2022, the Turin local health board was notified of a suspected foodborne outbreak involving six children who had consumed döner kebab purchased at take-away restaurant. Symptoms (vomiting and nausea) were observed 2-3 hours later. Microbiological analysis of the food samples revealed high levels (1.5 x 107 CFU/g) of coagulase-positive staphy-lococci (CPS). Immunoassay detected contamination with staphylococcal enterotoxins type B (SEB). Whole genome sequencing of isolates from the food matrix confirmed staphylococcal enterotoxin genes encoding for type B, which is in line with SEB detected in food. This toxin is rarely reported in staphylococcal food poisoning, however, because there is no specific method of detection. In-volvement of enterotoxin type P (SEP) was not confirmed, though the corresponding gene (sep) was detected in the isolates. Nasal swabs from the restaurant food handlers tested positive for CPS, linking them to the likely source of food contamination.

T1 - How to enhance production of recombinant proteins in *Yarrowia lipolytica*? Use secretory helpers!

Presenting Author - *Ewelina Celinska, Poznan University of Life Sciences, Poland*

Author/s – *Paulina Korpys-Woźniak, Piotr Kubiak, Ewelina Celińska*

Abstract Content

The non-conventional yeast *Yarrowia lipolytica* is an established platform for the biotechnological production of recombinant secretory proteins (rs-Prots). Since the worldwide demand for r-Prots is increasing, strategies for intensification of their production are sought. One possible approach is to co-overexpress the so-called secretory helpers (SHs) that assist the synthesis and/or transportation of the polypeptides. In this study, twelve SHs were tested for their potential towards enhancing rs-Prots production by *Y. lipolytica*. The SHs were previously identified as differentially expressed genes in the transcriptomes developed upon over-production of r(s)-Prot. The set of SHs covered those operating throughout a transcription-translation-folding-maturation-secretion pipeline, including a major transcription factor governing UPR activation (HAC1), a ribosome element (RPL3), cytosolic chaperones (SSA5 and SSA8), ER-residents (PDI1, SLS1, CNE1), as well as components of proximal (YET3, USO1), and distal (SEC1, SSO1, CWP11) vesicular transportation of the cargo protein outside the cell. The SHs were co-cloned with an easy-to-track reporter protein (secretory YFP; scYFP). The recombinant strains were tested under two temperatures (25 °C and 30 °C) for their secretory capacity, protein retention, and target gene expression. The SHs involved in polypeptide synthesis significantly enhanced the r-Prots amounts irrespective of the adopted temperature; however, for efficient secretion, the secretory pathway's capacity must be released by applying a decreased temperature. The other SHs (e.g. SSO1, CWP11) did not give such spectacular results in the amounts of the scYFP but allowed to maintain secretory capacity under unfavorable thermal conditions.

T3 - Constitutive glucose dehydrogenase elevates intracellular NADPH levels and luciferase luminescence in *Bacillus subtilis*

Presenting Author - *Ken-ichi Yoshida, Kobe University, Japan*

Author/s – *Yuzheng Wu, Honami Kawabata, Kyosuke Kita, Shu Ishikawa, Kan Tanaka, Ken-ichi Yoshida,*

Abstract Content

Genetic modifications in *Bacillus subtilis* have allowed the conversion of myo-inositol into scyllo-inositol, which is proposed as a therapeutic agent for Alzheimer's disease. This conversion comprises two reactions catalyzed by two distinct inositol dehydrogenases, IolG and IolW. The IolW-mediated reaction requires the intracellular regeneration of NADPH, and there appears to be a limit to the endogenous supply of NADPH, which may be one of the rate-determining factors for the conversion of inositol. The primary mechanism of NADPH regeneration in this bacterium remains unclear.

The gdh gene of *B. subtilis* encodes a sporulation-specific glucose dehydrogenase that can use NADP+ as a cofactor. When gdh was modified to be constitutively expressed, the intracellular NADPH level was elevated, increasing the conversion of inositol. In addition, the bacterial luciferase derived from Photorhabdus luminescens became more luminescent in cells in liquid culture and colonies on culture plates.

The results indicated that the luminescence of luciferase was representative of intracellular NADPH levels. Luciferase can therefore be employed to screen for mutations in genes involved in NADPH regeneration in *B. subtilis*, and artificial manipulation to enhance NADPH regeneration can promote the production of substances such as scyllo-inositol.

T4 - Gene expression dependence on spatial location in the genome

Presenting Author - *Guillermo Gómez García, Centro Nacional de Biotecnología (CNB-CSIC), Spain*

Author/s – *Víctor de Lorenzo*

Abstract Content

Background: Structural conformation of the chromosome and physical distance between genes and regulators in bacteria is sometimes overseen when regulation of expression of genes is discussed, deriving in gene expression noise, affecting how efficient can be an expression system. This idea was predicted by previous computational simulations, and experiments where the distance was brought to a minimal corroborated the predictions.

Objectives: Using as workstation the multi-faceted soil bacterium *Pseudomonas putida*, the main idea consists in the obtention of a library of strains where promoter and transcription regulator are located in different sites across the genome, in order to deepen what was got within the previous results, and to continuing supporting the hypothesis with more experimental data.

Methods: By attachment of the promoter to a fluorescent reporter, and, through random insertions with a suicide vector in *Pseudomonas putida* carrying a MiniTn5 transposon, we created a library of strains presenting different insertion locations for xylS and Pm-gfp. Finally, the resultant library of strains was analyzed using flow cytometry, that allowed us to observe different profiles of transcription noise patterns, and this data was correlated with the insertion locations determined previously.

Results: Previous experimental data showed that transcriptional noise was different when minimum distance distribution of xylS and Pm with maximum distance, when xylS is located outside the genome, were compared. So far, the results presented here manifest that the different locations inside the genome for both Pm and xylS lead to diverse signal patterns, supporting the idea of this work.

T6 - Multiomics data integrative approach for metabolic adaptation analysis of the extremophile *Chromohalobacter salexigens*

Presenting Author - *Montserrat Argandoña, Universidad de Sevilla, Spain*

Author/s – *Lourdes Martinez-Martinez, Manuel Salvador, Joaquín J Nieto, Carmen Vargas*

Abstract Content

Background: The halophilic bacterium *Chromohalobacter salexigens* is a natural producer of compatible solutes, such as ectoines, in response to increased salinity and temperature of the medium, adapting its metabolism to osmotic and thermal environmental stress. Ectoines present enormous biotechnological potential due to their stabilising and protective properties.

Objectives: For the rational design of ectoines over-producing strains, a holistic knowledge of the metabolism of *C. salexigens* is required. Thus, data from different omics techniques were analysed through an integrative approach.

Methods: Differential transcriptomic (RNA-seq) and proteomic (2D-DIGE) and metabolomic data (targeted metabolome) were obtained from cell samples cultured under the conditions of salinity and temperature, which drive minimum and maximum production of ectoines (0.6 and 2.5 M NaCl, both at 37°C; 2.5 M NaCl, at 45°C). Data of 1468 genes and 265 protein spots, differentially expressed, as well as intracellular concentration of 48 metabolites from central metabolism, were selected. The integrative approach was performed using a multivariate method in R, applying the multiblock (s)PLS-DA framework from the mixOmics package.

Results: Two relevance networks were obtained, in response to salinity or to temperature, which correlated the three types of data. Hubs analysis revealed key metabolites that were correlated with ectoines levels. Additionally, interesting correlations between nodes (genes/proteins) involved in metal homeostasis, signalling systems and ectoines metabolism were found depending on the environmental stress. Therefore, this multiomics data integration provided clues that will expand the knowledge about the metabolic adaptation of *C. salexigens* to different stress, related with ectoines production.

T7 - Physiological changes in *Pseudomonas aeruginosa* in response to prolonged exposition to carbon-based nanohybrid

Presenting Author - *Adrian Augustyniak, Poland*

Author/s – *Kamila Dubrowska, Joanna Jabłońska, Bartłomiej Grygorcewicz, Krzysztof Cendrowski, Rafał Rakoczy,*

Abstract Content

Background: Nanomaterials can be used as antimicrobials. However, once the dose of nanostructure drops to the sublethal level, it may cause specific changes in bacterial physiology. Evidence indicates that such interaction can have the stimulative potential for bacterial primary and secondary metabolism. While short-term contact between bacteria and nanostructures has been extensively studied over the years, little is known about the impact of these compounds after prolonged contact with the bacterial population.

Objectives: In this study, we monitored the physiological characteristics of *Pseudomonas aeruginosa* after multiple passaging with carbon-based nanocomposites.

Methods: The reference strain – *Pseudomonas aeruginosa* ATCC 27853 was passaged 50 times with a carbon-based nanohybrid and its components (i.e., nanotubes, cobalt nanoparticles, titanium dioxide, and graphene oxide). Afterwards, physiological parameters such as viability (by life/dead staining), metabolic activity (respiration), biofilm formation, secretion of pyocyanin and rhamnolipids, biochemical activity (VITEK2), antibiotic resistance, and expression of selected genes were examined. The potential genotoxicity was screened with pulse-field gradient electrophoresis while mass spectrometry (Biotyper) was used for strain typing.

Results: Multiple passaging has altered the physiology of the tested strains in a specific manner depending on the used nanomaterial. Differences were found in biofilm formation, pigment and rhamnolipids production, and growth dynamics. At the current stage of the project, the changes seem to be phenotypic. However, the observed physiological characteristics were stable after additional passaging without nanomaterials. The observed changes include the virulence of the initial strain, which should be considered when nanomaterials are proposed for use in medicine and biotechnology.

T8 - Biotransformation of carboxylic acids to alcohols: characterization of *Thermoanaerobacter* strain AK152 and 1-propanol production

Presenting Author - *Johann Orlygsson, Iceland*

Author/s – *Sean Michael Scully*

Abstract Content

Thermoanaerobacter strains have recently gained interest because of their ability to convert short chain fatty acids to alcohols using actively growing cells. *Thermoanaerobacter thermohydrosulfuricus* strain AK152 was investigated for its ethanol and other alcohol formation. The strain degraded a variety of compounds present in lignocellulosic biomass like monosaccharides, disaccharides, and starch. The strain is highly ethanologenic, producing up to 86% of the theoretical ethanol yield form hexoses. Strain AK152 was inhibited by relatively low initial substrate (30 mM) concentration, leading to inefficient degradation of glucose and leveling up of all end-product formation. The study presented shows that the strain produces alcohols from most of the tested carboxylic acids, with the highest yields for propionate conversion to propanol (40.7%) with kinetic studies demonstrating that the maximum conversion occurs within the first 48 h of fermentation. Various physiological tests were performed to maximize the acid conversion to the alcohol which reveals that the optimum pH for propionate conversion is pH 6.7 which affords a 57.3% conversion. Kinetic studies reveal that propionate conversion is rapid, achieving a maximum conversion within the first 48 h of fermentation. Finally, by using(13)C NMR, it was shown that the addition of propionate indeed converted to propanol.

T9 - Metabolic burden in *Pseudomonas putida*: from single proteins to entire pathways

Presenting Author - *Katharina Pflüger-Grau, Technical University Munic, Germany*

Author/s – *Ana-Sofia Ortega Arbulu, Andreas Kremling, Katharina Pflüger-Grau*

Abstract Content

The introduction of heterologous metabolic pathways in a bacterial host entails an increased demand for energy and cellular resources such as amino acids, ribosomes, or polymerases. This leads to a redistribution from normal cellular functions towards the additionally implemented task. Limitations in any of these resources usually result in a decrease in growth rate and cessation of heterologous production, a condition defined as metabolic burden. We recently constructed *P. putida* CAP, a strain that allows to follow the cellular capacity during heterologous protein expression. This was achieved by placing the fluorescent mCherry gene under the control of a constitutive promotor in the chromosome of *P. putida*. With this strain at hand, we aimed to analyze in detail the effect of the expression of single heterologous proteins with the aim to expand this towards the expression of an entire pathway. By analyzing the effects of heterologous protein expression on transcriptional and translational level we could conclude that the bottle neck most likely can be found on the post-transcriptional level. Interestingly, the addition of amino acids did not result in more protein production but had a positive effect on growth. As a next step, we shifted our interest towards an entire metabolic pathway and now focus on the metabolic burden during the production of the monoterpenoid geranic acid, which carries great potential for various industrially relevant applications, e.g., as a fragrance or antifungal agent.

T10 - Characteristic effects of gold nanoparticles on growth and H2 metabolism of *Ralstonia eutropha* H16 and *Escherichia coli*

Presenting Author - *Anna Poladyan, Department of Biochemistry, Microbiology, and Biotechnology, Yerevan State University, Armenia*

Author/s – *Tatev Manutsyan, Meri Iskandaryan, Syuzanna Blbulyan, Anait Vassilian, Tatiana Semashko*

Abstract Content

Background: *Ralstonia eutropha* and *Escherichia coli* possess different [NiFe]-hydrogenases (Hyd) enzymes involved in H2 metabolism. *R. eutropha* is a facultative chemolithoautotrophic bacterium that grows on organic substrates or H2 and CO2 in an aerobic environment. O2-tolerant Hyds are expressed under litho-autotrophic, energy-limited, or stressful conditions and can be used as anode biocatalysts in enzyme fuel cells (EFC).

Objectives: In the present study, the characteristic effects of gold nanoparticles (NPs) on growth, oxidation-reduction potential (ORP) kinetics, and Hyd activity of *R. eutropha* H16 and *Escherichia coli* BW25113 were investigated.

Methods: ORP was measured using titan-silicate/platinum redox electrodes, and H2-oxidizing Hyd activity was observed spectroscopically, by the reduction of methylene blue.

Results: Compared to the control (without AuNPs), after 24h of growth *R. eutropha* H16 the drop of ORP from positive to negative values was observed. Moreover, 20□l and 30□l concentrations of AuNPs stimulated the bacterial growth ~1.3 and ~1.4 fold, respectively. The H2-oxidizing Hyd activity was not observed in control samples, while it was detected after 24h when AuNPs were supplemented. In the presence of AuNPs, especially 20□l concentration, 12±0.01U (gCDW-1) Hyd activity was observed, which reached its maximum of 54±0.02U (gCDW-1) after 72 h of growth. In contrast to *R. eutropha*, AuNPs had a negligible or negative effect on *E. coli* BW25113 growth, H2 production, and Hyd activity. The decrease of ORP and increase of H2-oxidizing total-Hyd activity of *R. eutropha* H16 in the presence of Au NPs indicate a possible link between these mechanisms.

T11 - Efficient utilisation of waste concrete fines via microbial calcite precipitation

Presenting Author - *Hana Stiborova, University Of Chemistry And Technology Prague, Czech Republic*

Author/s – *Kristyna Klikova, Michael Borisov, Petr Holecek, Zdenek Prosek, Dana Konakova, Vaclav Nezerka, Katerina Demnerova, University of Chemistry and Technology, Prague, Czech Republic*

Abstract Content

There is a consensus that greenhouse gases contribute to increasing the average Earth's surface temperature, and the cement industry sector is a significant contributor, accounting for up to 8% of the total anthropogenic CO2 emissions. To address the issue of complete concrete recycling, efforts have been made to use waste concrete fines (WCF) meaningfully. We propose a strategy based on microbial calcite precipitation (MICP) technology, exploiting the ability of microorganisms to sequester atmospheric CO2 and deposit it in the form of CaCO3 crystals. Bacterial strains with ureolytic activity were isolated directly from WCF via enrichment in a low-nutrient medium with urea. The isolates were further clustered by MALDI-TOF MS and identified based on the full-length 16S rRNA gene sequence. The growth kinetics and ureolytic activity of new isolates were compared with the well-known ureolytic bacterium *Sporosarcina pasteurii* DSM 33. Furthermore, the precipitation of CaCO3 and the formation of different crystals were analyzed using X-ray diffraction (XRD) and scanning electron microscopy (SEM). Two types of WCF with different properties were used to examine the MICP efficiency and the formation of compact conglomerates by new isolates and *Sporosarcina pasteurii* DSM 33 upon the addition of urea and calcium ions.

T12 - Chitosan and chitosan-based materials as antimicrobials in fermentation and food industry

Presenting Author - *Sandra Regina Ceccato-Antonini, Universidade Federal De São Carlos, Brazil*

Author/s – *Sandra Regina Ceccato-Antonini, Isabella Carvalho Tanganini, Kelly Roberta Francisco, Andreia Fonseca Faria, José Machado Silva Neto, Lucas Nishino Cruz,*

Abstract Content

Chitosan is obtained through the deacetylation of chitin, which in turn can be extracted from naturally-chitin containing materials such as shrimp wastes, fungal cell walls and insect exoskeletons. Chitosan is a polymer with applications in different areas and has antimicrobial properties. In this work, the chitosan production process was optimized using lactic fermentation by *Lactobacillus* plantarum in sugarcane molasses medium with shrimp wastes to obtain chitin followed by chemical deacetylation, whose conditions were also optimized. The resulting chitosan was characterized and tested against a bacterium that contaminates the ethanolic fermentation process, *Lactobacillus fermentum*, and against an industrial yeast of *Saccharomyces cerevisiae* to verify its antimicrobial action. Additionally, chitosan microspheres were obtained aiming at their use as a carrier of antimicrobial substances for use in ethanolic fermentation, to replace sulfuric acid in the cell treatment step between fermentation cycles. With chitosan, the population of *L. fermentum* was reduced significantly and similarly to the result obtained with sulfuric acid. The effect on the yeast is much smaller. In mixed culture, the effect on the bacterium is smaller but still significant. The contact time with chitosan is 3 hours to obtain the antimicrobial effect. Fermentative tests are being carried out to evaluate the use of chitosan in conditions close to the industrial setup. Chitosan microspheres do not have antibacterial effect but have interesting characteristics for use in the encapsulation of substances. Assays with chitosan-based materials such as films for food packaging are also ongoing.

T13 - Cell surface display of trehalose synthase and its application to whole cell biocatalyst

Presenting Author - *Seung Hwan Lee, Chonnam National Universtiy, Republic of Korea*

Author/s – *Seon Jung Kim*

Abstract Content

Trehalose is a non-reducing disaccharide, which serves as an energy source, a protectant against various stress, a stabilizer of proteins, and a cell wall component. Because of these biological functions, it has been drawn much attention in the fields of food, agricultural, cosmetic, and pharmaceutical industries. In this study, we demonstrated surface expression of trehalose synthase from *P. putida* using YiaT outer membrane protein as an anchoring motif on the *E. coli* and bioconversion of maltose to trehalose via newly developed whole-cell enzyme. Functional immobilization of trehalose synthase on the cell surface was verified by measuring whole cell activity, western blot analysis, and immunofluorescence microscopy. To optimize whole-cell bioconversion condition, the effects of pH and temperature were investigated. Highest conversion yield was obtained 38.2% under the conditions of pH 8.00 and 30℃, and surface displayed TreS maintained over 50% of initial activity after consecutive 10 repeated reactions. These results suggested that trehalose synthase from *P. putida* was successfully expressed on the surface of *E. coli* via C-terminal deletion fusion with YiaT anchoring motif and TreS displayed *E. coli* can be used for the whole-cell production of trehalose.

T15 - Gut microbial impact of bio-active compound present in baby formula and their health repercussion: an *in vitro* study evaluation

Presenting Author - *Vineet Singh, Kyungpook National University, Republic of Korea*

Author/s – *HyunWoo Son, Jae-Ho Shin*

Abstract Content

Human gut harbors the huge number of gut microbes (~38 × 1012) comprising genetic material comparable to host itself. Gut-microbiome plays a crucial role in various functions including different gut-organ axes such as gut-brain axis, gut-liver axis, and gut-heart axis, by producing different metabolites (SCFAs, secondary bile acids, and biogenic amines). Microbes, especially health-benefiting butyrate-producing microbes such as Clostridiaceae, Lachnospiraceae, and Ruminococcaceae spp. are introduced to the gut at the early infant stage and along with other microbes get established in due course of time to mature as gut-microbiome. In recent times, it's been observed that baby formula milk is advertised as a healthy substitute for mother milk, and even in some countries, formula milk feeding can be started at a very early stage. The health claims of baby formula milk are based on the bio-active ingredients present in them, such as HMO, DHA, milk fat globule membrane (MFGM), and Lactoferrin. Though no detailed information is available about their health claims and possible impact on infant gut microbiome. In present study, impact of said bio-active compounds on gut microbiome is analyzed, using in-vitro digestion and fecal fermentation model. For the study, five healthy infants are selected to collect the fecal sample, and upon fecal-fermentation of bioactive compounds, metagenomic data (MiSeq), and metabolomic data are analyzed. Real-time shotgun metagenomic data will be analyzed to bolster the findings. Where metagenomic data is analyzed to predict the microbial variation and markers. Additionally, functional analysis is performed to relate their impact on infant health.

T16 - Novel cellulase derived from Jermuk hot spring metagenome

Presenting Author - *Ani Paloyan, Spc Armbiotechnology, Armenia*

Author/s – *Anna Krüger, Garabed Antranikian*

Abstract Content

Introduction: The transformation process from crude oil to biobased industry requires the supply of highly efficient microorganisms and enzymes for biomass conversion. The essential target for a sustainable biorefineries using lignocellulosic biomass is to obtain high-volume biofuels, high-value chemicals, and less waste.

Objective: Even after an intensive investigation of enzymes/enzyme cocktails for lignocellulosic biomass valorization, there is still a necessity to explore new efficient enzymes.

Methods: Metagenomic DNA was extracted from environmental samples taken from the Armenian hot spring Jermuk. Nickel affinity and size exclusion chromatography were applied for protein purification. Benchling was used for specific primer design, *in silico* PCR and assembly experiments. One-pot Golden Gate cloning and the CIDAR MoClo system were used for gene cloning.

Results: A 1672 bp length ORF was identified from Jermuk hot spring metagenome. The deduced gene product Jermuk-CelP cellulase appeared to have a signal peptide, a catalytic domain and a carbohydrate-binding module. The amino acid sequence showed the highest identity (49.1%) with an endo-b-1,4-endoglucanase of Thermotoga maritima. The purified enzyme exhibited the highest activity at 55°C and at pH 5.5. Jermuk-CelP was stable at temperatures up to 40°C after 24h of incubation.

The highest hydrolytic activity was detected with barley β-glucan as substrate, yielding a specific activity of 85 U/mg. The enzyme also showed activity towards lichenan, xyloglucan and carboxymethyl cellulose. Tri- and tetra-saccharides were found to be the major hydrolysis products. Accordingly, this enzyme is an attractive Candidate for use in the conversion of complex lignocellulosic biomass.

T17 - Bioleaching of chalcopyrite with inhibition of *Leptospirillum ferriphilum* using sodium chloride

Presenting Author - *Michael Schlömann, TU Bergakademie Freiberg, Germany*

Author/s – *Julius Kramer*

Abstract Content

Background: Leaching chalcopyrite concentrates with decent yields requires an oxidation reduc-tion-potential (ORP) of 410 – 530 mV vs. Ag/AgCl. However, *Leptospirillum ferriphilum*, abundant on natural ore bodies, due to a high affinity for Fe2+ and little inhibition by Fe3+, tends to increase the ORP to levels unfavorable for chalcopyrite leaching.

Objective: Since *Leptospirillum ferriphilum* is only moderately tolerant to elevated temperatures and chloride ions, the aim was to suppress this species by these parameters as compared to the more tolerant iron- and/or sulfur-oxidizing species *SulfoBacillus thermosulfidooxidans* and Acidi-thio*Bacillus* caldus.

Methods: Leaching experiments by a community of *Leptospirillum ferriphilum*, *SulfoBacillus thermosulfidooxidans* and *AcidithioBacillus caldus* were performed at different temperatures and NaCl concentrations using ICP-MS for copper analysis. Community analyses were performed by TRFLP (terminal restriction-fragment length polymorphism).

Results: First experiments varying both parameters (NaCl 0 – 600 mM; temperature 40, 45 and 50°C) led to the following results: at 40°C more chloride (550 mM) was needed to obtain moder-ate leaching results (Cu yield 31% in 8 weeks), while the ORP was approximately 570 mV. A tem-perature of 45°C increased the copper yield (49% in 8 weeks) and reduced the need of NaCl (150 mM) with an ORP of 540 mV. At 50°C the copper yield reached its maximum at 74% with less chloride (100 mM) needed to leach with an ORP at 510 mV. In summary, higher leaching temper-atures gave higher copper yields and lower NaCl concentrations needed to limit the ORP.

T18 - H_2 consumption characteristics of acetogens in microbial electrosynthesis

Presenting Author - *Laura Munoz, Aarhus University, Denmark*

Author/s – *Louise Groen, Tobias Jensen, Klaus Koren, Jo Philips*

Abstract Content

Some acetogenic bacteria have the capacity to use cathodes as electron donors for the reduction of CO_2 into more complex compounds like acetate. This capacity can be applied for the development of innovative technologies, such as Microbial electrosynthesis. This biotechnology combines the upgrading of CO_2 into organic compounds, such as biofuels, with the storage of excess renewable electrical energy. So far, however, the production rates of microbial electrosynthesis are too low to allow its use on an industrial scale.

The electron uptake by acetogens has been the subject of different research efforts to understand what makes some acetogens more efficient in the current consumption from cathodes than others. H_2 plays an essential role in the cathodic electron uptake by acetogens in MES. We hypothesized that the H_2 consumption characteristics of acetogens affect their performance in MES. Therefore, the H_2 threshold, which is the pressure at which acetogenesis halts, and the H_2-consumption kinetics at low H_2 concentrations were studied for different acetogenic strains. These characteristics were then correlated with the performance of the strains in bioelectrochemical reactors. The results showed that the strain with the lowest H_2 threshold and the highest H_2 consumption rates at low H_2 concentration produced the most acetate in the reactors. On the other hand, the strain with a higher H_2 threshold and a lower kinetic constant was not able to perform acetogenesis. We thus conclude that understanding the H_2 consumption characteristics of acetogens is important in selecting the best biocatalysts for specific applications.

T19 - Development of a non-propagative phage for delivering CRISPR-Cas9 targeting antimicrobial resistance genes

Presenting Author - *Thaysa Tagliaferri, Germany*

Author/s – *Tanja Schwab, Hans-Peter Horz*

Abstract Content

Background: In the light of the antimicrobial resistance crisis, the development of new strategies to target antibiotic resistant bacteria has been fostered. While conventional antimicrobial therapies select for antimicrobial resistant bacteria, CRISPR-Cas9 can be used to reduce resistance mechanisms in a target microorganism.

Objectives: We aim at developing a non-propagative phage-based system for delivering CRISPR-Cas9 into carbapenems-resistant Enterobacteriaceae. With that, CRISPR-Cas9 would be specifically delivered to the target bacteria, leading to resistance plasmid clearance while keeping the bacteria alive, preventing microbiome dysbiosis.

Methods: A T7 mutant was constructed based on homologous recombination and counter selection in the *E. coli* JW5856 host. The CRISPR-Cas9 plasmid was constructed using Gibson assembly and contains the T7 packaging origin, GFP and CRISPR-Cas9 with sgRNA targeting carbapenem genes. T7 packaging efficiency was evaluated with qPCR. Transduction efficiency was tested with different MOIs (10-1 to 10-8) and the respective bacterial viability was assessed concomitantly. Re-sensitization was first performed via electroporation to evaluate the efficiency of the designed sgRNA.

Results: The CRISPR-Cas9 plasmid was packed with a ratio of ~ 1:50. The highest transduction efficiency was obtained with the MOI 10-1, however it was associated with lower survival rates. At the MOI of 10-3, the transduction efficiency reached 105 and no difference in bacterial survival was seen. The re-sensitization rate of the CRISPR-Cas9 treated cells was around 90%. Future experiments aim at evaluating the re-sensitization rate based on phage delivery and the use of multiple CRISPR-Cas9 dosages.

T21 - Bioremediation of phenol-contaminated groundwater by small bioreactor chambers and CaO_2 through a continuous-flow model

Presenting Author - *Maryam Yavari Bafghi, University Of Tehran, Iran, Islamic Republic of*

Author/s – *Mahmoud Shavandi, Seyed Mohammad Mehdi Dastgheib, Mohammad Ali Amoozegar*

Abstract Content

Phenols are considered as the priority pollutant with high solubility in water and carcinogenic impacts on humans, animals, and plants. The entry of these contaminations into the environment is seriously hazardous and must be treated.

This study explored impacts of bioaugmentation and biostimulation on phenol removal from groundwater through a continuous-flow model system.

Four continuous up-flow plexiglas reactors with 100 cm length and 9 cm inner diameter were packed with underground originated sands and the phenol contaminated groundwater was passed through the columns. Chemical remediation, natural bioremediation, biostimulation and bioaugmentation efficiency were examined for 6 months. To investigate the impact of each process on the microbial biodiversity of the column's, next-generation sequencing (NGS) of the 16S rRNA gene was performed.

Simultaneous use of bioaugmentation (SBCs application) and biostimulation (CaO_2 injection) completely eliminated phenol during the first 42 days. In the biostimulation column, 90 % and 100 % of phenol removal was observed after 12 and 22 weeks of the experiment, respectively. The dissolved oxygen (DO) in the chemical column (I) effluent increased notably after the first injection and peaked on the 21st day, reaching 14.14 mg/L. By injection of nanoparticles into columns (III) and (IV), the dissolved oxygen was increased in comparison to the blank column (II). Microbial diversity was decreased by CaO2 injection while phenol-degrading orders such as Rhodobacterales and Xanthomonadales were dominated in biostimulation columns. In conclusion, the innovative use of SBCs in stimulated water provides evidence for the successful application of these methods in groundwater treatment processes.

T22 - Rep-WH1 amyloid domains as novel antimicrobials and tools for prion bioremediation

Presenting Author - *Rafael Giraldo, National Centre for Biotechnology (CNB-CSIC), Spain*

Author/s – *Leticia Lucero-López, María Luz Blasco-Santamaría*

Abstract Content

Amyloids, either pathogenic or functional, arise from the templated conformational conversion and assembly of soluble proteins into fibrillar aggregates. We have engineered a bacterial model of amyloid disease based on the WH1 domain in the plasmid replication protein RepA. RepA-WH1 amyloids naturally build complexes that pair plasmids together to negatively control DNA replication. *In vitro*, ligand-modulated structural transformation of RepA-WH1 results in fibre assembly. As expressed in *Escherichia coli*, RepA-WH1-mCherry aggregates propagate from mother to daughter cells as distinct cytotoxic prion-like strains. RepA-WH1 forms oligomeric pores in the inner membrane and triggers oxidative stress. This resembles the mitochondrial route in neurodegeneration, thus endorsing RepA-WH1 as a bacterial proxy to human amyloidoses. RepA-WH1 amyloids are biosafe, since their transmissibility in cultured mammalian cells is strictly dependent on pre-established repA-WH1 transgenesis.

We have developed several SynBio tools based on RepA-WH1 amyloids. An optogenetic device was built so that, on illumination, amyloidogenic oligomers assemble intracellularly as a new kind of antimicrobials (‘optobiotics’). We are exploring Rep-WH1 domains in plasmids from the emerging phytopathogen Xylella fastidiosa to test their cytotoxicity in disease control. Grafting a RepA-WH1 amyloidogenic peptide into an extracellular loop of the *E. coli* outer membrane porin OmpF enables bacteria to scavenge amyloids. We are adapting this tool to homotypically recognize amyloidogenic peptides from the mammalian protein PrP to bioremediate prion-contaminated environments.

T23 - Using *Lactococcus lactis* to produce small protein blockers of IL-23/IL-17 axis

Presenting Author - *Tina Vida Plavec, Jozef Stefan Institute, Slovenia*

Author/s – *Petr Malý, Aleš Berlec*

Abstract Content – IL-23/IL-17 pro-inflammatory axis has been shown to have a major impact on intestinal inflammation. Genetically engineered lactic acid bacteria have already been proposed for the therapy of intestinal inflammation by interfering with pro-inflammatory cytokines and the pro-inflammatory cascade.

In this study, we applied our recently constructed pNBBX plasmid to assemble multiple gene cassettes and achieve controlled simultaneous expression of proteins interferring with IL-23/IL-17 axis. Our aim was to simultaneously target IL-23 and IL-17, as well as their receptors, to potentially achieve a synergistic effect.

For inhibition of IL-23, we used previously prepared albumin binding domain (ABD)-derived antagonists of IL-23 receptor (REX-ligands) and ILP317 protein against the p19 subunit of IL-23, which inhibits binding of the IL-23 to its receptor. To inhibit IL-17-mediated signaling, we made genetic constructs and investigated the secretion of ABD-antagonists specific for human IL-17 receptor (ARS-ligands). We also displayed the IL-17 binding Fynomer on the surface of L. lactis. Both pairs of binders (ILP/REX and Fynomer/ARS) were co-expressed to achieve synergy in preventing IL-23/IL-17 signaling.

The expression and function of each protein was evaluated and confirmed by western blotting, flow cytometry and ELISA assay. We established a cell model based on HEKBlue IL-17 cells and demonstrated that engineered L. lactis are capable of interfering with IL-17 signaling axis. Efficient simultaneous expression and function of proteins that interfere with the IL-23/IL-17 inflammatory pathway through simultaneous expression of IL-23 and IL-17 binders, and their receptor antagonists in L. lactis, shows their potential application in managing inflammation in intestinal diseases.

T24 - Efficiency of non-thermal plasma on the suppression of filamentous fungal biofilms

Presenting Author - *Markéta Kulišová, University Of Chemistry And Technology Prague, Czech Republic*

Author/s – *Irena Jarošová*

Abstract Content – Microscopic filamentous fungi are ubiquitous microorganisms that adapt very easily to a variety of environmental conditions. Due to this adaptability, they can colonize various surfaces where they are able to start forming biofilms that could be harmful, especially by the production of mycotoxins. With increasing resistance of microorganisms to known disinfectants, there is an urge to search for new possibilities of biofilm elimination/suppression, and non-thermal plasma (NTP) could serve as promising disinfectant agent. The objective of our study was to evaluate the effect of NTP on filamentous fungi spores and biofilms of *Alternaria alternata* DBM 4004, *Aspergillus niger* DBM 4054, *Fusarium culmorum* DBM 4044 and *Fusarium graminearum* DBM 4344. A corona discharge in a tip-ring electrode system has been used for plasma generation, and the NTP efficiency has been evaluated by the MTT spectrophotometric method determining the metabolic activity of biofilms. In all experiments on 24-hour and 48-hour cultured biofilms, a decrease in metabolic activity was observed compared to control samples. The most efficient was NTP on spores of all filamentous fungal strains tested (almost 100% decrease in metabolic activity compared to the control).

T25 - Assessing the potential of *Bacillus subtilis* as a heterologous host for expression of biosynthetic gene clusters

Presenting Author - *Hanne Put, KU Leuven, Belgium*

Author/s – *Maarten Fauvart, Jan Michiels*

Abstract Content

Antimicrobial resistance poses a major threat to the future use of antimicrobials, leaving a pressing need for new compounds. Fortunately, the sequencing revolution and metagenomic data have revealed an enormous potential in cryptic biosynthetic gene clusters (BGCs). In order to gain access to these uncharacterized compounds, heterologous expression has been proven a valid strategy, although previous research has mainly focused on only few host species, in particular *Escherichia coli* and *Streptomyces* spp. We reason that *Bacillus subtilis* is a very promising extension to the current toolbox for multiple reasons, including its ease of genome engineering and the richness of BGCs in closely related species, thus expanding the range of unknown BGCs to characterize in the future.

This study explores the use of the model organism *Bacillus subtilis* as a host for the heterologous expression of BGCs. A new expression vector was constructed, providing increased stability and copy number flexibility. The polymyxin BGC was used as a proof of concept and cloned on this BAC/YAC, followed by heterologous expression in a *B. subtilis* host strain that we engineered for optimal production of secondary metabolites. Ongoing work studies the effect of promoter exchange on BGC transcription and polymyxin production in *B. subtilis*, and assesses the engineering potential of the polymyxin non-ribosomal peptide synthetase. In conclusion, this study has added a BGC expression system for *B. subtilis* and aims to further engineer the clusters on a genomic level to uncover new compounds against the ongoing antibiotic resistance crisis.

T26 - N-Carbamoyl-β-alanine amidohydrolases from *Rhizobium radiobacter* MDC 8606 strain as a biocatalyst for amino acid synthesis

Presenting Author - *Mariam Karapetyan, Spc Armbiotechnology, Armenia*

Author/s – *Ani Paloyan, Armen Sargsyan, Hasmik Grigoryan*

Abstract Content

Introduction: Unnatural amino acids are valuable building blocks in the manufacture of a wide range of pharmaceuticals. In biotechnology they can be applied to investigate the structure and dynamics of proteins, to study protein interactions, or to modulate the activity of proteins in living cells, etc [1]. We have cloned, and characterised the N-carbamoyl-β-alanine amidohydrolase (RrCβAA), from *Rhizobium radiobacter* MDC 8606 strain of Microbial Depository Center of SPC "Armbiotechnology" NAS RA.

Methods: Specific activity of RrCβAA was determined using an assay able to detect β-alanine concentration, upon conversion into an isoindole derivative by reaction with ortho-phthalaldehyde [2].

Results: The substrate spectrum of RrCβAA was investigated toward various N-carbamoyl-DL- L- and D-amino acids. It was found that RrCβAA showed stereo specificity toward N-carbamoyl-L-amino acids with greatest catalytic efficiency for N-carbamoyl-L-β-alanine.

Our results indicate that RrCβAA has a distinct preference for N-carbamoyl-L-amino acids with linear R-groups and that its active site does not readily accommodate branched hydrophobic or aromatic sidechains. Conversely, RrCβAA displayed very low activity against N-carbamoyl-L-valine and N-carbamoyl-L-leucine. This idea has further confirmed when we study the enzyme activity toward N-carbamoyl-L- allyl-glycine, allyl alanine, propargyl glycine, propargyl alanine, etc. Enzyme showed activity toward all of above listed substrates with greatest activity toward N-carbamoyl-L-allyl-glycine. RrCβAA displayed good activity toward N-carbamoyl-L-methionine, which implies that the sulfur containing sidechain is accommodated within the active site in some way. Thus, RrCβAA can be used to obtain amino acids and their derivatives.

T27 - Actinobacteria Isolated from marine sponges and their antimicrobial and anticancer potential

Presenting Author - *Mariana Luísa Baião Ramos de Oliveira, Interdisciplinary Center Of Marine And Environmental Research (ciimar), Portugal*

Author/s – *Celso Domingos, Ilário Lucas Timba, Isidro Tamele, Joana Xavier, Ralph Urbatzka*

Abstract Content

Actinobacteria, which are often part of the microbiome of marine sponges, are important sources of bioactive molecules for drug development. Taking advantage of the high bioactive potential of these microorganisms, we aimed to investigate the culturable biodiversity of Actinobacteria associated to five different species of Demospongiae, and to evaluate their antimicrobial and anticancer activity. Six sponges were collected in Southern Mozambique and identified as *Haliclona* (Reniera) sp. (n=2), *Psammocinia* sp. (n=3) and *Callyspongia perforata* Pulitzer-Finali, 1993 (n=1). Three sponges were collected in Northern Portugal and identified as *Cliona cf. celata*, *Axinella* sp. and *Desmacidon fruticosum*. In total, over 180 strains belonging to the phylum Actinomycetota were isolated. *Micromonospora* (n=38) and *Streptomyces* (n=12) were the most frequent genera retrieved from the sponges collected in Mozambique, while *Micromonospora* (n=36) and *Microbacterium* (n=8) were the most prevalent isolates obtained from the sponges collected in Portugal. The organic extracts of these Actinobacteria were tested using the disk diffusion assay against Gram-positive and Gram-negative bacteria, as well as against *Candida albicans* (ATCC 10231). A strain of *Streptomyces lannensis* has been shown to inhibit the growth of *Staphylococcus aureus* (ATCC 29213) (20 mm halo) and *Bacillus subtilis* (ATCC 6633) (22 mm halo). Four other strains of Actinobacteria also exhibited antimicrobial activity, but with smaller halos. The anticancer activity of these Actinobacteria is currently being tested, using different cell lines. This work evidences that the microbiome of the studied marine sponges shelters a high diversity of Actinobacteria, which may be a potential source of novel bioactive compounds.

T28 - Production and stability evaluation of *Saccharomyces cerevisiae* L-BC virus-Like particles

Presenting Author - *Enrika Celitan, Vilnius University Life Sciences Centre, Lithuania*

Author/s – *Elena Serviene, Saulius Serva*

Abstract Content

Background: Within the past decade, self-assembly of various viral proteins into virus-like particles (VLPs) have been extensively addressed. These nanosized structures mimic the native virus properties, while lacking the respective genetic material. Therefore, they are considered as non-infectious and by loading with various biomolecules used as nanocarrier system. We focus on *Saccharomyces cerevisiae* L-BC virus major capsid protein Gag to create a VLP-based nanodelivery system.

Objectives: The aim of this study was to prepare, evaluate and compare stability of L-BC virus Gag VLPs, produced in bacterial and yeast cells.

Methods: L-BC Gag VLPs were synthesized in both *E. coli* and *S. cerevisiae* cells by use of inducible expression strategies. Nanoparticles were purified in homogeneous form by sucrose cushion and CsCl gradient ultracentrifugation methods. Structure of obtained particles was verified by transmission electron microscopy (TEM) and stability evaluated by dynamic light scattering (DLS) method.

Results: Initial DLS measurements showed that the diameter of the particles obtained from both bacterial and yeast cells was essentially the same and comparable to that of the native L BC virus. To provide a comprehensive stability analysis of purified VLPs, the effects of buffering agent, salt concentration, magnesium ions, pH and storage temperature were evaluated. Stability analysis demonstrated that in several month period L-BC Gag particles withstand various chemical and physical conditions. These results illustrate the potential of these particles to be developed into nanodelivery system.

T29 - The role of glycine-betaine in the hydrogen metabolism of *Ralstonia eutropha* H16

Presenting Author - *Meri Iskandaryan, Department of Biochemistry, Microbiology, and Biotechnology, Yerevan State University, Armenia*

Author/s – *Liana Mnatsakanian, Anna Poladyan*

Abstract Content

Glycine-Betaine (GB) has a diverse role in the bacteria, it acts as an osmoprotectant and source of carbon and nitrogen, and also has a beneficial effect on the non-stress physiology of bacteria: GB can act as a protein stabilizer. Since GB is an abundant material in the soil, some soil microorganisms use it by involving it in the metabolic pathways. The chemolithoautotrophic *Ralstonia eutropha* H16 is ubiquitous in soils and is the subject of intensive biotechnological research as a model organism for the production of oxygen-tolerant [NiFe]-Hydrogenases (Hyds) production. This study examined the effects of various GB concentrations (7.0µmol/ml-300µmol/ml) on bacterial growth parameters (optical density, oxidation-reduction potential(ORP), and pH kinetics), as well as H2-oxidizing Hyd activity. The Hyds activity of the whole cells is monitored by H_2-dependent methylene blue reduction at 570 nm. Bacterial growth stimulation was registered at GB concentrations of 7.0µmol/ml-25µmol/ml by 1.2-1.4 fold, however, high concentrations (100µmol/ml-300µmol/ml) have a partial inhibitory effect (10-20%), after 48 h, compared to control (Fructose-Nitrogen medium). In all samples, a slight decrease of pH was recorded, which correlated with a slight decline of ORP values, recorded by Ti-Si electrode, after 24 h of bacterial growth. Upon GB supplementation, the minimal and maximal H_2-oxidizing Hyd activity of *R. eutropha* whole cells were 3.4±0.01 U(g CDW)−1 and 16.4±0.01 U(g CDW)−1, respectively at concentrations of 7.0µmol/ml and 300µmol/ml, while it was absent in the control samples. These results might lead to the development of new approaches to produce oxygen-tolerant Hyds with high activity.

T30 - Validation for reuse of Single-Use bags in fermenters for cultivation of *Brucella abortus*

Presenting Author - *Ricardo Jordao, Biological Institute, Brazil*

Author/s – *Claudia Ribeiro, Rene Neto, Priscila Shahriyar, Felipe Santos, Cinthya Okamoto,*

Abstract Content

The use of Single-Use in the production of biologicals such as vaccines and diagnostic kits has increased. With the COVID-19 pandemic, there was a worldwide priority for eradication. Productions were focused on COVID-19, then on human diseases and finally on veterinary diseases. Increasing costs and delivery times a lot. Countries like Brazil ran out of inputs for production, especially for veterinary products. Single-use equipment cannot be easily converted to use a conventional steel tank, for example. To guarantee the continuity of the production of immunobiologicals, the most viable solution would be the reuse of single-use bags. However, this reuse should be validated in terms of yield, safety and quality of the final product. Using a 300L single-use fermenter, standard bags and *Brucella abortus* bacteria, yields can be compared. After traditional cultivation with 300 L, the total volume was collected aseptically, and 1 L was kept as inoculum. And a second batch of culture medium is added.

The challenge was to collect the crop in an aseptic and safe way, but thinking that the hoses will be used again. Success was obtained in a second batch, the exhaust filters were critical, due to condensation, so as not to clog and increase the pressure in the bag. In conventional fermenters, the second batch has a higher yield, and the same happened with the Single Use reuse, but some aeration and agitation parameters had to be changed. For a third batch a hose welder is needed, or customize with more filters.

T31 - An investigation into the impact of the signal peptide of a biocatalytically active oxidoreductase

Presenting Author - *James Britton, Instituto Ramón Y Cajal De Investigación Sanitaria (irycis), Ireland*

Author/s – *Reeta Davis, David O'Connell, Tanja Narancic*

Abstract Content

Oxidoreductases are a class of biotechnologically important oxidizing enzymes with applications in bioremediation, biocatalysis and as biosensors. Oxidoreductases have been used to catalyze the hydroxylation of aromatic hydrocarbons to produce both monophenols and o-diphenols. A bacterial oxidoreductase from *R. solanacearum* has been identified with the capacity to convert monophenols into catechols. This enzyme contains an N-terminal signal peptide (SP) predicted to direct it to the twin arginine translocase (TAT), a widely conserved but seldom used system which allows transport of folded substrate proteins from the cytoplasm into the periplasm. However, this system is inefficient for biotechnological processes due to the low number of pores per cell and the long transport time required per protein, often resulting in build-ups of insoluble protein matter when a protein targeted to this system is overexpressed.

This study investigates the expression of the oxidoreductase, focusing on the impact of its SP. The gene encoding the oxidoreductase was expressed in *E. coli* BL21. Confirmation of the use of the TAT system by this enzyme was carried out by cell fractionation and affinity tagging the protein of interest. The impact of removing the SP, replacing the SP with SPs native to *E. coli* and expression of the enzyme in a host with the TAT translocase chromosomally deleted were all investigated to improve levels of soluble active enzyme produced.

T32 - Chimeric proteins for the study of protein interactions in the bacterial outer membrane

Presenting Author - *Álvaro Ceballos-Munuera, Centro Nacional de Biotecnología (CNB-CSIC), Spain*

Author/s – *Alejandro Prieto, Luis Ángel Fernández*

Abstract Content

Background: Dynamics of outer membrane proteins (OMP) in Gram-negative bacteria are not yet fully understood. OMPs do not diffuse freely, restricted to specific regions around their insertion point referred to as OMP islands. Studies suggest that this confinement could be due to interactions between the β-barrels, both homologous and heterologous. However, further research is required.

Objectives: We aimed to develop chimeric proteins that allow the study of protein-protein interactions in the outer membrane (OM). For this, we engineered chimeric OMPs to present functional domains both in the bacterial surface and the periplasm that could help determining such interactions.

Methods: Chimeric OMP were generated using the β-barrels from *E. coli* intimin and EhaA, as well as *Y. pseudotuberculosis* invasin. Extracellular passengers were substituted with a single-domain antibody (nanobody) while periplasmic sides were modified to contain the fragments of a split luciferase reporter. Bacteria expressing these chimeric proteins were analyzed by flow cytometry and Western blot to test their correct insertion in the OM, surface display and antigen-binding functionality of the nanobody. Protein-protein interactions were determined by measuring luminescence produced by *E. coli* bacteria co-expressing complementary proteins pairs carrying the luciferase fragments.

Results: We obtained chimeric proteins that correctly insert in the OM of *E. coli* while simultaneously displaying a functional nanobody in the bacterial surface and a luciferase fragment or peptide in the periplasm. Luminescence generated by complementary chimeras suggest that this system could be used for studying interactions between OMPs.

T33 - Quest for novel carriers for antisense oligonucleotides into bacteria using synthetic biology

Presenting Author - *Paramita Sarkar, University of Wurzburg, Germany*

Author/s – *Linda Popella, Jörg Vogel*

Abstract Content

Antisense antibiotics have proved to be viable alternatives to conventional antibiotics. However, they are limited by a lack of efficient carriers for the translocation of antisense oligonucleotides (ASOs) across the bacterial membrane. Additionally, there is no strategy to directly report their uptake. To address this, we develop a reporter to screen ASO carriers' penetration into the cell. Reporter systems wherein ASOs mimic the sRNA functions were developed resulting in up to ~60-fold upregulation in reporter protein (sfGFP) production. The dynamic range and applicability of the reporter was validated by screening activation of different bacteria-penetrating-peptide-ASO conjugates (BPP-ASO). Screening in different strains of *E. coli* and *S. enterica* showed differential efficacy upon treatment with different BPP-ASO conjugates. The reporter systems are expected to lead the way toward the discovery of species-specific ASO carriers.

T34 - How to steer pyocyanin production by *Pseudomonas aeruginosa* using zinc oxide nanoparticles?

Presenting Author - *Joanna Jablonska, West Pomeranian University of Technology in Szczecin, Poland*

Author/s – *Kamila Dubrowska, Adrian Augustyniak, Rafał Rakoczy*

Abstract Content

Background: Pyocyanin is a pigment produced by *Pseudomonas aeruginosa* and a well-known virulence factor that causes complications in wound healing or cystic fibrosis. However, recently pyocyanin is also recognised as a potentially utile chemical for energy generation and cancer therapy. It has been proven that nanomaterials, e.g., zinc oxide nanoparticles (ZnO NPs), can lower pyocyanin production. Nevertheless, it was also observed that low concentrations could cause opposite effects. However, it is not fully understood if such observations are intertwined with other changes in the culture.

Objectives: In this study, we tested the influence of zinc oxide nanoparticles on *Pseudomonas aeruginosa* culture on pyocyanin production and other physiological changes.

Methods: The experiments were conducted on *P. aeruginosa* ATCC27853 (PA) cultivated in King's A broth. Pyocyanin was extracted with chloroform, reextracted with HCl and quantified spectrophotometrically. The cultures exhibiting altered pyocyanin production than the control were monitored, including the growth, viability, and biomass production.

Results: Pyocyanin production is stimulated by the addition of low dosages of ZnO NPs and process temperatures, whereas higher nanoparticles' concentrations and temperature inhibited the production of this pigment but stimulated the production of the biomass and led to changes in the culture consistency. The interaction between ZnO NPs and PA may have double-edged consequences and stimulate or inhibit pyocyanin production and other physiological traits of PA, depending on the concentration and temperature. This outcome presents a chance to induce pyocyanin production for biotechnological purposes, as opposed to the possible ecological and medical threats arising from this phenomenon.

T35 - A biotechnology tool to detect integron gene cassettes

Presenting Author - *Filipa Trigo da Roza, Complutense University of Madrid, Spain*

Author/s – *Paula Blanco, Alberto Hipólito, Ester Vergara, Modesto Redrejo, Rocío Lopéz-Igual, José Antonio Escudero,*

Abstract Content

Background: Mobile integrons are genetic platforms that can capture and stockpile gene cassettes, such as antimicrobial resistance genes1. They have played a central role in the rise and increase of multidrug-resistant bacteria2, but despite their importance, our tools to detect cassettes are still inefficient. PCR methods are usually biased and deep sequencing is still not amenable to routine analysis.

Objectives: Here, we seek to develop a novel biotechnological tool to identify, for the first time, integron cassettes independently of their sequence and genetic background.

Methods and Results: We have re-engineered an integron and used a toxin as a reporter, in a way that captured cassettes will disrupt the toxin, only allowing recombinants to survive. This counter-selectable marker provides a broad dynamic range – 106 – for detecting cassettes. Through conjugation/recombination assays, we have verified that our tool captures cassettes above the limit of detection. We then tested this tool in a plasmid setup to capture chromosomal integron cassettes. Ectopically inducing the integrase produced a library that will be used to study the unknown functions of these chromosomal cassettes. Finally, we combined a genetically-modified naturally competent bacterium and DNA amplification using a hyper-processive polymerase to make this tool directly usable on DNA samples. We have demonstrated that this tool is capable of unveiling the integron content of different samples – in a highly-specific and sequence-independent manner – and will potentially become a ground-breaking diagnostic method for these antimicrobial resistance platforms, as well as a solid method to study genes that were until now inaccessible.

T36 - Exploiting the untapped potential of *Paracoccus* sp. as a novel chassis organism

Presenting Author - *Upasana Pal, Rwth Aachen University, Germany*

Abstract Content

In this study, we investigate the untapped potential of the genus *Paracoccus*, belonging to the family Rhodobacteraceae and the phylum Alphaproteobacteria. Strains of this genus possess a broad substrate range, osmotolerance, and good phenotypic robustness. Moreover, it is a fantastic choice in light of the circular bioeconomy, to use CO2, organic acids, and low-cost sugars as carbon sources to synthesize the biopolymer polyhydroxyalkanoate and the fine chemicals carotenoids.

In order to establish a new chassis, studies were performed exploring substrate utilization and tolerance and optimal growth temperature and pH values covering over fifty strains from this genus. In addition to the generation of physiological data, metagenomic studies including core- and pan-genome analyses were performed offering evolutionary relationships. In addition, to elucidate the metabolic flux *in vivo*, an in-depth analysis of flux distributions was performed in the type-strain *Paracoccus pantotrophus* DSM 2944T using 13C labeled glucose. The flux map showed that *P. pantotrophus* uses the pentose phosphate pathway and Entner–Doudoroff over glycolysis offering a surplus co-factor regeneration coupled with energy generation. A *Paracoccus*-specific genetic toolbox was designed comprising promoters with tunable strengths, gene integration and deletion strategies, and compatible origins of replication. Highlights of genetic engineering include growth on the non-native carbon-source terephthalic acid along with native carbon-source ethylene glycol, the two monomers from polyethylene terephthalate, for polyhydroxyalkanoate production. Finally, tailor-made fermentation strategies were established for *Paracoccus*, focusing on product upscaling.

The results show that the metabolically versatile *Paracoccus* deserves the title of being an upcoming chassis organism.

T37 - Stirred-tank reactor bioleaching options for the recovery of nickel and cobalt of Brazilian laterite ores

Presenting Author - *Stefanie Hetz, Federal Institute For Geosciences And Natural Resources, Germany*

Author/s – *Axel Schippers*

Abstract Content

Laterite ore deposits in Brazil and other tropical countries contain approximately 70% of the world's nickel and cobalt resources and other critical materials of commercial importance. Considering economic efficiency, developing an integrated low-energy and environmentally benign bio-hydrometallurgical process for recovering these metals from laterite ores in Brazil is the aim of the German-Brazilian project BioProLat. Bioleaching of the oxidized minerals is based on the published Ferredox concept using bacteria to reduce insoluble metal compounds to a water-soluble form at low pH. Suitable acidophiles include chemolithotrophic *AcidtihioBacillus* spp., mixotrophic *SulfoBacillus* spp., and obligate heterotrophic *Acidiphilum* sp., of which many can oxidize sulfur and gen-erate sulfuric acid, which maintains the acidic milieu needed. On a laboratory scale, 2 L stirred-tank bioreactor bioleaching experiments are used to optimize parameters, including, amongst others, bacterial consortia, pH, temperature, and aeration, for the recovery of nickel and cobalt. Mineralogical and geochemical analysis is used to estimate released metal portions by bioleaching, while molecular analysis is used to monitor cell numbers and composition of applied bacteria. Results of laterite bioleaching experiments, using a consortium consisting of different *AcidithioBacillus thiooxidans* strains, showed a recovery of 67-83% for cobalt and 32-83% for nickel, with lower pH increasing recovery rates for both metals. Eventually, the optimized process will be upscaled and reach pilot scale, transforming unexploited ores and limonite stockpiles into valuable resources and unlocking new reserves of raw materials through increasing metals recovery from existing mines.

T38 - Development of episomal plasmids for engineering of non-conventional yeast *Wickerhamomyces ciferrii*

Presenting Author - *Seong-Rae Lee, Ajou University, Republic of Korea*

Author/s – *Pyung Cheon Lee*

Abstract Content

Wickerhamomyces ciferrii is a non-conventional yeast with high tetraacetyl phytosphingosine (TAPS) productivity and secretion ability. Although *W. ciferrii* was well recognized as a TAPS-producer, there have been few studies for development of genetic engineering tools for the non-conventional strain *W. ciferrii*. Especially, the episomal plasmid system is one of the essential genetic engineering tools, but it has not yet been reported for *W. ciferrii*. In this study, a few antibiotics were screened for their efficacy as a selective pressure in the episomal plasmid system. Next, the functions of heterogeneous replication origins commonly used in other yeast species in *W. ciferrii* were investigated based on the colony-forming ability of recombinant cells on the selective agar plates and the growing of recombinant cells in a liquid medium supplemented with an antibiotic. Finally, the heterologous expression of several fluorescent proteins on the episomal plasmid system was analyzed in recombinant *W. ciferrii*. The established episomal plasmid system could play an important role in the system-level metabolic engineering of *W. ciferrii*.

T39 - The influence of carbon catabolite repression on polyhydroxyalkanoate (PHA) metabolism in *Pseudomonas putida* KT2440

Presenting Author - *Yixin Che, Instituto Ramón Y Cajal De Investigación Sanitaria (irycis), Ireland*

Author/s – *Tanja Narancic, Kevin O'Connor*

Abstract Content

Background: Polyhydroxyalkanoates (PHAs) are biocompatible and biodegradable polymers, which can be synthesized and degraded by a wide variety of microorganisms. As a valuable plastic alternative, the synthesis of medium chain-length PHAs has been extensively studied in *Pseudomonas putida*. It has been shown that several genes in PHA pathways are under the regulation of carbon catabolite repression (CCR), which allows a fast adaptation of bacteria to the changing nutrient supplies.

Objectives: To understand how CCR regulate PHA synthesis in *P. putida* KT2440, and to fine-tune PHA metabolism to enhance PHA production using synthetic biology tools.

Methods: CCR system components (Crc and Hfq proteins, and sRNAs CrcY/CrcZ) were deleted from KT2440 via CRISPR/Cas9, and CrcY/CrcZ were overexpressed from pBT'T vector. The growth profiles, PHA production and expression level of PhaC1 polymerase (the key enzyme of PHA metabolism) had been investigated in different culture conditions toward deletion and overexpressing strains.

Results: CrcY and CrcZ overexpression can enhance PHA production. The highest effect was observed with octanoate under nitrogen limitation, where the CrcY overexpression strain produced 62.51% of PHA of total cell dry weight (CDW), 1.2- and 2-fold higher compared to CrcZ overexpression and the control strain. However, this effect may be the result of sRNAs interaction with targets other than Crc regulatory protein, proposed to be their main target.

T40 - A proteomic investigation of polyhydroxyalkanoate granules in *Pseudomonas putida* KT2440

Presenting Author - *Jia-Lynn Tham, Instituto Ramón Y Cajal De Investigación Sanitaria (irycis), Ireland*

Author/s – *Gerard Cagney, Tanja Narancic*

Abstract Content

Polyhydroxyalkanoates (PHAs) are biodegradable polyesters composed of (R)-3-hydroxy fatty acids produced by a wide range of bacteria under conditions of nutrient imbalance. PHAs are stored intracellularly as water-insoluble granules, serving as carbon and energy stores that can be degraded in times of starvation. These granules are complex subcellular structures and electron microscopy data has shown that the hydrophobic polyester core is surrounded by a boundary layer of proteins known as granule associated proteins (GAPs). GAPs are believed to comprise PHA synthesis, structural and regulatory proteins. Phasins, a group of low-molecular-weight proteins, make up the majority of GAPs. Whilst there has been substantial research focused on understanding the PHA metabolism pathways, only 2 phasins (PhaF and PhaI) have been characterised in *Pseudomonas putida* KT2440 to date. Additionally, the exact composition and surface structure of PHA granules as well as the processes that take place during granule biosynthesis and degradation remain unknown, possibly because some proteins involved in PHA metabolism remain to be characterised.

This study completely characterised the PHA-ome, including GAPs using proteomic techniques. Multiple PHA-inducing conditions were used to stimulate PHA synthesis in KT2440 and changes that occur across the whole proteome were analysed in order to identify proteins that are significantly dysregulated or exclusively present under PHA-accumulating conditions. Since the proteomic analysis of isolated PHA granules is prone to false-positive results due to the artificial binding of proteins during cell disruption, we performed a whole cell comparative proteomics study using a PHA-negative mutant to identify background changes.

T41 - mRNA construction encoding the immunogenic fragment of omicron SARS-CoV-2 spike protein

Presenting Author - *Raman Kazakou, The Institute of Microbiology, NAS, Belarus*

Author/s – *Illia Kazlouski, Ina Belskaya, Anatoly Zinchenko*

Abstract Content

Background: Due to high ongoing incidence of COVID-19 cases the development of new effective types of vaccines as the main preventive measure against hospitalization and death from COVID-19 remains an area of special scientific interest. Among the wide range of currently approved vaccines, the mRNA platform shows promising results with proved high efficacy against severe cases of SARS-CoV-2 infection. However, obtaining the mRNA-based product mainly involves chemical synthesis.

Objectives: The work is aimed to present a cost-effective approach, in which mRNA is obtained by *in vitro* transcription of linear DNA template using chimeric T7-RNA-polymerase with the control of preventing mutation synthetized RNA.

Methods: To obtain vector DNA all the nucleotide sequences encoding RBD SARS-CoV-2 and the necessary regulatory elements were inserted into the pET-42a(+) plasmid using circular polymerase extension cloning. The mRNA synthesis was carried out by the chimeric SS07d-T7-RNA polymerase.

Results: Nucleotide sequences of regulatory elements such as 5'-UTR, 3'-UTR, signal peptide and polyA were inserted into the pET-42a(+) vector, where the final genetic construct was a universal vector for subsequent insertion of any nucleotide sequence encoding the target antigen. The next stage included RBD-encoding nucleotide sequence cloning and integration into the universal construct, so that the polypeptide could have been synthesized in human cells. Due to the presence of T7-RNA polymerase recognition sites mRNA construction containing all the necessary regulatory elements of translation and the nucleotide sequence of the RBD was obtained, which resulted in simple and relatively inexpensive technology for the modern generation vaccines development.

T42 - Establishing a high-throughput pipeline to speed up recombinant protein expression data generation by microbial cell factories

Presenting Author - *Aske Unger, Dtu Bioengineering, Denmark*

Author/s – *Aske Unger, Andreas Worberg, Anders B. Sørensen, José Luis Martinez Ruiz*

Abstract Content

Machine learning is a powerful tool in biology, particularly for predicting complex problems. However, to make accurate predictions, it requires large amounts of reliable data. While it has been used for predicting protein structures, protein expression has proven more complicated due to the large parameter space. To overcome this challenge, we have developed a standardized high-throughput method for gaining in-depth knowledge about protein expression and solubility in order to speed up the design and selection of efficient microbial cell factories with higher expression capabilities.

In this work we describe how to create a pipeline for obtaining protein expression and solubility data. We modified the ORF-selector ESPRIT system to create truncation expression libraries and implemented a novel barcoding system in combination with Nanopore's Flongle technology, which enables multiplexed sequencing while keeping low consumables costs and high throughput as the main factors. To enable this, split luciferase was used to yield data about both soluble and total protein expression.

The system is automated on a Hamilton robot with an incorporated plate reader and automated incubator for autonomous cultivation. The throughput of the growth and expression screening is approximately 1090* samples per day, yielding OD600 curves and data on total/soluble protein expression. The system has been tested using *E. coli* BL21(de3) and a pET-base vector plasmid as a proof of concept, but the cloning system has been set up for broadening into other plasmids, strains or organisms.

*16 96x plates with a 71% success rate when removing cloning and sequencing errors.

T43 - Changes in metabolites and gene expressions during electro-fermentation of *Citrobacter braakii* TB-96

Presenting Author - *Takuma Yanase, University Of Miyazaki, Japan*

Author/s – *Keiji Kiyoshi, Naoto Yoshida, Kengo Inoue*

Abstract Content

The *Citrobacter braakii* strain TB-96 produces 1,3-propanediol (1,3-PD) from glycerol but also produces a large amount of by-products such as lactic acid.

Electro-fermentation (EF) was applied to repress the by-products production. EF is a cultivation technique which changes the metabolism of microorganisms during fermentation by supplying extracellular electrons via electrode. However, the details of the effects of EF on microorganisms are not well understood and require further investigation. In this research, we attempted to investigate the effect of EF.

In strain TB-96, EF leaded to reduce lactic acid production, a major by-product, but not change 1,3-PD production. This suggests that EF caused some changes in the metabolism of strain TB-96.

To investigate the effects of EF more specifically, changes in protein and gene expression were analyzed. Comparing protein expression TB-96 with/without EF by SDS-PAGE were performed, but no significant changes were observed. However, expression levels of genes related to glycerol metabolism were measured by RT-qPCR. As a result, the expression of glycerol dehydratase and glycerol dehydrogenase were decreased in EF. Glycerol dehydrogenase oxidizes glycerol and produce NADH, which caused its expression to be suppressed by negative feedback under NADH-rich EF conditions. Further investigation is needed to clarify the mechanism of the effect of EF.

T44 - Use of solid, vegetable food waste as production medium for the metacycloprodigiosin producer *Streptomyces spectabilis*

Presenting Author - *Janina Krause, Osnabrück University, Germany*

Author/s – *Nicole Steiner*

Abstract Content – Sustainability has become an imperative in all areas of life including industrial production processes. According to the Food and Agricultural Organization of the United Nations immense losses arise in production- as well as in supply chains of produced food. Meanwhile, these wastes contain an abundance of valuable nutrients. Thus, the idea arose to use these losses to create media for the production of medically relevant secondary metabolites from microbes.

In our experiments we tested peels from potatoes, pineapples, bananas and apples as well as coffee grounds as sole ingredients for solid and liquid production media. *Streptomyces spectabilis* was used as test organism as it produces the antibiotic metacycloprodigiosin. Its presence can easily be observed due to red pigmentation. Metabolic activity of the bacterium (by dry cell weight, reducing sugars, pH) and production of metacycloprodigiosin was monitored during fermentation. The standard medium IPS2 was used as a control. Metacycloprodigiosin production could be observed on solid and in liquid ISP2- and potato peel media. The presence of the antibiotic was further verified via thin layer chromatography and spectroscopic measurements following extraction of the culture medium with ethyl acetate. After optimization of fermentation conditions, production levels in potato peel medium are above those of ISP2.

We demonstrated that potato peel medium is suitable for the production of metacycloprodigiosin by *S. spectabilis*. The possibility of bioconversion from potato waste to drugs should be evaluated for other medically relevant secondary metabolite producers and on large scale.

T45 - Valorization of lignocellulosic greenhouse residues into mycelium-based composite materials

Presenting Author - *Marie Bonduelle, Armand-Frappier Santé Biotechnologie Research Centre, Canada*

Author/s – *Audrey-Anne Durand, Simon Barnabé, Philippe Constant*

Abstract Content

Greenhouse vegetable growers generate about 290 tons of lignocellulosic residues per hectare per year. Landfilling is the most widespread management practices implemented, which depletes the soil of nitrogen and generates greenhouse gases. Thus, it is necessary to implement fast and sustainable alternative processes for a better management of lignocellulosic residues. The valorization of this biomass into mycelium-based composite materials, also known as “mycomaterials”, is a promising solution. This biotechnology relies on the growth of white rot fungi to transform crop waste into biodegradable materials. This project was aimed at producing mycomaterials with coconut fiber, used as a growing substrate for bell pepper plants. The objective was to produce materials with mechanical properties similar to those of expanded polystyrene, which is the main synthetic competitor. After three weeks of fungi colonization, the compressive strength at 10% sample (10.2 x 7.5 x 2 cm) deformation was 213.2 ±10.39 kPa and 190.5 ± 32.25 kPa for those obtained with *P. ostreatus* and *G. lucidum* fungi, respectively, and 392.9 ± 32.43 kPa for the expanded polystyrene samples. These mycomaterials also demonstrated hydrophobic properties following water absorption analyses. Further studies on the optimal growth conditions of fungi are underway to accelerate the production and improve the mechanical properties of the biomaterials. Isolation efforts and screening of environmental strains of fungi are combined with the assembly of synthetic bacterial communities and nutrient amendments. Optimization of mycomaterials process and mechanical properties is expected to strengthen their competitiveness against plastic polymers.

T46 - *In silico* binding predictions of enterolysin A to peptidoglycan fragments from Gram-positive and Gram-negative bacteria

Presenting Author - *Yared Bezabhe, Orebro University, Sweden*

Author/s – *Solomon Gebre-Selassie, Abraham Aseffa, Per-Erik Olsson, Jana Jass*

Abstract Content

Background: Antibiotic resistant infectious diseases are a global health emergency. The identification of new antibiotic targets within bacteria and development of new antibiotics is a priority research area. Natural bioactive substances remain an important source of new solutions to antibiotic resistance. Bacteriocins are a group being evaluated for potential use against multidrug resistant microbes. *In silico* screening is promising strategy for determining novel bacteriocin activity rapidly and efficiently.

Objective: To use in-silico prediction for binding of peptidoglycan fragments to Enterolysin A, a bacteriocin found in *LacticaseiBacillus paracasei* TI1258 with antimicrobial and antibiofilm activity.

Methods: Antimicrobial and antibiofilm activity of *L. paracasei* culture supernatants was done using microbroth dilution assay. Biofilms were examined by microscopy. Enterolysin A was identified from whole genome sequencing. *In silico* binding was predicted using SWISS-model and Internal Coordinate Mechanics (ICM 3.8.1).

Results: A crude extract containing Enterolysin A showed activity against *Pseudomonas aeruginosa* PAO1. The C-terminal sequence of Enterolysin A was modelled with C-chain of *Staphylococcus aureus* autolysin. Binding predictions of the model produced high-score interactions with N-acetylglucosamine (NAG) (ICM score, -22.6±5), N-acetylmuramic acid (NAM) (-22.3±3), penicillin-binding proteins (-28.4±1), and glycyl-l-alpha-amino-epsilon-timely-d-alanyl-d-alanine (-25.4±4) peptidoglycan ligands. An interaction model with NAG and NAM produced hydrogen-bonds and hydrophobic contacts that stabilize ligands in the active sites. These fragments are known targets for cell wall specific antibiotics. The *in silico* screening offered a rapid method for identification of a potential targets of the bacteriocin activity.

T47 - Isolation and characterization of probiotics from Egypt's Wadi El Natrun soda lake with superior levan productivity

Presenting Author - *Noura Abdelsamad, The American University in Cairo, Egypt*

Author/s – *Noura Abdelsamad, Rehab Abdallah, Mariam Elnahhas, Rania Siam*

Abstract Content

Bacterial antibiotic resistance is considered the next pandemic, and the need for alternatives is now a necessity. Probiotics represent eco-friendly alternatives to antibiotics. Probiotics isolated from saline and hypersaline environments are known to synthesize diverse classes of bioactive compounds. Previous studies have isolated antioxidant and antibacterial-producing microbes in Egypt's Wadi El Natrun soda lake. Yet, it remains an unexplored environment for levan-producing probiotics. Levan is a microbial exopolysaccharide with anti-inflammatory, anticancer, and antioxidant activities. Here we aim to isolate levan-producing probiotic bacteria from Wadi El Natrun sediments. Littoral sediments were collected in November 2021 and 40 different bacterial isolates were identified using casein nutrient agar and starch casein agar. Levan assay, casein hydrolysis, catalase, oxidase, fermentation tests, and 16 S rRNA gene sequencing were performed to characterize the isolates. Our data shows that 9 *Lactobacillus* isolates were able to produce Levan with a yield ranging from 96.4 g/L to ~117 g/L using only 100 g/L sucrose. The antibacterial and antitumor activity of the extracted Levan against a spectrum of bacterial species and cancer cell lines will be presented. The analysis of the consumption safety of the levan-producing bacteria, using blood hemolysis, acid and base tolerance, bile salt tolerance, antibiotic sensitivity, and cholesterol oxidase assays, will be presented. In conclusion, levan-producing bacteria from Egypt's Wadi El Natrun soda lake showed higher Levan productivity using lower sucrose concentration (100 g/L) compared to other previously reported Levan-producing bacteria isolated from soil and food and are potential probiotic Candidates.

T48 - Microbial therapeutics for lung cancer based on immune-checkpoint inhibitory scFv-producing engineered lactic acid bacteria

Presenting Author - *Fu Namai, Tohoku University / Shinshu University, Japan*

Author/s – *Haruki Kitazawa, Takashi Sato, Takeshi Shimosato*

Abstract Content

Background: Genetically modified lactic acid bacteria (gmLAB) can produce a variety of functional proteins by incorporating gene expression vectors and can be administered directly *in vivo* because they lack the endotoxins of *E. coli*, another common gene expression host. Therefore, gmLAB are expected to see use in microbial therapeutics to produce therapeutic proteins against diseases.

Objectives: To develop microbial therapeutics against cancer, we focused on immune checkpoint inhibitors (ICI). ICI can inhibit tumor immunosuppression and activate anti-tumor immunity. Here, we constructed a gmLAB strain that produces a single-chain fragment variable (scFv) that binds to programmed death ligand 1 (PD-L1), an immune checkpoint molecule, then examined the anti-tumor effects of intranasal administration using a metastatic lung cancer mouse model.

Methods: The expression vector with the designed anti-PD-L1 scFv (PDL1scFv) sequence was introduced into *Lactococcus lactis*. Expression of PDL1scFv was detected by western blot analysis. B16F1 cancer cells were injected through the tail vein of mice to create a mouse model of metastatic lung cancer. One week after model creation, gmLAB (1.0x10^9) were nasally administered daily to investigate the 35-day survival rate.

Results: Western blotting showed a 29.6-kDa band matching PDL1scFv in the gmLAB with the PDL1scFv expression vector, whereas no band was observed in the control gmLAB with an empty vector. In the *in vivo* study, significant improvement in survival at 35 days was observed in mice treated with intranasal administration of PDL1scFv-producing gmLAB (62.5%) compared with mice treated either with control gmLAB (0%) or PBS (0%) intranasally.

T50 - Discovery of the Pathway for 5-hydroxymethylfurfural Oxidation in *Pseudomonas umsongensis* GO16

Presenting Author - *Rhys Orimaco, Instituto Ramón Y Cajal De Investigación Sanitaria (irycis), Ireland*

Author/s – *Pauric Donnelly, Seán Sexton, Tanja Narancic*

Abstract Content

5-hydroxymethylfurfural (HMF) is a furanic aldehyde byproduct of chemically treated lignocellulosic biomass. HMF inhibits microbial metabolism, hindering usage of lignocellulose hydrolysates as fermentation substrates. Some bacteria have been described with the capacity to metabolise HMF, via upper pathway oxidation to furoic acid. Lower pathway ring cleavage of furoic acid terminates at α-ketoglutarate, a TCA cycle intermediate. This paves the way for a biological process to remove HMF toxicity from biomass derived substrates.

In silico analyses show that *Pseudomonas umsongensis* GO16, which can metabolise a range of aromatic compounds, has an HMF metabolising operon similar to the previously characterised *P. putida* ALS1267 [1]. This operon is functional but lacks homologs to known enzymes involved in HMF oxidation to furoic acid. The identity of these enzymes is being determined to better understand and harness HMF metabolism in *P. umsongenesis* GO16.

Present in the operon are PsfA and PsfG, aldehyde and alcohol dehydrogenases respectively. Both enzymes oxidise furfuryl alcohol, an HMF analog, into furoic acid. Hypothesising that they could also oxidise HMF, both enzymes have been overexpressed in trans in *P. umsongenesis* GO16 .

Overexpression of PsfG yields half the biomass of wild-type *P. umsongenesis* GO16 when HMF is the carbon substrate, while overexpression of PsfA leads to no growth whatsoever. Coexpression of the two enzymes together is being tested as it may be necessary to prevent the build-up of toxic intermediates. Knockout strains of both enzymes are also being constructed to further characterise their putative role in HMF metabolism.

T51 - Engineering of the pesticides removing recombinant *Escherichia coli* by displaying the specific binding peptide

Presenting Author - *SoonHo Hong, University Of Ulsan, Republic of Korea*

Author/s – *Jae Hoon Jeong, Ashokkumar Kumaravel, Soon Ho Hong*

Abstract Content

The constructed *Escherichia coli* efficiently adsorbed fenitrothion through the displaying a pesticide-binding peptide on it using the anchoring motif OmpC. Fenitrothion, or O, O-dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate, is a nitrophenolic pesticide that was widely used in agriculture. The inhibition of acetylcholine esterase activity and inhibition of acetylcholine breakdown in synapses result in the accumulation of acetylcholine in synapses, which causes convulsions, paralysis, and death of insects. However, under aerobic conditions, the major hydrolysis metabolite of fenitrothion, 3-methyl-4-nitrophenol, is toxic to many living organisms, raising environmental safety concerns. In this study, fenitrothion-adsorbing recombinant *Escherichia coli* was constructed through the display of pesticide-binding peptide, using OmpC as an anchoring motif.

T52 - Engineering and in situ monitoring of environmental parameters of structured phototrophic microbial communities in hydrogels

Presenting Author - *Christian Danneberg, Leipzig University, Germany*

Author/s – *Matthias Portius, Rohan Karande, Tilo Pompe*

Abstract Content

In nature, phototrophic microbial mats are a consortium of vertically structured microbial communities that share common living space in a self-produced polymeric matrix. Such microbial mats are a remarkable biological (eco) system that has evolved in nature for effective energy and material flow, virtually unexplored for biotechnological applications. In this context, an adequate toolbox is necessary to evaluate and optimize the process parameters of best-performing microbial composition, structure, and culture conditions with access to mass and energy fluxes. One strategy is the usage of immobilized cells in structured material scaffolds to modulate and analyse mass and energy balances with the goal of designing vertically structured phototrophic biofilms.

In this work, we have been engineering a synthetic layered hydrogel model based on agarose layers as a cultivation toolbox for embedding different types of phototrophic and non-phototrophic bacteria. The model system will allow a quantitative understanding of light, O2 and metabolite transfer, which is crucial to obtain control of growth, stability, and biotechnological performance. The microbial communities' structure, long-term stability, and developing metabolite gradients are investigated in a flow-cell system. First results show a stable cultivation of structured mixed-species phototrophic microbial communities of *Synechocystis* PCC 6803 and *Pseudomonas taiwanensis* VLB 120_eGFP with options for adjusting structural arrangement, cell growth and quantitative live-cell microscopic analysis. Here, it could be shown that the growth rate of the bacteria is influenced by the agarose concentration. Recent results demonstrate the applicability of implementing functionalized nanoparticles to detect O2 content in these hydrogel systems.

T53 - High yield expression of Norovirus GI.3 major capsid in *Escherichia coli*-based system

Presenting Author - *Illia Kazlouski, Belarus*

Author/s – *Raman Kazakou, Ina Belskaya, Natallia Paklonskaya, Tatsiana Yudziankova, Anatoly Zinchenko, Tamara Amvrosieva, The Republican Research and Practical Center for Epidemiology and Microbiology, Minsk, Belarus*

Abstract Content

Background: Human noroviruses (NV) are the main etiological agents of acute gastroenteritis outbreaks. Causing morbidity in all age groups laboratory diagnosis of norovirus infection is extremely relevant with predominant focusing on prevalent genotypes (GII.4, GII.17 and GI.3). It is recommended to use rapid tests to carry out diagnostics of norovirus infection. However, the development of express kits meets several challenges such as a high degree of antigenic variation, the uncultivable nature of NV and reported low antigen yield obtained in different expression systems, which affect the common market for the presented kits for NV detection.

Objectives: The aim of this study was to obtain the full-length NV GI.3 capsid protein (VP1) and its P-domain for the further creation of express kits for NV infection diagnostics.

Methods: Integration of genes encoding the VP1 GI.3 and its P-domain into expression vectors was performed by the Ligation Independent Cloning. The target polypeptides were expressed by *Escherichia coli* Bl21 (DE3).

Results: Cold shock-induced expression in *E. coli* (pColdI vector) resulted in high yield production of NV GI.3 VP1 while P-domain was obtained using the classical pET42 vector. All the target proteins were shown to exhibit immunogenic properties required for NV specific antibody production as the main component of expression kit. Prospective technologies based on the obtained antigen proteins would allow to expand the range of available diagnostic kits for the detection of NV genogroup I antigen.

T54 - Endosymbiont as chassis in synthetic biology: improving the culture media for *Bartonella quintana*.

Presenting Author - *Emilio Garrote-Sánchez, University Of Valencia, Spain*

Author/s – *Christian Seeger, Andrés Moya, Rosario Gil*

Abstract Content – Obligatory endosymbiotic organisms, whether parasitic or mutualistic, tend to have reduced genomes compared to their free-living relatives, because of the evolutionary process called 'genomic reduction syndrome'. Yet, these genomes present a group of genes that are essential to life and constitute what is called a minimal genome. Their characterization, as well as the possibility of optimizing them, by eliminating non-essential genes or by adding genes to complete impaired metabolic pathways, is highly relevant in the field of synthetic biology. However, most endosymbionts cannot be cultured in the laboratory, making it difficult to manipulate them.

Bartonella quintana Toulouse is a facultative endosymbiont that has the capacity to infect mammalian cells, making this bacterium a good model to design a chassis for potential biomedical applications. However, it has a very slow growth rate due to its complex nutritional requirements. Our objective is to define the ideal medium composition that improves growth efficiency,

which will also have an impact on the ease of performing genomic manipulation experiments for a better characterization of the model prior of its use as an endosymbiont chassis.

First, we generated a metabolic model of *B. quintana* from genomic data and, through flux balance analysis (FBA), we determined which compounds are limiting factors for its growth. Next, we established a protocol for culturing this bacterium using media supplemented with these compounds in different concentrations and measured its growth impact. Finally, we have performed proteomics studies to determine changes related to changes in media composition.

T55 - Insights into the susceptibility of *Pseudomonas putida* to industrially aromatic hydrocarbons that it can synthesize from sugars

Presenting Author - *Ana Ángeles García Franco, CSIC-estación Experimental Del Zaidín, Spain*

Author/s – *Patricia Godoy, Rocío Palacios, Estrella Duque, Juan Luis Ramos*

Abstract Content

Background: *Pseudomonas putida* DOT-T1E is a highly solvent-tolerant strain that has been engineered to produce L-phenylalanine, which in turn serves for the synthesis of styrene via trans-cinnamic acid (tCA); this approach represents a green alternative to petrochemicals.

It was shown that the *in vivo* production of this compound was limited by the tolerance to styrene of the producing strains, being this tolerance a key factor to construct a successful bacterial chassis to obtain chemical products.

Objectives: This study was conceived to provide insights on the tolerance of *P. putida* DOT-T1E to tCA and styrene and to unveil the mechanisms behind the resistance to them.

Methods: We have analyzed the physiological and genetic response of the wild-type and mutants to these compounds using a range of biochemical approaches.

Results: We found that 25mM tCA prevented growth of the strain but did not affect viability, while 0.1 % styrene initially decreased cell viability, but upon long incubation growth was restored. Physiological, omics and mutant behavior analysis revealed that in response to these compounds, the cell launches a multifactorial response to enhance membrane impermeabilization, via the conversion of cis unsaturated fatty acids to their corresponding trans isomers, stress response that involves the synthesis of chaperones and ROS removing enzymes, enhancement of the metabolism of glucose and induction of two efflux pumps to extrude the toxic chemicals.

The identification of these key molecular determinants for tolerance to these chemicals will allow us to develop a robust chassis for industrial production of aromatic chemicals.

T56 - Selecting sublethal doses of ferromagnetic nanoparticles on *Pseudomonas aeruginosa*

Presenting Author - *Kamila Dubrowska, West Pomeranian University of Technology in Szczecin, Poland*

Author/s – *Joanna Jabłońska, Adrian Augustyniak, Rafał Rakoczy*

Abstract Content

Background: Nanomaterials are predominantly tested as antimicrobial agents. Nevertheless, low concentrations of nanomaterials in bacteria may induce contradictory outcomes, often leading to the stimulation of secondary metabolism. *Pseudomonas aeruginosa*, due to its biotechnological potential, can be an interesting model for testing such a phenomenon. So far, we have confirmed the stimulative effect of sublethal concentrations of zinc oxide nanoparticles and multiwall carbon nanotubes on pyocyanin production. Still, little is known about the modulating effects of nanomaterials on rhamnolipids (Rh) production.

Objectives: The study aimed to determine the sublethal concentration of the ferromagnetic nanomaterial and its use to increase rhamnolipid production.

Methods: *P. aeruginosa* (PA) DSM 1128 (ATCC 9027) and iron (II) iron (III) oxide nanoparticles (FeNP) were used in the studies. The sublethal concentration was sought by 24h chronic toxicity test. The twofold dilutions of nanomaterial (15.625–1000 µg/mL) were incubated at 37℃ with PA for 24h, and optical density (OD) was measured (λ = 600 nm) at 0h, 6h, 12h, and 24h. Then the rhamnolipid concentration was obtained after 72h incubation with the sublethal FeNP dose. The concentration was quantified spectroscopically using methylene blue method.

Results: Biggest changes in OD were observed for sixth hour of the experiment, and a 500 µg/mL FeNP was selected as a sublethal dose. The Rh production was increased using the sublethal dose compared to the control.

T57 - Endolysins Targeting the IBD-associated bacterium *Ruminococcus gnavus*.

Presenting Author - *Ellen Murray, APC Microbiome Ireland, Ireland*

Author/s – *Ellen Murray, Ekaterina Khokhlova, Lorraine Draper, Andrey Shkoporov, Paul R. Ross, Colin Hill,*

Abstract Content

Background: *Ruminococcus gnavus* levels are strongly correlated with Inflammatory Bowel Disease (IBD). In IBD patients, an overabundance of *R. gnavus* is associated with increased inflammation. Endolysin therapy is a method of targeted microbiome editing. Endolysins are peptidoglycan hydrolases that attack the structure of their host cell wall and result in cell lysis. These phage-derived proteins can be used to eliminate specific bacteria in the gut without causing damage to overall microbiome composition.

Objectives: To assess the effectiveness of phage-derived endolysins for selective targeting of *R. gnavus* in the context of IBD.

Methods: Endolysin genes identified in temperate phages were cloned into *Escherichia coli* for recombinant expression. Lytic activity against *R. gnavus* was confirmed by spot assays, turbidity reduction assays, and kill curves. A narrow host range was established using a panel of commensal gut strains. However, endolysin-resistant mutants of *R. gnavus* arose at low frequencies. Illumina sequencing was used to compare mutant genomes to that of the sensitive parent strain.

Results: Host range analysis was performed on 20 relevant strains. The endolysins were specific for *R. gnavus* with some exceptions. We observed that lysin-resistant mutants appeared after 20h exposure to endolysins. Whole genome sequencing revealed point mutations in the mutant genomes in genes that are associated with the bacterial stringent stress response. Future work will assess the potential issues of endolysin-resistant mutants, and the potential of these endolysins as therapeutics to restore microbiome composition in IBD patients.

T58 - Evolution of ST38 *E. coli* following the conjugative acquisition of IncL plasmids carrying blaOXA-48

Presenting Author - *Pengdbamba Dieudonné ZONGO, Institut Pasteur - Paris, France*

Author/s – *Nicolas CABANEL, Philippe GLASER, Isabelle ROSINSKI-CHUPIN*

Abstract Content

Carbapenems are last resort ß lactams used to treat multidrug resistant Gram negative bacteria. However, the increased incidence of carbapenem resistance is threatening their therapeutic efficacy. The emergence of carbapenemase producing *E. coli* (CPEC) and their dissemination in the community are particularly worrisome. Sequence Type (ST) 38 is the most prevalent ST among CPEC isolated in France and in other European countries, characterized by chromosomal integrations of the blaOXA 48 gene. We are aiming to characterize the benefit of chromosomal integrations and the involved mechanisms.

pOXA 48 plasmids were transferred by conjugation into naïve ST38 *E. coli* strains. Growth curves for fitness cost assessment and 10-day passages without selective pressure for plasmid stability were performed. Transconjugants were evolved for 28 days in the presence of meropenem to look for fitness improvements and potential blaOXA-48 chromosomal integrations. Evolved strains were whole-genome-sequenced.

pOXA 48 plasmids were unstable in all tested ST38 *E. coli* genetic backgrounds. Particularly, growth curve analyses revealed a pOXA-48 associated fitness cost in all tested strains. blaOXA 48 chromosomal integrations were observed during experimental evolution and led to fitness recovery. Alternatively, an increase in both the fitness of evolved strains and in plasmid stability was recurringly associated with mutations in an uncharacterized operon encoding a novel antiplasmid system. How the proteins encoded by this operon contribute to pOXA 48 instability is under study. Altogether our results provide new clues on the emergence of these prevalent carbapenemase producing lineages.

T59 - Biosynthesis and characterization of Palladium-Based Nanoparticles produced by *Escherichia coli*

Presenting Author - *Ana Campana, University of Oslo, Norway*

Author/s – *Nadeem Joudeh, Mohamed L. Merrun, Jaime Gomez-Bolivar, Dirk Linke, Pavlo Mikheenko*

Abstract Content

Palladium (Pd) is a rare chemical element from the platinum group of metals with unique catalytic properties [1]. The use of Pd in the form of nanoparticles (NPs) increases the surface-to-volume ratio for catalysis, and confers unique electronic, magnetic, and photonic properties [2]. Different chemical and physical methodologies are now used on industry for NPs production however; they require large amounts of energy, use toxic precursors or result in highly contaminant residues. Some bacteria can reduce Pd ions into NPs form during metabolic processes as consequence of mechanisms developed to protect their integrity under stressful environments. Therefore, the biological synthesis of NPs using bacteria could present a simple, cost-effective, and more environmentally friendly alternative to the conventional synthesis methods.

Biosynthetic NPs are bound to biological material, surfaces and biopolymers during and after their formation. Such biological scaffolding avoids the problems of NP agglomeration and results in more homogeneous particle populations. These NPs have interesting catalytic and magnetic properties yet the exact mechanism of Pd reduction and Pd NPs formation in bacteria is still unknown.

In this work, a systematic characterization of biogenic Pd and Pd-Fe NPs produced by *Escherichia coli* K-12 BW25113 strain is presented. By means of TEM combined with EDX spectroscopy, the composition, size distribution, and localization of the biosynthesized NPs were analyzed. In addition, AFM and MFM were used as a novel methodology for the direct study of the low magnetic moment of intracellular and extracellular Pd-based NPs within well-preserved cells.

T60 - Biosynthetic potential and antifungal activity of *Streptomyces* strains isolated from coal related environments.

Presenting Author - *Piotr Siupka, Poland*

Author/s – *Klaus R. Westphal, Trine Sørensen, Frederik T. Hansen, Weronika Kąsek, Teis Søndergaard, Zofia Piotrowska-Seget*

Abstract Content

Actinobacteria, especially *Streptomyces* are known for their biosynthetic potential and are major source of bioactive compounds used by humans. However, increasing resistance of pathogens to known agents requires constant search for novel chemicals. For that reason, researchers' attention is turning to the previously neglected and extreme environments. Coal related environments (CRE) are example of them. Our goal was to isolate Actinobacteria strains from the CRE, evaluate their biosynthetic potential, bioactivity, and metabolic profiles. Biosynthetic potential was evaluated by genome sequencing and biosynthetic gene clusters (BGCs) detection using antiSMASH software. Plate assays were used for bioactivity test. Metabolic profiles were obtained with use of liquid chromatography with high resolution mass spectrometry (LC-HRMS). We were able to isolate 19 strains of Actinobacteria from CRE and obtained. Two of them were already shown to have high biosynthetic potential of strong antifungal activity against fungal phytopathogens. One strain, *Streptomyces* sp. S-2 was isolated from black soot after hard coal combustion, and the second one, *Streptomyces* sp. MW-W600-10 from underground, coal mine water. The antifungal activity varied between strains as well as was dependent on culture medium used for test. At least partially the activity was related to production of volatile organic compounds. The biosynthetic potential revealed large number of BGCs in genomes of both strains, many showing no similarity or <30% of similarity to functionally known clusters. Ongoing LC-HRMS analysis showed so far that both strains producing surugamide derivate, however other compounds are being identified.

T61 - Non-vaccine-serotype-specific identification of *Streptococcus pneumoniae* using the LAMP method

Presenting Author - *Jun Sakai, Saitama Medical University Hospital, Japan*

Author/s – *Takahiro Iijima, Dai Kanamori, Tomonori Hoshino, Shigefumi Maesaki, Mitsuko Seki, Takano C, Hayakawa S, Nihon University, Japan; Chang B, National Institute of Infectious Diseases, Japan; Kilgore P, Wayne State University, USA; Kim DW, Hanyang University, Korea*

Abstract Content

Background: Reported invasive pneumococcal disease have declined since the introduction of pneumococcal conjugate vaccine (PCV7/PCV13). The incidence of invasive diseases due to pneumococcus that were not included in the vaccines, however, has increased not only in children but also adults. It's a public health problem globally. Previously, we established the loop-mediated isothermal amplification (LAMP) method for PCV13 and PPSV23 serotype-specific assays. In this presentation, we aimed to develop a rapid, simple, and cost-effective assay to detect non-vaccine serotypes.

Methods: The LAMP assays targeting non-vaccine-serotype-specific genes were developed. We designed LAMP primer sets on the sequences available for the serotypes, 6C, 7C, 13, 15A, 16F, 23A, and 23B. Each assay was evaluated to determine test reactivity, specificity, and sensitivity, and compare its performance to that of conventional PCR.

Results: The specificity of the LAMP assays were confirmed using 41 serotypes of pneumococcal strains. The sensitivity of the LAMP assays was 100 copies per reaction while that of the PCR assays was 10E+3 to 10E+6 copies per reaction.

Conclusions: A rapid and simple LAMP-based non-vaccine serotype detection method has been developed for use across a variety of countries globally. Further evaluations of this assay are now needed in the context of surveillance and vaccine effectiveness studies.

T62 - Modulation of ultrasound-assisted extraction effect on the molecular composition of fungi

Presenting Author - *Wan-ting Tam, Hong Kong Metropolitan University, Hong Kong*

Author/s – *Ching-ching Hui, Ching-ho Lung, Yi-Ching Cheung*

Abstract Content

Background: Ultrasound-assisted extraction (UAE) has been widely evaluated for isolation of food and medicinal products. However, the knowledge in the UAE to extract polysaccharide-protein complexes (PSPs) from fungi is lacking.

Objectives: The study aims to optimize the UAE method to extract PSPs from edible fungi so as to attain the optimized bioactivity effect.

Methods: Four variables in the UAE process of two edible fungi were investigated. The polysaccharide and protein contents of the extracts versus time of UAE were measured and fitted to models by linear regression. Antioxidant activity of the extracted PSPs were also tested in cell culture.

Result: The optimum condition for the UAE of polysaccharides and proteins in both fungi was found to be 16.25W/cm2 ultrasound (US) power intensity with 300.5 μm fungal particle sizes and 1:30 solid-to-liquid ratio. However, the optimum temperatures to extract polysaccharides required higher temperature (70℃) whereas proteins' preferred lower temperature (55℃). For both fungi, the polysaccharide and protein contents (wt%) in the extracted PSPs were found to be correlated to the US energy density (MJ/m3) by the Power Law of extraction: , where y is the polysaccharide/protein content and x is the US energy density. However, such correlation was only valid below 20 W/cm2 US power intensity. Within the same US power intensity range (< 20 W/cm2), it was also observed the constant ratio of polysaccharide to protein content (wt% of polysaccharide/ wt% of protein) in the extracted PSPs against the increasing US power intensity.

T63 - Cost effective production of desferrioxamine B in *Streptomyces pilosus*

Presenting Author - *Shalini Singh, Helmholtz Institute Freiberg for Resource Technology (HIF), Germany*

Author/s – *Rohan Jain, Katrin Pollmann*

Abstract Content

Secondary metabolites such as siderophores produced by microorganisms and plants have shown to bind commercially important elements such as Ga3+, Ge4+ and Ti4+ in addition to Fe3+.As suggested by many complexation studies, siderophores are a potential Candidate for sustainable and environmentally friendly metal recovery technologies. However, the native and heterologous production of these siderophores is limited due to many reasons such as iron inhibition, highly regulated production, tendency of recycling siderophores, and simultaneous production of various siderophores. The most studied siderophore for metal recovery is desferrioxamine B, but due to its commercial production by only chemical means, its industrial application has been limited. To overcome the application, we have chosen to produce it biologically in native host. In this project, we have carried out media optimization experiments for desferrioxamine B production in the native host, *Streptomyces pilosus*, using minimal media as well as complex media. *Streptomyces pilosus* have filamentous growth and thus form clusters especially in minimal media, which becomes a challenge in effective media optimization. Initially efforts were made to obtain homogenous growth of *Streptomyces pilosus* especially in minimal media without iron for production of Desferrioxamine B using batch reactor. On changing, the growth of *S. pilosus* from cluster to homogenous, phenotypic switching in siderophore production has been observed. It is interesting to note how morphology affects the siderophore production in *Streptomyces pilosus*. These studies will help in deeper understanding of factors that play role in large scale production of secondary metabolite such as siderophore in *Streptomyces* species.

T64 - Production of cellulose using Antarctic bacteria

Presenting Author - *Alberto Vassallo, University Of Camerino, Italy*

Author/s – *Marco Zannotti, Maria Chiara Biondini, Rita Giovannetti, Sandra Pucciarelli*

Abstract Content

Background: Cellulose is a polysaccharide formed by glucose units linked by β-1,4 glycosidic bonds, and it represents the most abundant biopolymer on earth, as it is the main constituent of the cell wall in plants, algae, and fungi. Cellulose has a relevant importance in daily life: for example, cotton fibers are almost completely made by cellulose and one of its most known uses is the production of paper. Cellulose is also produced by some bacteria, such as those belonging to the acetic acid bacteria group, and *Komagataeibacter xylinus* is recognized as the highest producer of bacterial cellulose. One of the main advantages of bacterial cellulose in comparison to its vegetal counterpart is its purity grade, because it does not contain hemicellulose and lignin; however, scale up of bacterial cellulose production is hindered by lack of high yield production and use of expensive and pure nutrients in culture media.

Objectives: To improve the production of cellulose using Antarctic bacterial strains and cheaper experimental setups.

Methods and Results: Bacteria isolated from the Antarctic psychrophilic ciliate *Euplotes focardii* were isolated and preliminarily identified through sequencing of their 16S rDNA. Two of them belonging to the genera *Bacillus* and *Brevundimonas*, respectively, showed the ability to produce a biofilm composed mainly by cellulose. Thus, their genomes were sequenced using Nanopore technology and different growing conditions were tested and compared. Interestingly, they were able to produce cellulose even when cultivated in seawater containing only glucose as additional nutrient.

T65 - Development of a new *in vitro* model of inflammatory bowel syndrome gut microbiome

Presenting Author - *Stéphanie Blanquet-Diot, Laboratory Microorganisms: Genome Environment, France*

Author/s – *Ophélie Uriot, Sylvain Denis, Nicolas Kerckhove, Julien Scanzi, Lucie Etienne-Mesmin,*

Abstract Content

Background: Irritable bowel syndrome (IBS) is associated in human with alterations of gut microbiota, highlighting its importance in the disease onset or evolution. *In vitro* gut models can offer a great alternative to *in vivo* assays in IBS preclinical assays. However, there is up to now no relevant *in vitro* system reproducing the colonic ecosystem of IBS patients.

Objectives: We aimed to develop and validate, through comparison with *in vivo* data in human, a new *in vitro* colonic model adapted to diarrheic IBS (IBS-D) conditions, by using the Mucosal Artificial Colon (M-ARCOL) system, previously set-up under healthy situation.

Methods: An in-depth literature review was performed to adapt the main nutritional (composition of ileal effluents) and physicochemical parameters (pH, transit time) to the specific conditions found in IBS-D colon. Then, each stool from four healthy donors was used to inoculate two bioreactors ran in parallel, set-up with healthy or IBS-D parameters. Microbiota composition and activity were followed during fermentation.

Results: Under IBS-D conditions, a tendency to produce less short chain fatty acid was shown. A decrease in Archaea and Ruminococcaceae, together with a rise in *Akkermansia*, Veillonellaceae and Proteobacteria were observed in accordance with *in vivo* data. This study brings first evidence that nutritional and physicochemical parameters of IBS-D colon play a key role in shaping disturbed microbiota. Once fully validated (further studies using IBS-D stools for inoculation), this *in vitro* model may help to test strategies such as pre- or probiotics or faecal microbiota transplantation to restore gut microbiota eubiosis.

T66 - Characterization of autotrophic bacterial nanocellulose production by *Starkeya* sp. STN1A

Presenting Author - *Rocio Fernández González, CSIC-estación Experimental Del Zaidín, Spain*

Author/s – *Inés Castillo-Rodríguez, Sophie Martirani-von Abercron, Daniel Pacheco-Sánchez, Patricia Marín, Silvia Marqués,*

Abstract Content

Background: Bacterial nanocellulose (BNC) is a polymer with nanofiber characteristics directly produced by some bacteria. We have isolated the strain *Starkeya* sp. STN1B, capable of using a great diversity of carbon sources to grow, including CO2, and producing nanocellulose from them [1, 2]. We also isolated a spontaneous mutant of this strain, STN1A, with an overproducing capacity of the polymer. CO2 is the most influential pollutant in global warming, so its capture and especially its possible recovery in value-added compounds, such as the cellulose produced by this strain, is of great interest.

Objectives: The aim of this work is to study the gene determinants involved in CO2 fixation and in obtaining the reducing power for the process, as well as to determine the characteristics of the cellulose produced.

Methods: A number of knockout mutants in the different pathways involved in CO2 fixation and cellulose production were constructed and analyzed. Cellulose was characterized by scanning electron microscopy (SEM), Fourrier-transformed infrared (FTIR) spectroscopy, X-ray diffraction (XRD) and atomic force microscopy (AFM).

Results: BNC produced with CO2 as carbon source had similar properties to that synthesized from organic carbon sources. A mutant in the cbbL gene coding for the RuBisCO large subunit was severely impaired in grow with CO2 as sole carbon source. The strain has two sulfur oxidation pathways to support CO2 fixation, as well as a complete hydrogenase cluster. Site directed mutants in these pathways allowed the definition of the elements required for CO2 fixation and cellulose production in this strain.

T67 - Impact of microalgae as biostimulator on juvenile coffee plants

Presenting Author - *Nina Böning, Costa Rica Institute of Technology, Germany*

Author/s – *Fabian Villalta, Kattia Núñez-Montero, Olman Gómez-Espinoza, Maritza Guerrero, Francinie Murillo*

Abstract Content

Chemical fertilizers production is responsible for 50 % of the carbon footprint of agricultural goods, causing detrimental impacts on the environment due to CO2 production, water usage, and eutrophication. In several studies, biofertilizers containing microalgae have shown beneficial performance for different crops and have been proposed as an eco-friendly, sustainable, and cost-effective alternative to synthetic chemical fertilizers. Since coffee is the second-highest traded commodity in the world, in this study, we aimed to determine the influence of microalgae treatments on coffee plants as biostimulants at an early phase of growth. Different treatments, consisting of living or lysis of microalgae cells of an Arthrospira maxima species, were applied in a time range of six weeks on coffee nursery plants. Plants' growth parameters were recorded over time. Microbiome soil changes were also studied by 16SrRNA amplicon metagenomics, bioinformatic analysis, and total protein extraction. Our results showed a significant decrease in the leaves damages caused by leaf cutter ants using a microalgae lysate treatment. Also, this treatment significantly increases the total protein in the soil, whereas living microalgae tend to decrease the soil proteins. Moreover, metagenomics showed an increase in the five most abundant genera for both microalgae treatments. Notably, the higher abundance in the genera *Bacillus* and *Planifilum* might contribute to biostimulation, as they are known for being fungicidal and providers of nitrogen and carbon in the soil.

T68 - Recombinant *Escherichia coli* strain for simple extraction of *Bacillus* licheniformis keratinase

Presenting Author - *Mariya Chindareva, Institute of Microbiology of the National Academy of Sciences of Belarus, Belarus*

Author/s – *Illia Kazlouski, Anatoly Zinchenko*

Abstract Content

Background: Currently, there are abundant amount of genetically engineered *Escherichia coli* strains that expressed keratinases of various microorganisms. However, the main drawback of them is necessity to disintegrate bacterial wall (e.g. using ultrasonication, french-pressing) for enzyme extraction and purification, so it hardly could be used for the biotech scale.

Objectives: Bacterial strains *E. coli* XJb (DE3), *Bacillus licheniformis* BIM B-400; plasmid pET42a(+)

Methods: Plasmid pET42.kerA carrying gene, that encodes *B. licheniformis* BIM B-400 keratinase was made by using circular polymerase extension cloning method. Sanger sequencing confirmed that we got plasmid with target gene. That plasmid was used to transform *E. coli* XJb (DE3) competent cells. Recombinant strain was grown on LB medium with 3 mM arabinose (for cell lysis) and 0.2 mM IPTG (for protein expression). Cells with the resuspension buffer were freezed and thawed, and obtained lysate was centrifuged. Cell-free liquid with recombinant keratinase was assessed by SDS-PAGE electrophoresis and keratinase was purified by Ni-NTA-agarose column. Keratinolytic activity of the recombinant enzyme was assayed using keratin-azure as a substrate. One unit of activity was defined as the amount of enzyme required to increase the OD595 value by 0.01 per hour.

Results: Autolytic recombinant *E. coli* strain that express *B. licheniformis* BIM B-400 keratinase was made. Producing ability of the strain was 62500 units per liter of the cultural fluid. The strain could be used for effective extraction and purification of keratinase for different biotechnological applications.

T69 - Developing easily-implementable Pir-based yeast surface display by systematic reshuffling of Pir2-reporter constructs

Presenting Author - *Bojan Zunar, Faculty of Food Technology and Biotechnology, Croatia*

Author/s – *Tea Martinić-Cezar, Mateja Lozančić, Ana Matičević, Vladimir Mrša, Renata Teparić*

Abstract Content

Background: Yeast *Saccharomyces cerevisiae* can be used for surface display, i.e. to covalently bind proteins to its cell wall, thus anchoring them to the outer cell surface. Such a mechanism could be used to retool yeast's cell wall into a living catalyst. Namely, cells could be engineered to bind commercially attractive enzymes to their exterior, which would remake the entire cell surface into a catalytically active living material. To immobilize such enzymes near their N-terminus, the enzymes are often fused with or inserted within one of five Pir proteins (proteins with internal repeats), which bind covalently to β-1,3-glucan.

Objectives: Currently, the smallest part of Pir protein required for surface display and the optimal location for inserting proteins of interest into Pir proteins remain unclear, making Pir-based surface display reliant on guesswork and intuition, and thus inefficient.

Methods: To address this issue, we used β-lactamase as a reporter enzyme, inserted it at five positions in Pir2 (Hsp150) protein, and constructed and tested eight additional truncated Pir2-β-lactamase variants. Next, we followed β-lactamase activity and Pir2-binding efficiency through enzymatic and immunochemical methods, while using Alphafold2 to *in silico* predict the structure of the thirteen constructs.

Results: We present and rationalize a new set of practical guidelines for a reliable, easy, and effective yeast surface display using Pir proteins. Moreover, by truncating the original Pir2 protein, we engineered the Pir-tag, i.e. the minimal Pir2 portion needed to display enzymes of interest on the cell wall efficiently.

T70 - Fibrillar and globular protein-degrading proteolytic enzymes from entomopathogenic micromycetes

Presenting Author - *Anna Bogomolova, , Russian Federation*

Author/s – *Daria Basalaeva*

Abstract Content

Background: The search for enzymes that degrade fibrillar and globular proteins effectively is one of the most important questions in biotechnology today. Proteolytic enzymes are used in a broad variety of applications, for example medicine, cosmetology and food processing. Considering that entomopathogenic fungi require a wide range of proteins to obtain nutrients from the host, they represent a promising source of proteolytic enzymes.

Objectives: The objectives of these studies were to analyse the proteolytic potential of entomopathogenic micromycetes for fibrillar and globular proteins, pH of fungi's extracellular metabolites.

Materials & Methods: 10 fungi from Russia and Vietnam have been studied. The proteolytic potential evaluation was performed using agar media containing collagen and haemoglobin as protein substrates. The hydrolysis of the substrates around the colonies was observed. The areas were visualised and enzyme indices (EIs) (the diameter of the hydrolysis area to the diameter of the strain colony) were measured. Additionally, strains were tested for the secretion of acidic and alkaline metabolites by growing in Creatine Sucrose Agar (CREA) and red phenol agar, respectively.

Results: The proteins tested were digestible by more than half of the entomopathogenic micromycetes studied. All screened fungi are capable of hydrolyzing collagen, 70% degrade haemoglobin. As a result, these micromycetes secrete proteases cleaving both fibrillary (collagen) and globular (haemoglobin) proteins. The most active strains are *Akanthomyces cf. aculeatus* on collagen (EI=2.27) and *Akanthomyces muscarius* on haemoglobin (EI=1.37). Finally, a half of the studied fungi secrete acidic enzymes and only *Purpureocillium takamizusanense* - alkalic metabolites.

T71 - Selective production of human milk oligosaccharide lacto-N-fucopentaoses in *Escherichia coli* with engineered fucosyltransferases

Presenting Author - *Kento Koketsu, Kirin Holdings Company, Limited, Japan*

Author/s – *Tomotoshi Sugita, Shun Endo, Sayaka Kamai, Kazuki Nakamura, Sotaro Sanpei, Fuhito Yamazaki,*

Abstract Content

Human milk oligosaccharide lacto-N-fucopentaoses (LNFPs) are the member of fucosylated pentasaccharides contained in human breast milk and shows potential health benefits for infants and adults, including immune modulation and antimicrobial/antivirus effects in intestinal environment. Recently, commercial-scale fermentative production of various HMOs, such as 2'-fucosyllactose (2'-FL), by engineered microorganisms have been developed. However, the large-scale fermentative production of LNFPs was limited in only few example due to their complex structures. One of the problem of this process development is difficulty controlling the generation of unexpected byproducts including FLs and undesired isomers of LNFP.

To solve this issue, we focused on the fucosyltransferases (FucTs) and improved the substrate specificity via the combination strategy of homolog screening and protein engineering.

As one of the example we have been demonstrated, we present the construction of LNFP III-producing *E. coli* with α1,3-FucT, which shows substrate specificity to lacto-N-neotetraose (LNnT) rather than lactose. LNFP III is synthesized as follows; fucose moiety of GDP-fucose is transfered to GlcNAc moiety of LNnT catalyzed by α1,3-FucT. But known α1,3-FucTs from Helicobacter pylori or Bacteroides fragilis also transfer fucose to reducing terminal glucose moiety of LNnT or lactose, resulting in the generation of various byproducts.

As a result of screening of α1,3-FucTs homologs, we found FucT from Parabacteroides goldsteinii (PgsFucT). The strain expressing PgsFucT showed a significant accumulation of LNFP III (3.19 g/L) while 3FL and LNFP VI byproducts were undetectable. Also, we will introduce another example for selective production of LNFPs by engineered *E. coli*.

T72 – Platinum group metals recovery by modified bacterial polymers

Presenting Author - *Leonor Matos, University of Coimbra, Portugal*

Author/s – *Beatriz Rito, Diogo Margato, Ana Paula Chung, Romeu Francisco, Paula V. Morais*

Abstract Content

Background: Platinum (Pt) and iridium (Ir) belong to the platinum group metals (PGM) and integrate into the European Union's Critical Raw Materials list due to their economic importance and supply risk. PGMs mining is limited due to the scarcity of viable sources, costly and prone to cause considerable environmental impacts. Therefore, it is essential to optimize PGM recovery from recyclable wastes, to meet future demand and reduce dependency on the mining sector.

Objectives: This study aimed to produce a modified biopolymer able to extract PGM from a solution, with high affinity and efficiency.

Methods: To achieve this objective, a metallophore produced by strain B was extracted by FPLC and cross-linked to two biopolymers: Carboxymethylcellulose (CMC) and a biopolymer produced by strain A. The modified biopolymers were used in PGM uptake assays.

The metallophore extract's fluorescence behavior in presence of PGMs was studied, and a concentration-dependent quenching effect was observed.

The assays for Pt and Ir extraction were performed using modified biopolymers and non-modified controls. Assays were incubated for 1 hour in a shaker. The biopolymers modified with strain B's metallophore were compared to biopolymers modified with Deferoxamine B mesylate (DFOB).

Results: Both biopolymers modified with strain B metallophore showed a significantly higher extraction ability in comparison to the controls. Strain A's biopolymer modified with strain B's metallophore was able to remove 94.5% and 92.2% of the Pt and Ir in solution, respectively.

Conclusion: This work demonstrated the superior performance of PGM polymer A-metallophore B biofilter when compared to commercial DFOB.

T73 - Revealing antimicrobial activity of silver nanoparticles synthesized via green approach using grapevine waste

Presenting Author - *Anna Miškovská, University Of Chemistry And Technology Prague, Czech Republic*

Author/s – *Olga Maťátková, Alena Čejková*

Abstract Content

Background: Metal and metal oxide nanoparticles, including silver nanoparticles (AgNPs), are regarded promising inhibitors of microorganism growth, and their activity has been reported against many pathogenic bacteria and yeasts. The attractiveness of nanosized metals lies in their large specific surface area, which leads to unique properties. The advantage of AgNPs compared to classic antibiotics is that they display antimicrobial activities by several mechanisms.

Objectives: The current study explores the antimicrobial potential of AgNPs prepared in a one-step eco-friendly way using Vitis vinifera cane extract.

Methods: Characterization of nanopartilecs was performed using UV-Vis spectroscopy, scanning electron microscopy (SEM-EDS) along with Fourier transform infrared spectroscopy (FTIR). AgNPs were tested for their antimicrobial activity against different types of yeasts and bacteria that are important from a clinical point of view. The effects were observed against microbes that grow in suspension and in a form of biofilm. Furthermore, the hypothesis of synergistic action of AgNPs and V. vinifera extract was tested. The experiments were performed in three independent replicates, with a minimum of five parallels each.

Results: Our AgNPs with an average size of 17 ± 7 nm were found to be capable of inhibiting the growth and formation of biofilms of G- and G+ bacteria, as well as yeasts from *Candida* sp. The revelation of synergistic activity of AgNPs and grapevine phytomolecules was dependent on the microorganism tested and its form of growth, a uniform trend was not observed.

T74 - Potential of inorganic nanoparticles to fight biodeteriogens of tangible cultural heritage objects

Presenting Author – *Andreea Stefania Dumbrava, University of Bucharest, Romania*

Author/s – *Irina Gheorghe-Barbu, Viorica Maria Corbu, Ilda Czobor Barbu, Ionut Petece, Marcela Popa, Ioana Cristina Marinas, Nicoleta Ianovici, Denisa Ficai, Anton Ficai, Mariana Carmen Chifiri, Tatiana Eugenia Sesan*

Abstract Content

Background: Biodeterioration of cultural heritage objects, involving both aesthetic and structural alteration, is an urgent problem that requires the rapid development of safeguarding strategies.

Objectives: The study aimed to demonstrate the antimicrobial efficiency of Ag, Au, Cu and ZnO NPs against a significant number of microfungi and bacterial strains isolated from wooden and stone cultural heritage objects from different Romanian regions (17th-19th centuries) as new strategies to prevent the biodeterioration of artifacts.

Methods: Six types of NPs: Ag (1, 2, 3), Au, Cu and ZnO synthesized by classical, solvothermal and Turkevich methods and characterized by FTIR and SEM were investigated for their efficiency (antimicrobial and antibiofilm activity, ecotoxicity, phytotoxicity and the ability to reduce the enzymes/ organic acid production) against a total number of 75 filamentous fungi and 17 bacterial strains.

Results: The highest antimicrobial efficiency has been recorded for the AgNPs (58.7%), followed by CuNPs (42.67%) and ZnO NPs (25.33%) in the case of microfungi strains (Aspergillus montevidensis, Penicillium commune and P. corylophylum). In the case of Bacillus megaterium and B. cereus bacterial strains the Ag2 and Ag3 NPs proved to be the most active. The AgNPs, CuNPs and ZnONPs decreased the capacity of the microbial strains recovered from stone objects to adhere to the inert substratum. The influence of the tested NPs against the enzymes/organic acids production varied depending on the NP types and by species.

The obtained results prove the potential of the tested NPs to develop efficient solutions for the conservation of the cultural heritage.

T75 - Antibacterial activity of pigmented bacteria producing astaxanthin and lutein

Presenting Author - *Anuttree Inyoo, Chiang Mai University, Thailand*

Author/s – *Chayakorn Pumas, Jeeraporn Pekkoh, Thidarat Nimchua, Thararat Chitov*

Abstract Content

Bacterial pigments have a wide range of biological activities that make them valuable compounds in the food industry. Bacteria producing pigments receive increasing attention because they are fast-growing and their pigment production is not affected by seasonal changes. The aims of this study were to isolate pigmented bacteria from natural and food environments and to characterise the pigments and their antibacterial activities. Pigmented bacteria were isolated from soils, seafood, biofertilizers, and water from paddy fields and reservoirs. A portion of each pigment (extracted with methanol or acetone) was determined for its maximum absorbance wavelength (λmax) and total carotenoid content (TCC) by observing the absorbance at 480 nm. Another portion was subjected to antibacterial activity testing and HPLC analysis. Moreover, the pigment-producing bacteria were identified using 16S rRNA gene sequencing. In total, 50 isolates of pigmented bacteria were obtained. Some of the isolates were potential producers of astaxanthin and lutein, with λmax in the range of 464–477 nm. The TCC of the isolates was between 82.92 and 1766.63 µg/g dry cells. Some pigment extracts were found to contain astaxanthin and lutein by HPLC analysis. The pigmented bacteria were identified as *Brevundimonas* sp. and *Rhodococcus corynebacterioides*. They showed antibacterial activity against *Staphylococcus aureus* TISTR 746, *Escherichia coli* O157, and *Salmonella enterica* subsp. *enterica*.

T77 - Characterization of SusD from Bacteroidota as putative plastic binding proteins

Presenting Author - *Myllena Pereira Silverio, Universität Hamburg, Germany*

Abstract Content – SusD-homologs are glycan binding proteins and part of the operon sus, well described in the Phylum Bacteroidota. The objective of the present work was to screen three SusD-like proteins (named SusD1, SusD38489 and SusD70111) from metagenomes, in respect to their synthetic polymer adsorption. The N-terminal secretion signal Sec/SPII was deleted and the C-terminal of each protein was fused with the fluorescence label superfolder GFP (sfGFP). The fluorescence measurements detected putative binding to the synthetic polymers polyethylene terephthalate (PET), polyamide 6 (PA6), polyvinylchloride (PVC), polyethylene (PE), polypropylene (PP) and Epoxy. Besides, the proteins might possibly bind to microcrystalline cellulose (MC). Data collected by pull-down assay and surface plasmon resonance (SPR) confirmed the hypothetical adsorption to PET, MC and carboxymethylcellulose. SusD1 also bound to the polyurethanes Impranil DLN and DAA. We believe that these proteins might represent a powerful tool for the identification of micro- and nanoplastics in certain environments.

T78 - Production of granular biofilms for the recovery of gel-forming extracellular polymeric substances

Presenting Author - *Abdo Bou-Sarkis, LBAE, France*

Author/s – *Sidonie Durieux, Nerea Chiramberro, Nicolas Derlon, Etienne Paul, Yolaine Bessiere, Elisabeth Girbal-Neuhauser*

Abstract Content

Aerobic granular sludges (AGS) used in wastewater treatment are dense spherical and self-aggregating biofilms made of bacteria embedded in a matrix of extracellular polymeric substances (EPS). AGS were grown in sequenced batch reactors (SBR) to allow the application of selection pressures based on the amount of total organic substrates, the Carbon to Nitrogen ratio, and the duration of aerobic and anaerobic phases. This allowed the aggregation of inoculated consortia in dense granules containing up to 50 % EPS in mass.

EPS extracted from granules by alkaline treatment and recovered through acidic precipitation can form hydrogels. The gel-forming EPS were fractionated according to their charge using anionic exchange chromatography and the gel formation capacity of the collected fractions was compared using a miniaturized method based on calcium-induced gelation. Globally, the gelation capacity increased with charge. The size distribution of fractions was analyzed showing that high molecular weights were implied in the gelation whatever the charge of the polymers (between 65% and 100%) while low molecular weights had limited participation (<40%). Analysis of the biochemical composition indicated that the gelling capacity was mainly correlated with the presence of anionic polysaccharides containing uronic acids.

These results confirm the utility of SBR technology for the production of granule-type bacterial aggregates. As EPS extracted from AGS are eliciting a high commercial interest, the results show that modulation of extraction and purification methods towards the selection of EPS with relevant charge and molecular weight are important factors for their further valorization as biohydrogels.

T79 - Search and identification of slime-forming bacteria - biopolymer producers

Presenting Author - *Aisulu Zhuniszhan, Federation of European Microbiological Societies (FEMS), Kazakhstan*

Author/s – *Togzhan Mukasheva, Ramza Berzhanova, Gulshara Abay, Mariya Ahmetova, Alibek Kudabayev,*

Abstract Content

Bacterial polymers are biotechnology products that are of tremendous interest due to their rheological properties. Many extracellular biopolymer-producing bacteria synthesise capsular or water-soluble slimes.

Slime-forming bacteria were discovered in the rhizosphere of plants growing in the Zailiysky Alatau's foothills and foothill plains and may be found in practically all plants (up to 50%). The distribution of SLYM in plant species and excretion source was inconsistent. The most were discovered in plant rhizospheres, whereas the fewest - in plant roots.

SLYM has been isolated in high amounts from the rhizosphere of the following plants: small-flowered yarrow (*Achillea micrantha* Wild), peppermint (*Mentha piperita*), and lemon balm (*Melissa officinalis*). A considerable number of slime-forming isolates were found among the endophytic bacteria in peppermint (*Mentha piperita*).

The isolates were categorised based on their capacity to form slime, ranging from less thick (+) to mucoid slime. Forty-six isolates produced thick slime (mucoid). When bacteria were cultured in a liquid culture media, the viscosity of the culture liquid increased with growth. The medium viscosity varied across cultures of different isolates. On the fifth day of incubation, the greatest values of kinematic viscosity (over 25 mm2 s-1) were recorded for the same 46 strains. The viscosity of the remained cultures was substantially lower. Eighty-five isolates displayed emulsifying activity, with 35 isolates forming a thick slime, mucoid, at the same time.

Forty-six isolates were investigated for morphological and physio-biochemical features. More than 70% of bacteria found are gram-negative.

T80 - Purification, characterization and prospects for application of high potential protease of *Bacillus amyloliquefaciens* strains L

Presenting Author - *Anna Mkhitaryan, Spc Armbiotechnology, Armenia*

Author/s – *Lev Khoyetsyan, Mariam Karapetyan, Ani Paloyan, Artur Hambardzumyan*

Abstract Content

Introduction: Proteases are among the most valuable enzymes, which are widely used in biotechnology. The proteases of microbial origin are among most required because of high enzyme yield in production, less time and space consumption in production, lofty possibilities of genetic manipulations, and cost-effectiveness of production, which have made them suitable for different applications.

Objectives: The aim of this research was isolation and characterization of new protease. The main attention was paid on the deepness of proteolytic cleavage of waste material (curd and cheese whey).

Methods: Standard microbiological methods were used to isolate strains from soil samples on Petri dishes containing medium prepared based on whey. For protease production the strain was incubated on a rotary shaker in 500 ml flasks containing 200 ml of curd whey at 30°C for 48 hours. Protease was purified from the culture liquid by CM-cellulose chromatography and was characterized.

Results: A new protease-secreting strain was identified as *Bacillus amyloliquefaciens* LK1 based on the 16S rDNA sequence. From culture fluid, the target *B. amyloliquefaciens* LK1 protease was purified by elution with a linear pH gradient from a CM-cellulose column in one step. The protease showed about 7200 U/mg specific activity at 65oC, pH 9.0. The enzyme was efficiently used for whey protein hydrolyses. In the case of curd whey Leu concentration was highest followed by Ser, Lys, Tyr, Phe and Ile, whereas in cheese whey fractions Lys concentration was high, followed by Leu, Tyr, Phe and Glu.

T81 - Systems Biology investigations into the value of waste generated by biopharmaceutical drug processes

Presenting Author - *Laura Murphy, University College Dublin, Ireland*

Author/s – *David O'Connell, Gerard Cagney*

Abstract Content

The circular bioeconomy and its implementation in industrial waste management is an emerging concept to aid in the fight against climate change. There is a daily global bioprocess capacity of 17.4 million litres, and yeast cell culture is a substantial facet of this. Several major therapeutics are currently produced in yeast, including recombinant human insulin. The central question of this project relates to what is happening on a molecular level to the media in which yeast bioprocess occur, and whether items of value can be extracted from this used fermentation media to be reused, remanufactured, or recycled into other processes, thereby implementing the circular bioeconomy. Volatile metabolites could provide a wealth of potential value as there are several applications for the small molecules.

To begin, an elemental composition analysis and a metabolic analysis of yeast fermentation media pre- and post- growth was analysed to give an understanding of what is happening at a fundamental level with yeast fermentation. Results revealed that several key elements are utilised to a significant ($p < 0.0001$) degree for a variety of processes. This provides a foundation for the hypothesis that different yeast processes, such as the variety that occur in industry, will produce unique volatile metabolite profiles. Following on from this, biopharmaceutical yeast models have been created to simulate industrial bioprocesses. In the creation of these Industrial Models, representative industrial waste will be generated and can then be analysed in the future for items of value and potential valorisation.

T82 - Pooled CRISPRi screen in *Saccharomyces cerevisiae* reveals genes important for tolerance to acetic acid and formic acid

Presenting Author - *Ibai Lenitz Etxaburu, Chalmers University of Technology, Sweden*

Author/s – *Maurizio Maurizio, Anders Blomberg, Vaskar Mukherjee, Yvonne Nygård*

Abstract Content

More robust and tolerant yeast cell factories are needed for biorefinery applications where plant biomass is used as the substrate for production of different biocommodities. Increasing our understanding on yeasts' tolerance to formic acid and acetic acid, two of the main inhibitors present in lignocellulosic hydrolysates, can contribute to the development of cell factories with improved tolerance. Here, we employed competitive growth assays (CGAs) and an acetic acid biosensor, followed by barcode sequencing to screen a CRISPRi strain library targeting >98% of essential or respiratory growth essential genes in *Saccharomyces cerevisiae*. Fluorescence-activated cell sorting of biosensor containing cells allowed enrichment of strains with higher acetic acid retention whereas the CGAs allowed determining strains with an improved or hampered tolerance to acetic or formic acid. Both methods led to identification of strains with gRNAs targeting genes previously reported to be involved in weak acid tolerance, as well as some genes that were not previously reported to be involved in formic or acetic acid tolerance, namely HIP1 and SMC4. Our study identified genes encoding for proteins of the 19S particle of the proteasomal complex or involved in chromatin regulation as important for tolerance of both acids. Moreover, targeting genes encoding proteins involved in glycogen synthesis demonstrated to affect acid sensitivity. The strains with highest enrichment in acetic and formic acid medium were further characterized for their role in oxidative stress regulation. Collectively, our data provides a better understanding on the role of gene regulation in tolerance to weak acids and oxidative stress.

T83 - Mechanical stability analysis of an *Acinetobacter* adhesin by steered molecular dynamics simulation

Presenting Author - *Jun Sasahara, Nagoya University, Japan*

Author/s – *Kazushi Fujimoto, Shogo Yoshimoto, Dirk Linke, Andrei Lupas, Katsutoshi Hori,*

Abstract Content

Background: Most bacteria adhere to surfaces to acquire niches for their survival via adhesins. Adhesins are subjected to mechanical loads such as shear stress and tensile forces, making their inherent toughness important for maintaining cell adhesion. *Acinetobacter* sp. Tol 5 shows high adhesiveness to various material surfaces as well as to biological surfaces through the cell surface protein AtaA. AtaA is a member of the trimeric autotransporter adhesin family and a tether molecule 250 nm in length that possesses toughness to prevent easy structural collapse. The C-terminal head domain of AtaA (Chead) is located at the base of the AtaA fiber, and the crystal structure suggests its complex structure contributes to the toughness. However, the mechanism underlying AtaA's toughness remains unclear.

Objectives: This study aimed to elucidate the mechanical stability of Chead and its molecular mechanism.

Methods and Results: Steered molecular dynamics simulations were conducted to analyze the mechanical response of Chead to tensile stress. The mechanically fragile Ylhead domain in Chead unfolded after the unfolding of the stable headCap domain, suggesting that the headCap protects the Ylhead from the applied force. The mechanical transmission network during the elongation was analyzed, visualizing mechanical propagation paths in Chead. The force transmission paths were not only in the direction of the tensile axis, but also spread perpendicularly through the headCap. These findings indicate that the intradomain and interdomain interactions effectively prevent the structural collapse of Chead by dispersing the mechanical loads when external forces are applied.

T84 - Expression profile of selected transcription factors under internal and external stress conditions in yeast *Yarrowia lipolytica*

Presenting Author - *Maria Gorczyca, Poznan University of Life Sciences, Poland*

Author/s – *Ewelina Celińska*

Abstract Content

Transcription factors (TFs) regulate the expression of subservient genes in response to external and internal signals. The external stimuli may elicit signaling of stress conditions encountered by a cell during culturing, like substrate limitation, or its too-high load accompanied by osmotic stress, pH fluctuations, etc. The internal signals may result from e.g. cellular stress awakened upon recombinant protein (r-Prot) over-production. Such high-level r-Prot synthesis may lead to the Unfolded Protein Response or heavy oxidative stress, .

Yarrowia lipolytica is a recognized platform for r-Prots production. Out of 125 TFs identified in this species' genome, only several are well described, and their function was experimentally confirmed.

Knowledge about the implication of specific TFs in the elicited cellular response to internal or external stimuli may be used in engineering complex traits like over-production of r-Prots or stress resistance.

We examined the expression profile of selected TFs in *Y. lipolytica* strain over-synthesizing intracellular fluorescent r-Prot, challenged by external stress factors (pH and hyperosmolarity). The selected TFs were recently shown to be associated with resistance to osmotic stress (SKN7), responsive to pH fluctuations (YAP-like TF), involved in increased r-Prot synthesis (GZF1,HSF1,TF126), downregulated under r-Prot secretion (MGF2,MHY1,TF024), suppressing r-Prot synthesis (Mig1), or, based on the literature data, involved pH-induced cellular response (Rim101).

Our results present the expression profile of the TFs under different environmental conditions in the *Y. lipolytica* strain over-producing r-Prot. These results give an indication of the TF's implication in the response to the investigated external stimuli in the context of r-Prot over-synthesis.

T85 - Insight into the effects caused by the co-overexpression of hac1 on heterologous protein production by *Yarrowia lipolytica*

Presenting Author - *Paulina Korpys-Woźniak, Poznan University of Life Sciences, Poland*

Author/s – *Ewelina Celińska*

Abstract Content

The major transcriptional activator of unfolded protein response (UPR), Hac1, is responsible for the deregulation of over 100 different genes involved in the processes of protein formation, folding and secretion, but also in lipid synthesis and membrane expansion. It is natively activated upon excessive synthesis of proteins (including recombinant secretory proteins (rs-Prots)) and specifically - by the accumulation of incorrectly folded polypeptides in the over-loaded endoplasmic reticulum (ER). Its activation encompasses elevated expression and unconventional splicing event.

In our previous study we demonstrated a positive effect of the co-overexpression of HAC1 (YALI0B12716g) on the synthesis and secretion of rs-Prot in *Yarrowia lipolytica* cells. Here, we investigated molecular mechanisms by which the co-over-expression of HAC1 improves secretion of r-Prot (scYFP) in a steady-state maintained *Y. lipolytica* cells, using global transcriptomics (RNAseq). Additionally, unconventional splicing rate of the HAC1 mRNA was counted through transcript sequencing. Co-overexpression of the HAC1 with the rs-Prot contributed to significant up-/down-regulation of 316 / 237 genes over the prototrophic control strain, and resulted in massive changes a multiple biological processes including ribosome biogenesis, nuclear and mitochondrial events, cell cycle arrest, attenuation of gene expression by RNA polymerase III and II, as well as modulation of proteolysis and RNA metabolism. Interestingly, "conventional" downstream Hac1 targets (KAR2, PDI) were not deregulated under HAC1 over-expression.

T86 - *Bifidobacterium bifidum* BL6BA and *Lactobacillus fermentum* 90-TS4 supernatants inhibit biofilm formation and pyocyanin production

Presenting Author - *Mbarga Manga Joseph Arsene, Russian Federation*

Author/s – *Goriainov Sergei, Anyutoulou Kitio Linda Davares, Podoprigora Irina V., Yashina Natalya V., Pikina Alla, Vasilieva Elena A., Ermolaev Andrey V., Smolyakova Larisa A., Girich Valentina S., Maruf Razan., Das Shommia.,Sharova Irina N., Gizinger Oksana A.*

Abstract Content

Background: Biofilms and pyocyanin production are two key virulence factors in *Pseudomonas aeruginosa*(PA) that can lead to the aggravation of the symptoms of the diseases caused by this bacterium.

Objectives: Evaluate antimicrobial activity and the ability of two probiotics (*Lactobacillus fermentum* 90-TS4 (LF) and *Bifidobacterium bifidum* BL6BA(BF)) to inhibit biofilm and pyocyanin production in PA and to investigate the molecules involved in this activity.

Methods: LF and BF were grown in MRS-broth for 1week/37°C/200rpm. The antimicrobial-activity of the supernatants was tested against a PA strain using the well-diffusion (WDM) and microdilution (MDM) methods. The crystal violet attachment-assay was used to assess probiotics ability to inhibit biofilm formation and 4 mixtures supernatant/nutrient-broth (v/v:µL,100/100;75/125;50/150;25/175 and 0/200) were prepared to qualitatively assess the ability of the supernatant to inhibit pyocyanin production. The short-chain fatty acid content of the supernatants was determined by gas-chromatography with flame ionization (GC-FI).

Results: No inhibition zone was observed with WDM, but the MDM showed bacteriostatic activity only at the first dilution (50%) for both supernatants. Biofilm-inhibition was observed from the 100/100 to 50/150 mixtures and only for 100/100 mixture respectively with BF and LF supernatants. With both supernatants, visual inhibition of pyocyanin was observed with all mixtures except 25/175 and 0/200. Respectively in BF and LF supernatants, GC-FI revealed the presence of propionic acid (1119.34 and 1135.435µg/ml), acetic acid (665.7 and 670.35µg/ml), valeric acid (62.93 and 54.14µg/ml), butyric acid (5.78 and 5.66µg/ml), isobutyric acid (4.825 and 5µg/ml), and isovaleric acid (4.26 and 4.42µg/ml).

T87 - Tumor spheroids as a tool to evaluate tumor colonization and cytotoxicity of synthetic bacteria

Presenting Author - *Alba Cabrera-Fisac, Centro Nacional de Biotecnología (CNB-CSIC), Spain*

Author/s – *Carmen Mañas-Torres, Eva Pico-González, Elena M Seco, Luis Ángel Fernández*

Abstract Content

The use of bacteria for the treatment of solid tumors takes advantage of the natural characteristics of anaerobic bacteria, which allow them to colonize and grow preferentially in the hypoxic and immunosuppressed tumor microenvironment. These natural properties of bacteria can be enhanced with synthetic biology to increase specificity and efficacy of bacterial tumor therapies. In our laboratory, we have developed synthetic adhesins for *E. coli* that enable the specific adhesion of bacteria to mammalian tumor cells expressing a cell-surface antigen (1). We have engineered *E. coli* strains with synthetic adhesins binding the human cell surface receptors EGFR and Her2, which are frequently dysregulated in cancers of epidermal origin, such as colon and breast carcinomas, and armed them with different cytotoxic strategies. This work focuses on the study of the behavior of these bacteria in tumor spheres or spheroids, an *in vitro* 3D model that resembles more closely solid tumor masses and their complex metabolic microenvironment. By labeling with different fluorescent proteins bacteria with synthetic adhesins binding EGFR, Her2 and non-relevant control antigens we have been able to demonstrate that recognition of the tumor cell by the adhesin strongly enhances the colonization of tumor spheroids, both from colon and breast carcinomas. These results allow us to use spheroids as a potent *in vitro* model to test the efficacy of engineered *E. coli* strains with synthetic adhesins and therapeutic cargoes against human tumors prior to further *in vivo* studies.

T88 - Immobilization of biocatalysts on solid surfaces using the bacterionanofiber protein

Presenting Author - *Shogo Yoshimoto, Nagoya University, Japan*

Author/s – *Minami Sawada, Kosaku Noba, Masahito Ishikawa, Katsutoshi Hori*

Abstract Content

Background: Immobilization of biocatalysts plays a crucial role in their efficient utilization. The gram-negative bacterium Acinetobacter sp. Tol 5 shows noteworthy adhesiveness independent of biofilm formation through a trimeric autotransporter adhesin (TAA), namely AtaA. The AtaA polypeptide is composed of 3630 amino acids and is one of the largest TAAs. AtaA is unique in terms of its nonspecific adhesiveness to various abiotic surfaces including hydrophobic plastics, hydrophilic glass, and stainless steel. We previously invented a method for bacterial cell immobilization using AtaA.

Objectives: The purpose of this study is to evaluate the ability of AtaA as an immobilization tool for biocatalysts.

Methods and Results: In-frame deletion mutants were used to perform functional mapping, revealing the essential domain for the adhesive feature of AtaA. The biocatalysts fused to recombinant proteins of AtaA exhibited high adhesiveness to material surfaces, indicating that AtaA has the potential for use in the immobilization of biocatalysts.

T89 - Synthetic bacterial secretion system for the cytosolic delivery of anti-tumor agents into tumor cells

Presenting Author - *Jongho Kim, Hankuk University Of Foreign Studies, Korea, Republic of*

Author/s – *Hyunbi Kim, Minje Kim Kim, Yeram Ahn, Daejin Lim, Miryoung Song*

Abstract Content

Bacteria-based cancer therapy is a promising alternative approach to current cancer therapy due to its tumor-targeting characteristics and immune-stimulating activity. Such targeted delivery has raised hope for cures for many types of cancer with fewer side effects. Our study aims to deliver anti-cancer proteins directly into the cytoplasm of tumor cells using an engineered bacterial secretion system. To this end, we developed a new version of the synthetic type 3 secretion system (SynT3SS, V2.0) by incorporating tip and translocon parts into the previously engineered SynT3SS , which was achieved by refactoring the genes of *Salmonella* pathogenicity island-1 for extracellular delivery of proteins. Since the tip (SipD) and translocon (SipBC) are the parts responsible for injection into the eukaryotic membrane, both parts should be incorporated for cytosolic delivery of the proteins. When the native sequences of SipBCD were introduced, translocation of the reporter protein SptP was detected from the cytosol of HeLa cells. Next, native sequences were replaced by synthetic sequences, together with synthetic RBSs, to increase the delivery efficiency. Finally, an apoptosis-inducing protein, Noxa, was examined for cytosolic delivery through SynT3SS V2.0. By adding the secretion signal of SptP at the N-terminus of Noxa, the recombinant protein was delivered into HeLa cells through SynT3SS V2.0. And HeLa cell death was observed after the delivery of SptP-fused Noxa through SynT3SS V2.0. The results prove that intracellular delivery of anti-cancer protein can be established by our SynT3SS for cancer treatment.

T90 - Engineering of *Escherichia coli* Nissle 1917 for the delivery of immune modulators as vaccine adjuvants

Presenting Author - *Yeram Ahn, Hankuk University Of Foreign Studies, Korea, Republic of*

Author/s – *Yeram Ahn, Jongho Kim, Hyunbi Kim, Minje Kim, Daejin Lim, Miryoung Song,*

Abstract Content

An outbreak of infectious disease by new pathogens can threaten our daily lives at any time, as seen with the COVID-19 pandemic. Thus, a new vaccine platform is required that can be applied safely with high efficacy in a short time. Our study is focused on the development of an engineered probiotic as a vaccine platform where multiple antigens and immune modulators can be presented to maximize efficacy and ensure safety. Since the flagellin of bacteria is well known for immune-stimulating activity via the Toll-like receptor (TLR) 5-mediated signaling pathway, Salmonella flagellin (FliC) was engineered and introduced into *Escherichia coli* Nissle 1917, which can serve as a vaccine vector. The engineered FliC was detected in the bacterial culture supernatant, where it was secreted through the flagellar type 3 secretion system of the *E. coli* Nissle strain. And the engineered FliC could activate TLR5-mediated immune stimulation when it was tested using HEK-Blue™-hTLR5 cells. Currently, multiple antigens are being introduced into the *E. coli* Nissle strain carrying the engineered FliC, and the antigenicity of those will be tested using ex vivo cells and a mouse infection model. This study will prove the feasibility of the engineered probiotic strain as a vaccine platform, where bacterial or viral antigens can be easily presented, and immune modulators such as cytokines can stimulate immune responses through concise genetic manipulation.

T91 - Specific target expression of cytotoxic anticancer proteins delivered by tumor-colonizing bacteria

Presenting Author - *Seyeon Hong, Kangwon National University, Republic of Korea*

Author/s – *Song Miryeong, Shin Minsang, Lee dogeun, Jeon Insu, Lim daejin*

Abstract Content

Bacterial cancer therapy depends on the innate system of tumor-colonizing bacteria. Tumor-colonizing bacteria promote tumor elimination to transferring cytotoxic anticancer protein. However, the deficiency of the previous study was the specific targeting of anticancer protein not only to solid tumor but also to the reticuloendothelial system (RES), the liver and spleen. This study examined the fate of the *Escherichia coli* and an attenuated strain of *Salmonella enterica* serovar Gallinarum with knockout pp(G)pp after intravenous injection into tumor-bearing mice. This result observed that the bacteria, targeted the tumor tissue, activated aggressively, whereas the bacteria, colonized the RES, was metabolically inactivated and cleared by innate immune cells. Based on this observation, this study was measured that the activity of the exponential phase promoter rrnB P1 in ΔppGp. RNA analysis showed that tumor-associated strain activated rrnB operon genes encoding the rRNA components of the ribosome during the exponential stage of growth, whereas those in the RES substantially decreased levels of this gene. We engineered ΔppGpp *S. Gallinarum* to express constitutively a recombinant immunotoxin comprising TGFα and Pseudomonas exotoxin A (PE38) using a constitutive exponential phase promoter, the ribosomal RNA promoter rrnB P1. And we evaluated the antitumor effects of the immunotoxin on mouse colon cancer and breast cancer cells implanted into BALB/c mice. The construct effected anticancer effects on mice. Therefore, our results suggest that a cytotoxic anticancer protein gene engineered to a constitutive promoter was expressed only in the bacteria residing in the tumor tissue, resulting in tumor suppression.

T92 - Screening unconventional transport mechanisms from the termite gut microbiome for innovative microbial cell factories

Presenting Author - *Laurens Lambrecht, Belgium*

Author/s – *Inge Van Bogaert, Magdalena Calusinska*

Abstract Content

Background: Biotechnological production processes, based on microbial cell factories to convert lignocellulosic biomass and other waste streams into high-quality products, are increasingly replacing less sustainable and labor-intensive chemical production processes. These microbial cell factories often require in-depth pathway engineering and fine-tuning to bring microbial production efficiency up to an industrial scale. An often neglected strategy is the optimization of the transport (e.g., pentose sugar import) across the crucial boundary, the biological membrane.

Objectives: The objective consists of optimizing the import process of arabinose, xylose and cellobiose from *E. coli* by searching for novel and improved transport proteins and mechanisms. To this end, candidate transport proteins are selected from a metagenomic dataset created from the termite gut microbiome. Termites are considered one of the most important lignocellulosic degrading natural systems as they survive on this biomass and have therefore developed a specialized gut microbiome.

Methods: The selected transport proteins are codon optimized and expressed at different expression levels. Subsequently, characterization experiments are conducted to establish the transporter sugar specificity, kinetic parameters (such as the uptake rate and sugar affinity) and to what extend their sugar uptake is repressed by the presence of glucose.

Results: A putative cellobiose importer was selected as a first candidate from the termite gut microbiome metagenomic dataset. Different transport protein expression levels are being tested and establishing the specific transporter parameters is ongoing.

T93 - Selection and engineering of channels with the help of microbial biosensors – a platform for microbial cell factory optimization

Presenting Author - *Lobke Sips, Belgium*

Author/s – *Liam Richard Jenkins Sánchez, Inge Van Bogaert*

Abstract Content

Background: Due to the global shift from chemical to biotechnological industry, there is an increasing interest in the optimization of microbial cell factories for the conversion of biomass into high-value products. One aspect of these cell factories that remains largely unexplored is the transport of metabolites. However, by increasing substrate influx and product efflux, the metabolic reaction equilibrium can be shifted towards the compound of interest and bottlenecks can be relieved. Consequently, transporters contain a large potential for the optimization of cell factories. Especially diffusion channels are strong candidates, due to their energy independence, structural conservation and functional diversity.

Objectives: By developing a platform technology for the selection and subsequent engineering of channels for the transport of molecules, the implementation of channels in microbial cell factories will be facilitated. We mainly focus on highly hydroxylated molecules, which have numerous applications in many different fields, such as biofuels and the pharmaceutical, food and cosmetic industry, and even synthetic compounds.

Methods: Using the bidirectionality of channels, microbial biosensors will be developed to measure the intracellular, and thus imported, concentration of a product. In this way, a library of naturally occurring channels will be evaluated. The best candidates will be further engineered using semi-rational design and this new library will again be evaluated using the beforementioned biosensors.

Results: Biosensors, able to measure specific highly hydroxylated compounds, have been successfully designed. A library of natural channels and the first group of engineered channels have been established and are being tested.

T94 - Impact of flow and nutrients on biofilm growth dynamics in simple microfluidic channel

Presenting Author - *Massinissa Benbelkacem, France*

Author/S – *Gabriel Ramos Perroni, Terence Desclaux, Christine Roques, Yohan Davit*

Abstract Content

Bacterial biofilms are structures of attached cells embedded in a matrix of extracellular polysaccharides. Biofilms represent the dominant lifestyle for prokaryotes on Earth and play a fundamental role in the environment, in engineering applications and in medicine. In a majority of systems, flow and transport mechanisms shape many aspects of biofilm development, most of which are still poorly understood. Here we present a novel microfluidic system to study biofilm development under flow in a channel with square cross section. This system uses, in particular, UV-C LEDs to control the spreading of bacteria and to limit contaminations. Using this device in combination with time-lapse optic microscopy, we study the impact of the flow on the growth and detachment of *Pseudomonas aeruginosa* PAO1 biofilms. We find that the pattern of biofilm detachment and the fluctuations in pressure drop strongly depend upon the flow conditions. We hypothesize that this is the consequence of a competition between hydrodynamic stresses and growth. We also evidence a coupling between hydrodynamic conditions and nutrient transport and a strong impact of initial conditions on detachment, even after several days of biofilm growth. We anticipate that this may lead to the development of new strategies to control biofilm growth under flow and solve engineering issues due to clogging caused by biofilms in water processes.

T95 - Blue light-inducible photoproduction of gasoline-like hydrocarbons in a bacterial strain

Presenting Author - *Angel Baca Porcel, France*

Author/S – *Bertrand Légéret, Florian Veillet, Stéphan Cuiné, Yonghua Li, Frédéric Beisson, Damien Sorigué, Pascaline Auroy-Tarrago, Cécile Giacalone*

Abstract Content

Background: Fatty Acid Photodecarboxylase (FAP) is a photoenzyme that converts fatty acids into hydrocarbons (HCs) in presence of light. Naturally present in algae, this enzyme is known to produce long-chain hydrocarbons (≥15 carbons). Unfortunately, such compounds are not suitable for gasoline application mainly constituted of medium-chain HCs (5-9 carbons). Recently our group showed that, *in vitro* as well as *in vivo*, FAP can use exogenous medium-chain fatty acids more efficiently than its natural substrate (C16-C18 fatty acids). This opens new biotechnological perspectives for FAP.

Objectives: This study aims to evaluate the possibility to synthesize medium-chain fatty acids and convert them to HC in a bacterial strain expressing FAP.

Methods: The gene encoding the FAP of *Chlorella variabilis* was co-expressed with different thioesterases (TES) that generate medium-chain fatty acids. A blue light-inducible promoter was used to limit the addition of chemical inducers. The fadE gene was deleted to block the β-oxidation pathway and increase the medium-chain fatty acid pool. The HC productivity was evaluated using bacteria cultivated in a photobioreactor.

Results and Discussion: The co-expression of FAP together with different TES genes resulted in the photoproduction of heptane (7 carbons) as main HC. The TES from the plant Cuphea palustris was found to be the best for heptane production. A yield of 2 mg HC L-1 h-1 was obtained. This study provides a proof of concept that illuminated bacterial cultures expressing FAP can be used to produce medium-chain HCs.

T96 - Integrated -omics uncover *S. cerevisiae* CENPK2-1C yeast cellular adaptation mechanisms to CBD exposure

Presenting Author - *Erin Jordan, Germany*

Author/s – *Ramin Shirali Hossein Zade, Thomas Abeel, Oliver Kayer*

Abstract Content

Background: Yeast metabolism can be engineered to produce other compounds, such as cannabinoids, the principle isoprenoids of the plant Cannabis sativa, through heterologous metabolic pathways. However, yeast cell factories (YCFs) continue to have low cannabinoid production.

Objective: This study employed an integrated -omics approach to investigate the physiological effects of cannabidiol (CBD) on *S. cerevisiae* CENPK2-1C yeast cultures, using transcriptomics and metabolomics.

Methods: We monitored CENPK2-1C cultures' biomass and treated the experimental group with 0.5 mM CBD. We observed a latent-stationary phase growth spurt in the experimental group and harvested samples in the inflection point of this phase shift for RNA sequencing and metabolomic analysis. We then assembled the transcriptome to our strain specific genome and compared the CBD-treated yeast to the positive control, identifying 16 significantly overexpressed genes with an LFC of at least two and a p-value of less than 0.05.

Results: Notable genes included TAR1 (mitochondrial regulator), ALR1 (Mg(2+) transporter), PDR5 (ABC-steroid and cation transporter), HRK1 (activator of H(+)-ATPase Pma1p), and PSB1 (sphingoid exporter). Metabolomic analysis revealed 21 compounds, 13 of which were identifiable as non-CBD compounds, including fatty acids, glycerophospholipids, and phosphate-salvage indicators. Our results suggest that mitochondrial regulation, ion transport regulation, and plasma membrane remodeling play a role in yeast's response to CBD. We hypothesize that cellular adaptation to CBD exposure in yeast cells may involve plasma membrane remodeling and re-regulation of ion transporters. Further research on plasma membrane and mitochondrial membrane dynamics is necessary for a better understanding of these processes.

T97 - Potential for natural products discovery from antarctic rare Actinobacteria

Presenting Author - *Leticia Barrientos, Universidad de La Frontera, Chile*

Author/s – *Pablo Bruna, Kattia Nuñez-Montero, Maria José Contreras, Karla Leal, Ana Zarate, Andres Santos, Jonathan Alarcón, Rodrigo Salazar, Dayaimi Gonzalez, Danae Flores, All from Universidad de La Frontera, Temuco Chile*

Abstract Content

Actinobacteria have been a valuable source of secondary metabolites as novel natural products (NPs). Most microbial metabolites are products of the metabolic pathways encoded by biosynthetic gene clusters (BGCs), and now we know that actinobacteria could harbor silent or cryptic BGCs capable of producing new NPs yet to be discovered. Rare non-explored Actinobacteria isolated from Antarctica could allow the discovery of new BGCs with biotechnological potential. In this work, we aimed to isolate rare Antarctic Actinobacteria and mined their genomes to study BGCs. To do this, we applied different protocols for the selective isolation of rare-Actinobacteria from Antarctic soil samples. The complete genome of eight isolates was obtained and analyzed for taxonomic identification and BGCs predictions. The phylogenetic analysis and taxonomic classification based on 16S rRNA comparison ANI, AAI, and dDDH values suggest that seven out of eight strains are new species belonging to the genera *Lapillicoccus*, *Pseudarthrobacter*, *Micrococcus*, *Allobranchiibius*, and *Paenarthrobacter*. The identified species was *Janibacter terrae*. Forty-five BGCs belonging to the identity of the sequenced strains, of which three groups showed 100% similarity, four groups showed >=50% genetic similarity, and 38 BGCs showed <50% similarity to other known secondary metabolites. All the strains presented BGCs whose metabolites could be Candidates for NPs discovery with antimicrobial, antitumoral, and antioxidant properties. Our results confirm the relevance of selective isolation protocols for rare-Actinobacteria during bioprospection. Also, here we provide evidence of novel actinobacteria taxa inhabiting the Antarctic environment.

T99 - Assessing the performance of X-SEED® yeast extracts on *Bacillus* fermentations

Presenting Author - *Nina Mittelheuser, Ohly GmbH, Germany*

Author/s – *Carmen Mandel, Abhishek Somani, Moritz Radomski*

Abstract Content

Bacillus are bacteria that can form spores allowing them to tolerate harsh environmental stress, such as (but not limited to) nutrient limitation, heat or freezing. *Bacillus* is commonly used as a probiotic supplement, as its spores can survive the harsh conditions of the digestive tract. During *Bacillus* fermentations, exposure to specific nutrients can cause spores' germination. After subsequent vegetative cell accumulation, nutrient depletion typically triggers *Bacillus* spore formation. For the industrial production of *Bacillus* spores, high spore yields with short batch times are preferred. Therefore, it is crucial to optimize the media components, including the correct yeast extract, to ensure maximal batch outputs.

With the aim of improving germination and consequent spore formation of *Bacillus* strains, various existing yeast extracts and new prototypes were tested at lab scale. For spore-to-spore *Bacillus* fermentations, a method was developed and conducted using DASGIP® Bioreactors (Eppendorf). Additionally, a method for spore measurement was developed using the BactoBox® (SBT Instruments).

In all experiments, fermentation was initiated by using *Bacillus* spores. Online monitoring of standard parameters such as DO, pH will be presented in response to use of different yeast extracts. End-of-fermentation analysis for spore titers, percent sporulation and a new method for spore counting using BactoBox will also be shared.

Overall, the impact of different yeast extracts on *Bacillus* spore production and its crucial will be demonstrated. It will be shown that we were able to develop a method to showcase the benefits of incorporating yeast extract in spore-to-spore fermentation of *Bacillus*.

T100 - Investigating the impact of different X-Seed® yeast extracts on culturing of lactic acid bacteria via high throughput screening

Presenting Author - *Alessandro Ciranna, Ohly GmbH, Germany*

Author/s – *Abhishek Somani, Dina Krueger*

Abstract Content

Probiotic microorganisms like lactic acid bacteria (LAB) are fastidious strains, which often times show auxotrophy leading to the incapability to produce de novo amino acids, small peptides or nucleobases. Since this can inhibit or slow down the growth of these organisms, an external addition of nutrients such as yeast extracts is recommended in order to improve fermentation processes. The aim of this study was to elucidate the nutritional needs of various LAB strains and whether one specific yeast extract or preferably a combination of products would lead to an improved fermentation performance.

Therefore, we conducted a high-throughput fermentation screening with various X-seed® yeast extract products and the combinations thereof. The fermentation of the following LAB strains: *Streptococcus thermophilus*, *Lactobacillus helveticus*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus* was executed in the Biolector II® from Beckman Coulter with real-time monitoring of biomass and pH profiles as well as offline measurement of intact and total cell counts.

Results show that strain growth is highly dependent on the yeast extract being used. Not only differences in species but also different strains can be influenced by different yeast extracts. Thus, optimal yeast extract selection can be paramount for effective culturing of lactic acid bacteria.

T101 - Cell-surface display of pancreatic lipase inhibitor peptide on *Saccharomyces boulardii* probiotic yeast

Presenting Author - *Mozhgan Raigani, Institut Pasteur, Islamic Republic of Iran*

Author/s – *Mozhgan Raigani, Vahid Khalaj, Farzaneh Barkhordari, Somayeh Enayati*

Abstract Content

Background: Obesity has become an increasingly serious public health problem. Pancreatic lipase (PL) is an ultimate target for the treatment of obesity. In this regard, peptides with potential inhibitory activity to PL are used. As proteins and peptides of various kinds can be displayed on the yeast cell surface, the system is expected to allow the preparation of tailor-made functional proteins. In this sense, new drug delivery systems for the treatment of obesity is desired.

Objectives: In the present study, surface display system of *S. boulardii* is used to express PL inhibitor peptide.

Methods: The peptide was chosen based on previous published articles and patents. After evaluation of peptide properties and docking simulation studies, the peptide in the AGA2- EGFP -c-Myc-G4S-peptide sequence was cloned into pYES2 vector. The original GAL1 inducible promoter of pYES2 vector was replaced with TEF1 constitutive promoter and two plasmids, pYES2-GAL1-display peptide and pYES2-TEF1-display peptide, were separately transformed into ura3 auxotroph mutant of *S. boulardii*. Following the analysis of protein expression by SDS-PAGE and western blotting, flow cytometric and *in vitro* pancreatic lipase inhibition assay were performed.

Results: According to results, our constructs were successfully expressed. Flow cytometry analysis of *S. boulardii* expressing AGA2-EGFP-peptide confirmed *S. boulardii* surface displayed desired recombinant protein. Furthermore, analysis of pancreatic lipase inhibitory showed that its activity could be efficiently inhibited by surface displayed peptide. In conclusion, the yeast surface display could be a promising approach in the use of peptides for the treatment and management of some diseases like obesity.

T102 - Turning up the Heat: Investigating polyhydroxyalkanoate metabolism in *Cupriavidus necator* H16 using thermal proteome profiling

Presenting Author - *Kate McKeever, University College Dublin, Ireland*

Author/s – *Gerard Cagney*

Abstract Content

Polyhydroxyalkanoates (PHAs), naturally occurring bioplastics produced by many bacteria, are promising substitutes for petrochemical plastic. The chemolithoautotroph *Cupriavidus necator* is a carbon dioxide consumer capable of producing PHA. PHA synthesis, involving enzymes PhaA, PhaB, PhaC and PHA granule associated proteins, is induced by stress (e.g. low nitrogen). Key enzymes are under coordinate genetic control however, how these pathways are integrated into wider cell metabolism is unclear. Metabolic pathways regulated at mRNA and/or protein expression level can be studied globally using transcriptomic or proteome technologies, respectively. However, allosteric and related regulation mechanisms are challenging to study. Here, we implement at new mass spectrometry approach, thermal proteome profiling (TPP).

The overall aims for this research are to adapt the TPP approach to *C. necator*, and investigate protein conformation effects arising from growth in low nitrogen.

The cell lysate of cultures grown in normal and low nitrogen conditions were treated with a thermal gradient, labelled using TMT isotope labelling, and analysed by LCMS.

Pilot studies, testing various different methods, revealed an optimal TPP protocol which allowed for the identification of 2, 000 proteins. Preliminary results show over 1, 400 proteins were identified in a comparison of normal and low nitrogen conditions. These included proteins of interest PhaA, PhaB, PhaC, PhaP4 and PhaZ2. Melting curves for each protein will be generated and compared statistically for evidence of differential protein stability in low nitrogen conditions. It is anticipated that the approach will have value in identifying conformation change at single protein and pathway levels.

T103 - Engineering *S. erythraea* to enhance erythromycin biosynthesis

Presenting Author - *Anna Liuzzi, University of Leicester, United Kingdom*

Author/s – *Helen O'Hare, Martin Sim,*

Abstract Content

Actinobacteria are a diverse group of bacteria particularly important for theproduction of commercially important secondary metabolites, such as antibiotics. Optimisation of fermentation yield has traditionally involved optimising the feedstock, and recent advances in understanding of how primary metabolism influences secondary metabolite production bring the potential to improve productivity by genetic approaches. Building on work on metabolic regulation in *Mycobacterium* and *Corynebacterium*, we hypothesised that genetic engineering of the metabolic regulator GarA in antibiotic-producing Actinobacteria could enhance antibiotic yield, thus making strains more attractive for novel antibiotic development.

To test this hypothesis, erythromycin-producing *Saccharopolyspora erythraea* was engineered to express a second copy of garA under the control of a constitutive promoter. An additional strain was created in which this second copy lacks regulatory phosphorylation sites and should be constitutively active towards TCA cycle enzymes. The concentration of erythromycin produced by these strains was assessed by Liquid Chromatography coupled with Mass Spectrometry. In addition, the level of amino acids was measured to provide an understanding of how GarA affects metabolism.

Our findings indicate that GarA was present throughout fermentation, with a truncated form predominating during the antibiotic production phase. Expression of phosphoablative GarA increased erythromycin yield, demonstrating a promising method for yield enhancement for pharmaceutically important natural products.

T105 - Increasing mevalonate production using CRISPR/Cas9 system combined with Omics tools in *Saccharomyces cerevisiae*.

Presenting Author - *Alejandro López Barberá, Spain*

Author/s – *Nuria Canela, Helena Torrell, Nerea Abasolo*

Abstract Content

Mevalonate (MVA) is a high value molecule, used as a precursor of isoprenoids with importance in a broad spectrum of industry applications. In this study CRISPR/Cas9 system and Omics tools were combined to increase the natural mevalonate production of the baker's yeast *Saccharomyces cerevisiae*. To achieve this, metabolomic analysis over the mevalonate pathway and global transcriptomics were performed in yeasts that had been grown in minimal medium and in a rich medium focused in increase growth and mevalonate production. The results from these analyses combined with bibliographic information allowed us to define the best targets and way to apply the CRISPR/Cas9 system modifications on the MVA metabolic pathway. A double modification was performed in the MVA pathway to produce a bottleneck, increasing its production and reducing its consumption. Then, a post-mutation Multiomics analysis was used to confirm the absence of off-target mutations that can produce unintended alterations over the yeast's phenotype helping us to confirm the specificity of the mutations. This study shows the possibilities of integrating omics tools into the CRISPR/Cas9 system to help in a better design of the mutations and analyze possible off-target mutations effects.

T106 - Characterization of a cyanobacterial rep protein with broad-host range and its utilization for expression vectors

Presenting Author - *Yutaka Sakamaki, Tokyo University of Agriculture, Japan*

Author/s – *Kaisei Maeda, Kaori Nimura-Matsune, Taku Chibazakura, Satoru Watanabe*

Abstract Content

Cyanobacteria is expected to the ecology-friendly host for producing biomaterials, such as biofuels. Genetic engineering is one approach to promote utilization cyanobacteria, however, cyanobacteria still lag behind the more advanced *E. coli* toolkit. Especially, there is limited information on expression vectors available for gene overexpression in cyanobacteria. Currently, RSF1010-based vector systems are used for bioproduction using cyanobacteria, although expression levels of exogenous genes is limited due to the low copy number. Thus, high-copy vector systems that can be used with a variety of cyanobacteria are required.

Our comprehensive screening using a genomic library of *Synechocystis* sp. PCC 6803 revealed that a certain region encoding a Rep-related protein (here named Cyanobacterial Rep protein A2: CyRepA2) exhibits high autonomous replication activity in a heterologous host cyanobacterium, *Synechococcus elongatus* PCC 7942. A reporter assay using GFP showed that the expression vector pYS carrying CyRepA2 can be maintained in not only S. 6803 and S. 7942, but also *Synechococcus* sp. PCC 7002 and *Anabaena* sp. PCC 7120. In S. 7942, GFP expression in the pYS-based system was tightly regulated by IPTG, achieving 10-fold higher levels than in the chromosome-based system. Furthermore, we made use of genes encoding 1,8-cineole synthase (cnsA) to demonstrate functional gene expression in S. 7942 from the pYS *in vivo*. These findings suggest that pYS is useful for genetic engineering, such as modifying metabolic pathways, and is expected to improve the performance of cyanobacteria as bioproduction chassis.

T107 - Repetitive fermentation of *Pichia pastoris* as an efficient expression system for unspecific peroxygenase from *Agrocybe aegerita*

Presenting Author - *Florian Kelsch, Hamburg University Of Technology, Germany*

Author/s – *Victoria S. Büschler, Niklas Teetz, Giovanni V. Sayoga, Frank Hollmann, Dirk Holtmann, Andreas Liese, Daniel Ohde, Hamburg University of Technology, Institute of Technical Biocatalysis, Hamburg, Germany & Paul Bubenheim, Hamburg University of Technology, Institute of Technical Biocatalysis, Hamburg, Germany*

Abstract Content

The synthetic industry's demographic shift towards biocatalysis requires elaborate enzyme production pathways. Unspecific peroxygenase derived from *Agrocybe aegerita* (AaeUPO) is an enzyme with a rapidly rising number of applications. The cytochrome-P450-like enzyme can be obtained from a recombinant yeast, *Pichia pastoris* X33 strain, using methylotrophic fermentation. *P. pastoris* is a well-established and Generally Recognized As Safe (GRAS) workhorse for recombinant protein biosynthesis, which was even used in the first pilot-scale production. Yet, the enzyme remains not commercially available in significant amounts. Therefore, AaeUPO must be produced in sufficient quantities at lab-scale in research facilities working with it.

Since the enzyme is expressed extracellularly and can be easily separated from the yeast during downstream processing, the cells remain viable. The overall objective of this contribution is to demonstrate the feasibility of recycling the yeast cells three to four times in batch operation, regardless of the fermentor set-up. The biomass, enzyme activity, and protein concentration of the ferment were measured and evaluated. Based on these process data, the productivities of the respective cycles were compared.

The results demonstrate that reusing biomass over multiple fermentation cycles provides advantages in terms of time, capacity, and resources for the production of AaeUPO. Normalization of productivity by total fermentation time and biomass concentration allows the comparison of different fermentation systems, showing the same overall trend for all investigated fermenters. The potential applications of the results showcased extend to both laboratory-scale and small-scale productions of extracellular proteins with *P. pastoris* or similar organisms.

T108 - Enabling the dynamic interplay between complex microbiota and host in the small intestine though *in vitro* modelling

Presenting Author - *Inez Roegiers, Center for Microbial Ecology and Technology (CMET), Belgium*

Author/s – *Tom Van de Wiele, Marta Calatayud Arroyo*

Abstract Content

Background: The small intestinal environment is a crucial site in the human body, which due to the complexity of interactions between host and microbes, remains challenging to study. We tackle this difficulty by proposing an *in vitro* model for this ecosystem using a triple co-culture, combining a microbial community, epithelial cells and immune cells.

Objective: Our main objective is to create a model that is responsive to a certain bacterial load or community.

Methods: In the first experiment, we add 103 or 105 CFU/mL of a synthetic microbial community (*L. fermentum*, *S. parasanguinis*, *P. intermedia*, *V. atypica*) resembling the small intestinal microbiota on top of epithelial cells (Caco-2 and LS174T) and macrophages (THP-1) in a Transwell® set-up. In a second experiment, we expose the cells in the same set-up to a more complex, SHIME-derived community. In both experiments, we monitor barrier integrity by measuring transepithelial electrical resistance (TEER), look at paracellular transport over the epithelium, measure cytotoxicity with the lactate dehydrogenase (LDH)-assay, quantify gene expression of ZO-1 (tight junctions), MUC2 (mucus secretion), VIL1 (brush border) with RT-qPCR, and determine cytokine release from intestinal cells with the Luminex® assay.

Results: Results indicate that the bacteria do not impose any cytotoxic effects on the intestinal cells and show the model's responsiveness to a certain load of bacteria under non- and proinflammatory conditions, as well as to a more complex microbial community. This proves its future potential to research host-microbe interactions in the small intestinal, and in general, the gastrointestinal microenvironment.

T109 - Screening of the complex formation inhibitors of Survivin and HBXIP using a yeast two-hybrid method and evaluation of the properties

Presenting Author - *Yasuhiro Iida, Kanagawa Institute of Technology, Japan*

Author/s – *Yoshiho Akiyama, Takumi Fujita, Mao Hayashi*

Abstract Content

Using the yeast two-hybrid method, we constructed a system to evaluate the binding ability of survivin and HBXIP. The system was used as an index to screen extracts of herbal medicines with binding-inhibiting abilities. Apoptosis was evaluated using tumor cells for the crude drug extracts that were evaluated to have the binding-inhibiting ability, and all of the crude drug extracts were shown to induce apoptosis in tumor cells. On the other hand, none of the crude drug extracts induced apoptosis in normal cells. In this study, we focused on marine sand, a herbal medicine, and tried to isolate and identify inhibitors of Survivin-HBXIP complex formation contained in the extract of *Lygodium japonicum*.

The yeast two-hybrid method is commonly used to clone cDNAs for proteins that bind to the protein of interest. However, we have tried to screen substances that inhibit the binding using the activity of galactosidase produced by binding as an index. The proteins of interest are Survivin and HBXIP. Survivin belongs to the IAP (Inhibitor of apoptosis protein) family and is highly expressed in cancer cells. Survivin suppresses apoptosis by interacting with HBXIP and forming a complex. As a result of screening using this two-hybrid method, it was suggested that a substance that induces apoptosis in tumor cells could be found.

T110 - Effect of culture medium and salinity on biosurfactant production by moderately halophilic bacteria

Presenting Author - *Alba Arranz, Spanish Society for Microbiology, Spain*

Author/s – *Tania Antón Rodríguez, Joaquín José Nieto Gutiérrez, Carmen Vargas Macías, Montserrat Argandoña Bertrán*

Abstract Content

Background: Nowadays, the replacement of fossil-based products with cost-effective and environmentally friendly bioactive compounds is of great interest. Owing to their metabolic versatility, low nutritional requirements, and adaptability to harsh conditions, halophilic microorganisms constitute an interesting reservoir to detect and identify the production of biosurfactants. Due to their amphiphilic character, containing both polar and apolar moieties in their structure, these molecules possess bioactive properties of high interest in pharmaceutical, cosmetic, food and agrochemical industries.

Objectives: This study aims to screen the ability of 40 moderately halophilic bacteria to produce biosurfactant and to evaluate the effect of medium composition and salinity on biosurfactant production on a subset of strains from the initial strain collection.

Methods: All strains were initially cultured in complex medium at their optimal salinity conditions. Biosurfactant activity of the supernatant fractions was determined using the following screening methods: oil displacement assay, drop collapse, emulsification activity and CTAB/methylene blue plate assay.

Results: A collection of 40 moderately halophilic bacteria were selected based on bibliography, databases, and *in silico* analysis to predict the presence of biosynthetic gene clusters using antiSMASH1. Among them, some strains that exhibited different biosurfactant production profiles for the detection methods were selected. Further analysis to assess biosurfactant production in complex and minimal medium at different salinities, revealed that both factors play a crucial role on the production of these bioactive compounds and the profiles observed differ significantly depending on the strain.

T111 - Endophytic selenobacteria as bio-factories of SeNPs: a comparative study of their synthesis and characterization

Presenting Author - *Eulàlia Sans, Universidad de la Frontera, Chile*

Author/s – *Carla GallardovBenavente, Paola Durán, María de La Luz Mora*

Abstract Content

In the current study, two endophytic selenobacteria (*Bacillus paranthracis* and *Enterobacter ludwigii*) were used to biosynthesize and characterize selenium nanoparticles (SeNPs) that will be used for biofortification and/or biotechnological applications.

We showed that both strains were suitable "cell factories" for manufacturing SeNPs (B-SeNPs from *B. paranthracis* and E-SeNPs from *E. ludwigii*), with varied characteristics, by controlling growth conditions and selenite exposure duration.

According to studies using dynamic light scattering (DLS), transmission electron microscopy (TEM), and atomic force microscopy (AFM), intracellular E-SeNPs (56.23±4.85 nm) had a smaller diameter than B-SeNPs (83.44±2.90 nm), and both formulations could be found in the surrounding medium or bound to the cell wall. Particularly in the instance of *B. paranthracis*, AFM images revealed the absence of pertinent fluctuations in bacterial volume and shape as well as the presence of layers of peptidoglycan encasing the bacterial cell wall under conditions of production.

SeNPs were found to be surrounded by proteins, lipids, and polysaccharides of bacterial cells, as revealed by Raman spectroscopy, Fourier transform infrared spectroscopy (FTIR), energy dispersive X-ray (EDS), X-ray diffraction (XRD), and X-ray photoelectron spectroscopy (XPS). B-SeNPs also contained more functional groups than E-SeNPs. Our further efforts must be concentrated on the evaluation of their bioactivity as well as on the determination of how the various features of each SeNPs modulate their biological action and their stability.

These findings suggest that these two endophytic stains are suitable as potential biocatalysts to produce high quality Se-based nanoparticles.

T112 - Searching for new siderophores of industrial interest produced by halophilic bacteria combining *in silico* and *in vivo* screening

Presenting Author - *Tania Antón Rodríguez, Universidad de Sevilla, Spain*

Author/s – *Alba Arranz San Martín, Carmen Vargas Macías, Joaquín José Nieto Gutiérrez, Montserrat Argandoña Bertrán*

Abstract Content

Background: Nowadays, natural amphiphilic products are highly requested due to their ability to form supramolecular structures with potential biotechnological applications, such as micelles and vesicles. The amphiphilic properties of some siderophores, which are produced by bacteria to scavenge iron from the environment, makes them interesting in several scientific fields such as medicine, agriculture, or food industry.

Objectives: The objective of this work is to find novel siderophores with interesting industrial properties produced by moderate halophilic bacteria, whose metabolic versatility, low nutritional requirements and adaptability to adverse conditions, makes them ideal for this aim.

Methods: More than 900 moderate halophilic strains have been compiled from scientific publications and databases. The available genomes have been analysed for the prediction of biosynthetic gene clusters (BGCs) responsible for siderophore synthesis using the bioinformatic tool antiSMASH. Then, a first selection of strains covering both taxonomical and siderophore type diversity was made and an *in vivo* analysis using overlaid CAS (O-CAS) methodology was performed.

Results: Among the 472 analysed genomes, more than 160 BGCs codifying for siderophores were identified. High taxonomical diversity has been achieved as 70 different bacterial genera were identified. Gammaproteobacteria and Bacilli were the most representative bacterial classes; and *Halomonas*, *Halobacillus* and *Marinobacter*, the most abundant genera. Structural and genetic variability has been performed as almost 30 different types of BGCs were detected. All the strains analysed with O-CAS so far have produced siderophores *in vivo*. Each strain has shown a different production level according to the results inferred from O-CAS.

T113 – Biosynthesis, characterization, and evaluation of antimicrobial activity of AgNPs functionalized with photosensitizing agents (PS) for application in endodontics

Presenting Author - *Pablo Betancourt, Universidad de la Frontera, Chile*

Author/s – *Gonzalo Bernal, Eulália Sans-Serramitjana, Mónica Pavez, Olga Rubilar, Alvaro Cerda,*

Abstract Content

Endodontic infections are generally caused by bacterial biofilms. Silver nanoparticles (AgNPs) have antimicrobial activity at low concentrations, which can be synthesized biologically. Conversely, photosensitizing agents (PS) stand out for their antimicrobial activity, e.g., Rose Bengal (RB). The aim of this work was to synthesize and characterize AgNPs functionalized with RB, and to evaluate their antimicrobial activity.

The biogenic synthesis of AgNPs was performed using *Galega officinalis* extract and direct functionalization of PS using 30 µl of RB, forming the AgNPs/RB complex. Both AgNPs and AgNPs/RB were characterized by UV-Vis spectroscopy and dynamic light scattering. The antimicrobial activity was evaluated by minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and kill curve assays against *Enterococcus faecalis* ATCC 29212 and *Escherichia coli* ATCC 25922.

We reported bioproduction of AgNPS and AgNPs/RB complex with an average size of 47.64±0.90 and 85.37± 2.49 nm, respectively, and with optimal Z potential and PDI values in both cases. Both formulations showed bactericidal activity against *E. coli*, but not against *E. faecalis*, which only showed growth inhibitory activity (*E. faecalis* (AgNPs-MIC 0.20 mg/ml and AgNPs/RB-MIC 0.25 mg/ml); *E. coli* (AgNPs-MIC 0.40 mg/ml and AgNPs/RB-MIC 0.25 mg/ml). However, it showed a growth decrease of 64% for *E. coli* and 50% for *E. faecalis* against both NPs at an initial 30 min of total exposure time (240 min).

Thus, this work shows promising results, which will be complemented by further antimicrobial photodynamic therapy-based experiments.

T114 - Design, activity and expression of a novel antimicrobial peptide – R10

Presenting Author - *Marta Rubina, University of Latvia, Latvia*

Author/s – *Zane Lasa, Inese Strazdina, Gints Kalnins, Uldis Kalnenieks, Anna Ramata-Stunda, Martins Boroduskis, Reinis Rutkis, Institute of Microbiology and Biotechnology, University of Latvia,*

Abstract Content

Background: Antimicrobial drug resistance is widespread and well identified problem, therefore there is a great interest in the discovery of novel therapeutics and production strategies. Since antimicrobial peptides are less prone to the bacterial resistance, they have been suggested as a viable class of novel antimicrobials.

Objectives: Here we design a novel antimicrobial peptide – R10, and determine its antimicrobial activity against ESKAPE pathogens and evaluating cytotoxicity using Balb/c 3T3 and dermal fibroblast cells. Additionally, a possible bacterial expression system is examined.

Methods: The structure of the peptide was predicted by APPTEST and later confirmed via circular dichroism measurements. Mode of action was hypothesized on the basis of FITC-tagged peptide confocal microscopy images. Balb/c 3T3 cells were used to determine cytotoxicity. The MIC concentration were determined by quantification of pathogen growth in a 96-well plate microplate reader.

Results: The size of R10 peptide is 2.74 kDa and the conformation is α-helical. The FITC-tagged peptide was localized in virtually intact bacterial cell membrane, thus suggesting that R10 is inhibiting bacterial growth rather by pore formation than complete cell membrane disruption. The MIC value for *Escherichia coli* and *Pseudomonas aeruginosa* is 2 μg/mL, for *Enterococcus faecium* and *Staphylococcus aureus* - 4 μg/mL and for *Cutibacterium acnes* - 0.5 μg/mL. Low concentrations of R10 were nontoxic to Balb/c 3T3 cells. As, *Zymomonas mobilis* bacteria, plausibly due to uncoupled growth phenomenon, possessed an increased resistance against R10 (31 μg/mL), we examined *Z. mobilis* as an expression platform for this peptide.

T115 - NxtGenWood: Converting wood-based phenols to value added products

Presenting Author - *Mauricio Troncoso, Instituto Ramón Y Cajal De Investigación Sanitaria (irycis), Ireland*

Author/s – *Kevin O'Connor, Tanja Narancic*

Abstract Content

Lignin, one of the most abundant natural polymers on the planet, is also one of the most underutilized due to its recalcitrant nature. Our project aims to investigate novel ways to valorise lignin into value-added products utilizing bacterial cultures as biocatalysts. In order to achieve this, different bacterial species were selected from the literature due to their reported ability to use lignin derived phenols. The bacteria were tested for growth on the three main phenolic acids present in lignin (ferulic, sinapic and p-coumaric acid). The results from these experiments showed that Pseudomonas species showed the most promise for growth on phenolic acids.

We then improved the growth of some of the strains on ferulic and p-coumaric acids using Adaptive Laboratory Evolution (ALE). Here we systematically increased the concentration of p-coumaric acid in the medium the strains were exposed to. After three weeks we obtained an adapted strain that was able to grow at a faster growth rate and was tolerant to higher concentrations of ferulic and p-coumaric acid. This bacterium also shows promising growth with sinapic acid (which is the most recalcitrant out of the three phenols) after a further ALE experiment. It is therefore a promising candidate for revalorization of three lignin based phenols. Future experiments will be focused on developing a process using the optimized bacterial strains for the biotransformation of lignin derived phenols into value-added products.

T116 - Prevalence of multidrug-resistant (MDR) *Klebsiella pneumoniae* causing bloodstream infections in hospital settings

Presenting Author - *Amela Dedeic Ljubovic, OU Clinical microbiology, Bosnia and Herzegovina*

Author/s – *Đana Granov, Daria Bekić*

Abstract Content

Background: *Klebsiella pneumoniae* is the second most prevalent gram-negative rod that causes nosocomial infections in hospitalized or otherwise immunocompromised patients. It has emerged as a major clinical problem due to the rising prevalence of the infections caused by emerging multidrug-resistant strains. This study aimed to analyze prevalence of MDR *Klebsiella pneumoniae* isolated in blood samples collected from patients with bloodstream infections.

Methods: The retrospective study included *Klebsiella pneumoniae* isolates that were recovered from blood culture samples in a period 2018-2022. Samples were incubated in a BD Bactec 9120 automated blood culture system (Becton Dickinson, Sparks, MD, USA). Final identification and antimicrobial susceptibility was determined by VITEK 2 Compact System (bioMerieux, France). ESBL and carbapenemase detection was confirmed by double disc synergy test and combined-disk test containing meropenem ± various inhibitors (ROSCO Diagnostica A/S, Denmark). Results were interpreted according to EUCAST guidelines.

Results: A total od 200 *Klebsiella pneumoniae* isolates were detected from blood culture samples from various hospital departments: ICU 17,5% (35), pediatric ICU 40,5% (81) and non-ICU department 42% (84). 43,5% (87) were ESBL producers, while 17% (34) of isolates showed resistance to carbapenems (CRKP). The prevalence of ESBL-positive isolates for the period 2018-2022 was 14,5%, 9%, 7%, 9% and 3%, respectively. These strains were sensitive on carbapenems and colistin, while 38% of isolates showed resistance to amikacin. The prevalence of CRKP isolates in the same period was 0%, 3,5%, 5,5%, 5,5% and 2,5%, respectively. All these isolates were sensitive on colistin, while 41,2% were resistant to amikacin.

T118 - Asymptomatic colonization of methicillin-resistant *Staphylococcus aureus* in upper respiratory tract of younger children

Presenting Author - *Margaret Ip, The Chinese University of Hong Kong, China*

Author/s – *Liuyue Yang, Priyanga Dharmaratne, Chendi Zhu, Dulmini Sapugahawatte, Nannu Rahman, Nilakshi Barua, Carmen Li, Kin On Kwok,, Mingjing Luo, Veranja Liyanapathirana*

Abstract Content

Background: The burden of asymptomatic methicillin-resistant *Staphylococcus aureus* (MRSA) colonization is ambiguous, especially among young children. Available studies lack data on a global scale.

Objectives: This systematic review and meta-analysis aimed to estimate the global prevalence of asymptomatic colonization and associated risk factors and antibiotic resistance in the upper respiratory tract of younger children.

Methods: MEDLINE, Embase, Web of Science, and CINAHL databases were searched between 2010 and 2022, following the registered protocol in PROSPERO (CRD 42022328385), and articles were screened using Covidence.

Results: A total of 12,974 articles were screened, and 35 studies (34 peer-reviewed papers and one letter to editor) were selected for the analysis. The studies' pooled prevalence of *S. aureus* and MRSA was 25.1% (95% CI, 21.4 - 28.8; I2 = 97.9%) and 3.4% (95% CI, 2.8 - 4.1; I2 = 96.7%), respectively. MRSA prevalence was high in Asia (5.8%) and South America (5.6%) than that in Europe (0.6%). MRSA prevalence in community and hospital settings was 7.2% and 5.6%, respectively, while in children's institutions was 2.2%. Previous antibiotic exposure is a significant risk factor ($p < 0.05$) in asymptomatic colonization, while over 60.0% pooled resistance rates were reported in cefoxitin (100.0%), penicillin (98.9%), oxacillin (95.1%), ampicillin (93.3%), and erythromycin (63.0%).

Conclusion: The increasing colonization rate and considerable heterogeneity of prevalence estimation highlighted the need for more cross-regional studies, which would provide a more precise understanding on the burdens of asymptomatic colonization of both HA-MRSA and CA-MRSA among the susceptible population.

T119 - *Leptospira* spp. and recombinant proteins attachment to host cells

Presenting Author - *Ana Lucia Nascimento, Butantan Institute, Brazil*

Author/s – *Maria Takahashi, Aline Teixeira*

Abstract Content

Background: One of the main mechanisms of pathogens during infection is adherence to host tissues. Although adhesion to some host cells was shown, the ligands involved in this adherence are not fully understood.

Objectives: Thus, we aimed to extent these studies to other cell lines and to verify the involvement of Leptospiral proteins in these interactions.

Methods: Evaluation of the adhesion of virulent, culture-attenuated and saprophyte leptospires to endothelial, epithelial and fibroblasts was performed by ELISA. Interaction of recombinant proteins to immobilized cells was evaluated by ELISA or when in suspension by Western blotting.

Results: Pathogenic strain was able to adhere to all cells, however when culture-attenuated was used, adherence was observed only to 293T, EA.hy926 and HULEC5a. Saprophytic strain adhered only to HULEC5a. OmpL1 showed binding to all immobilized and in suspension cells. LipL41 interaction was also observed to all cells, however it did not show binding to immobilized HULEC5a and to in suspension HMEC-1 cells. LipL46 and OmpL37 showed binding to all cells in suspension; the binding to immobilized cells were observed only to epithelial cells and HULEC5a for LipL46, and 293T cells for OmpL37. LipL21 showed no binding to cells in suspension, but interacted to immobilized 293T cells. *Leptospira* virulent strain binds to mammalian cells more efficiently than culture- attenuated and saprophytic strains. As OmpL1 and LipL41 interacted with all the immobilized or in suspension cells, and both are present in pathogenic strains, it is anticipated a contribution of these proteins during Leptospiral infection.

T121 - Antimicrobial and antibiofilm properties of polypeptide-enriched extracts from the Mediterranean seagrass *Posidonia oceanica*

Presenting Author - *Domenico Schillaci, University Of Palermo, Italy*

Author/s – *Diletta Punginelli, Valentina Catania, Mirella Vazzana, Manuela Mauro, Vincenzo Arizza, Maria Vitale*

Abstract Content

Due to the rapid increase of antimicrobial resistance to conventional antibiotics and the diffusion of multidrug-resistant pathogens worldwide, it is urgent to develop alternative class of therapeutic molecules. Antimicrobial peptides (AMPs) are considered potential therapeutics in the treatment of bacterial and fungal infections. We focused on Mediterranean seagrass *Posidonia oceanica* as a source of new bioactive molecules with antimicrobial and antibiofilm properties. Polypeptide-enriched fractions of rhizomes and green leaves of the seagrass were tested against Gram-positive reference ATCC strains (*Staphylococcus aureus*, *Enterococcus faecalis*) and Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*) and towards the yeast *Candida albicans*. The above mentioned extracts showed MIC values ranging from 1.61 µg/mL to 7.5 µg /mL, against tested pathogens. Peptide fractions were further analyzed through a high-resolution mass spectrometry and database search, identifying twelve novel peptides. Some peptides and their derivatives were chemically synthesized and assessed *in vitro* against bacterial and yeast strains. In particular, two synthetic peptides, derived from green leaves and rhizomes of *P. oceanica*, revealed an interesting antibiofilm activity towards *E. coli* and *P. aeruginosa* (BIC50 equal to 17.6 µg/mL and 70.7 µg/mL). In conclusion, these results support the potential use of discovered peptides as platform for the development of novel synthetic AMPs with improved pharmaceutical potential against relevant pathogens.

T122 - Nosocomial foodborne disease outbreak of *Salmonella* Enteritidis, South Africa, 2022

Presenting Author - *Anthony Smith, National Institute For Communicable Diseases, South Africa*

Author/s – *Brian Brummer, Phuti Sekwadi, Linda Erasmus, Juno Thomas*

Abstract Content

Background: In September 2022, health authorities were notified of a suspected outbreak of foodborne disease in a hospital in South Africa. Multiple medical staff reported acute onset of abdominal cramps, diarrhoea, fever and rigors after eating a chicken pasta meal.

Objectives: To use whole-genome sequencing (WGS) analysis of bacterial isolates to support an epidemiological investigation of the nosocomial foodborne disease outbreak.

Methods: Epidemiological investigation of the outbreak was led by the Infection Control Manager of the hospital and supported by an outbreak response team. Standard microbiological procedures were used to process stool samples and culture/identify diarrhoeal pathogens. Bacterial cultures were investigated using WGS performed using Illumina NextSeq technology. WGS data were analyzed using multiple bioinformatics tools, including those available at the Center for Genomic Epidemiology and EnteroBase. Core-genome multilocus sequence typing (cgMLST) was used to investigate the phylogeny of isolates.

Results: Forty-nine cases were identified. Stool samples were collected from 21 cases and nontyphoidal *Salmonella* was isolated from 19/21 (90%) of the samples. Isolates were identified as *Salmonella* Enteritidis. Isolates differed from each other by ≤2 allele differences on cgMLST, indicating that isolates are highly genetically related. Delays in testing of food retention samples rendered the negative test results of limited value. A case-control study was conducted; eating chicken pasta was associated with developing gastroenteritis (odds ratio, 39; 95% confidence interval 1.57-969.24). All evidence suggests that the chicken pasta was the likely vehicle of transmission in this outbreak. The source of *Salmonella* Enteritidis in the implicated meal remains unknown.

T123 - Impact of the partially purified bioactive extracts from *Euclea crispa* on cell membrane integrity of microbial pathogens

Presenting Author - *Kazeem Alayande, North-West University, South Africa*

Author/s – *Rasheed Adeleke*

Abstract Content

Antimicrobial extracts from the leaf and stembark of *Euclea crispa* was partitioned into different components based on polarity of the active contents using solvent partitioning system. Antimicrobial potentials of the potent fractions were determined through MIC and time-kill kinetics. Degree of compromise on the cell membrane integrity was determined by measuring leakages of proteins and nucleotides from the intracellular compartments, while the extent of physical damage was observed through scanning electron microscopy imaging. The functional groups of the bioactive components were determined using FTIR. The leaf extract partitioned into n-butanol and ethyl-acetate exhibited the lowest MIC of 0.16 mg/ml against most bacteria and 0.31 mg/ml against *Candida albicans*, while the lowest MIC (0.31 mg/ml) exhibited by n-butanol fraction partitioned from the stembark was against *Enterococcus faecalis*. N-Butanol fraction from leaf extract achieved absolute mortality against *Bacillus pumulis* and *Klebsiella pneumoniae* after 90 and 120 min contact time respectively at 1xMIC. N-Hexane fraction achieved the same feat against *Candida albicans* after 120 min. After 120 min of contact time at 1xMIC, ethyl-acetate fraction partitioned from the stembark achieved total mortality against *Listeria* sp. and S. Typhimurium. Maximum quantity of proteins (0.566 mg/ml) was released from *K. pneumoniae* by n-butanol fraction at 2xMIC after 120 min while the maximum nucleotides released (4.575 mg) was from *B. pumulis* by n-hexane fraction under similar condition. SEM imaging revealed conspicuous cell membrane disruption. This study suggests *Euclea crispa* a source of bioactive compound with membrane attack as one of the mechanisms of its biocidal action.

T124 - Septic arthritis in a child caused by 19a serotype *Streptococcus pneumoniae*: a case report

Presenting Author - *Preslava Hristova, Department of Microbiology and Virology, Medical University Pleven, Bulgaria*

Author/s – *Hristina Y. Hitkova, Stefan V. Trifonov, Nikolay K. Balgaranov, Raina G. Gergova, Alexandra S. Alexandrova,*

Abstract Content

Background: In the last years, the pneumococcal septic arthritis in children is extremely rare. The application of pneumococcal vaccines reduces dramatically the incidence of invasive pneumococcal disease but also result in the spread of new emergent and adaptive non-vaccine serotypes.

Objectives: We report a cases of knee infection in a 3-year-old boy caused by 19A serotype *S. pneumoniae* and present data from molecular typing of this strain.

Methods: The synovial fluid was positive for *S. pneumoniae*, identified by Vitek2 Compact (bioMérieux, France). PCR for the major pneumococcal autolysin was conducted to distinguish *S. pneumoniae* from other streptococci. Serotyping of the isolate was performed using the latex agglutination factor antisera provided by Statens Serum Institut (Pneumotest-Latex kit; SSI). Susceptibility testing was performed by the broth microdilution method using the Sensitre custom plate format (TREK diagnostic systems). The presence of macrolide resistance was determined by PCR for the expression of ermB and mefE genes. MLST was carried and genetic relatedness to reference pneumococcal clones was confirmed in the Pneumococcal Molecular Epidemiology Network.

Results: The lytA-based PCR identified *S. pneumoniae*. The latex agglutination test determined a 19A serotype isolate. The strain showed dual macrolide resistance, in presence of both ermB and mefE genes with high MIC values (MIC>256 mg/L) and susceptibility to all other tested antibiotics. The MLST analysis disclosed ST695, which is categorized as GPSC type 27.

T125 - A novel autotransporter Adhesin identified in atypical enteropathogenic *E. coli* serotype O2:H16 mediates biofilm formation

Presenting Author - *Rodrigo Hernandes, Universidade Estadual Paulista (UNESP), Brazil*

Author/s – *Henrique Orsi, Guilherme F R de Souza, Rosa M Chura-Chambi, Mark A Schembri, Tânia A T Gomes, Waldir P Elias*

Abstract Content

Background: Atypical enteropathogenic *Escherichia coli* (aEPEC) causes diarrhea affecting children and adults worldwide. In a comparative genomic analysis of 106 aEPEC, we have identified a set of 31 genes unique to the serotype O2:H16, among which we found a gene encoding an uncharacterised autotransporter adhesin.

Objectives: The goal of this study was to evaluate the contribution of this novel autotransporter adhesin (ATA) to biofilm formation.

Methods: The recombinant plasmid pIC (pBAD Myc His-A harboring the autotransporter adhesin-encoding gene) was electroporated in MS427 (an *E. coli* K-12 MG1655 derivative with a deletion in the agn43 gene), as well as in the atypical and typical EPEC strains 1711-4 and C209-2, respectively. In addition, the passenger domain was cloned in the pET-28a vector, and this construct was used to express and purify the recombinant protein for the production of a polyclonal antibody. ATA expression in the *E. coli* isolates was confirmed by immunoblotting; biofilm formation was evaluated in polystyrene plates using crystal violet staining after 24, 48, and 72 hours of incubation.

Results: The novel ATA increased the ability of MS427 to form a biofilm by ~1.5-fold at all time points tested. In aEPEC 1711-4 and EPEC C209-2, expression of ATA led to a statistically significant difference in biofilm formation (~1.5-fold) after 72 hours incubation. Together, our data suggest the novel ATA identified in this study may contribute to biofilm formation in both typical and atypical EPEC isolates.

T126 - Application of fluorescence microscopy for the early determination of meropenem activity against *Klebsiella pneumoniae*

Presenting Author - *Kamilla Alieva, Gause Institute of New Antibiotics, Russian Federation*

Author/s – *Anastasia Kuznetsova, Maria Golikova, Elena Strukova, Yuri Portnoy, Stephen Zinner*

Abstract Content

Background: To increase the likelihood of successful treatment of bacterial infections, antibiotic activity should be defined as quickly as possible so therapy can be modified accordingly.

Objectives: We evaluated the applicability of fluorescence microscopy with acridine orange for the early determination of *Klebsiella pneumoniae* susceptibility to meropenem.

Methods: Two meropenem-susceptible (MIC 2 µg/ml) carbapenemase non-producing and OXA-producing clinical isolates of *K. pneumoniae* were used. Test tubes with Mueller-Hinton broth with no antibiotic and with 1 (0.5×MIC), 2 (1×MIC) and 8 µg/ml (clinical MIC breakpoint) of meropenem were inoculated (5×10^5 CFU/ml) with an 18h bacterial culture and incubated at 37°C. Each tube was sampled at 0, 2, 4, 5, 6, 7, 8, 24 h. Samples for microscopy (20 µL) were air dried on a microscope slide and resuspended in 5 µL of 0.01 % acridine orange stain, samples (100 µL) were also plated on Mueller-Hinton agar. The limit of detection (LOD) was 5.7 and 3.0 log CFU/ml, respectively.

Results: Without antibiotic, bacterial counts obtained with microscopy (green plus red fluorescence) and with agar plating were almost equal throughout the observation period. In the presence of meropenem, cell regrowth was consistent with the antibiotic concentrations. The significant relationships between the ratio of meropenem concentration to MIC and the bacterial counts at 6, 8, 24 h determined by microscopy (r^2 0.80-0.83) or agar plating (r^2 0.75-0.9) were established. Fluorescence microscopy is applicable for the early MIC determination. It is desirable to reduce the LOD to increase the accuracy of this technique.

T127 - Comparing a covid-19 rapid antigen assay (colloidal gold) from saliva samples with RT-PCR results in patients with symptoms of upper respiratory tract infections

Presenting Author - *Gulfem Ece, University Of Health Sciences Turkey, Turkey*

Author/s – *Dogukan Pira*

Abstract Content

Covid-19 describes a spectrum of disease obtained by inhaling SARS-CoV 2. Covid-19 is predominantly diagnosed in the laboratory, either with fast antigen tests or nucleic acid amplification procedures like RT-PCR. This study was conducted to compare the performances of a Covid-19 RT-PCR assay and a colloidal gold based Covid-19 Rapid Antigen Test applied on saliva samples in patients presenting with signs of upper respiratory tract infections; and evaluate the clinical correlation. An analysis of the results obtained after testing two separate samples collected from 300 patients revealed the sensitivity of the rapid antigen test to be 91.84% when compared to RT-PCR results as a gold standard. There was high concordance between the testing techniques (kappa coefficient 0.938). The rapid antigen test was deemed fit for use as an auxiliary test for the screening of symptomatic individuals.

T129 - Antibacterial properties of various cationic steroid antibiotics against *Enterococcus* spp.

Presenting Author - *Mayram Hacioglu, Istanbul University, Turkey*

Author/s – *Fatima Nur Yilmaz, Eda Altiner, Cagla Bozkurt Guzel, Nese Inan, Paul B. Savage*

Abstract Content

Background: Enterococci, are common indigenous flora members of the gastrointestinal tracts and can also normally present in the oral cavity, vagina, oropharynx, and urethra of humans. Today, the most important feature of *Enterococcus* is the increasing resistance rates to antibiotics, especially vancomycin-resistant *Enterococcus* (VRE). Attempts have been made to discover new antimicrobial agents that target new sites that can overcome resistance. The cationic steroid antibiotics (CSA) designed to mimic the activities of antimicrobial peptides, are a new class of antimicrobial agent.

Objectives: In this study, the *in vitro* effect of various CSAs and antibiotics were investigated against fifty *Enterococcus* spp. (18 of them VRE) isolated from various clinical speciemens submitted to the Synevo Laboratories Ankara Central Laboratory in Turkey (2021-2022).

Methods: The minimum inhibitor concentration (MIC) and minimum bactericidal concentration (MBC) of CSA-13, CSA-44, CSA-90, CSA-131, CSA-138, CSA-142, CSA-192, vancomycin, linezolid and daptomycin were determined according to the recommendations of the Clinical and Laboratory Standards Institute.

Results: According to the findings, the MIC50/90 of CSA-13, CSA-44, CSA-90, CSA-131, CSA-138, CSA-142, CSA-192, vancomycin, linezolid and daptomycin were 16/16 µg/ml, 2/4 µg/ml, 4/8 µg/ml, 16/16 µg/ml, 32/32 µg/ml, 8/16 µg/ml, 2/4 µg/ml, 32/512 µg/ml, 0.5/1 µg/ml and 1/1 µg/ml, respectively. The values noted for MBC were equal to or greater than those for MIC.

T130 - Healthcare-associated infections during the coronavirus pandemic

Presenting Author - *Branka Petrovska Basovska, Institute Of Public Health Of Republic Of North Macedonia - Skopje, North Macedonia*

Author/s – *Zlatko Arsenievski, Dugagjin Osmani, Shaban Memeti*

Abstract Content

Background: Nosocomial infections are acquired infections in patients during their stay in an acute or long-term healthcare institution. The prolonged length of stay in hospital contributes to the occurrence of healthcare-associated infections (HAIs). The European Centre for Disease Prevention and Control (ECDC) reveals there is an increase in HAIs in European hospitals. Objectives: This study will reviewed incidence of hospital-associated infections during the Coronavirus disease 2019 (COVID-19) pandemic.

Methods: This research includes epidemiological and microbiological data from public and private acute care hospitals in the capital town - Skopje. The study includes findings for two and half years (from 11 March 2020 – beginning the COVID-19 to 11 September 2022). Material report was available from reported HAIs data from 3 public state hospitals and 2 private healthcare facilities (one hospital and one specified health institution).

Results: The examined period showed 171 data findings. In 2020 were reported 27 nosocomial infections from public hospitals and 12 from private health care institutions. Year of interest was 2021 because there was no report from private hospitals for health-associated infections and only 10 HAIs from public state hospitals. There was a twist in intra-hospital reporting in 2022. Public state hospitals reported 94 nosocomial infections, while the private 28. Infection prevention measures had been necessarily collapsed during pandemic; patients went for a hospital care after delaying primary health visits and they went to health care institutions even when diseases were in progress. HAIs didn’t go away when COVID-19 came and their incidence increased.

T131 - Seroprevalence of torch IgG antibodies in pregnant women

Presenting Author - *Zhinzela Qyli, Fan S Noli University, Korca, Albania*

Author/s – *Zhinzela Qyli*

Abstract Content

Background: ToRCH includes *T. gondii*, others (*T. pallidum*, Varicella-zoster virus, Parvovirus B19, HIV), Rubella, Cytomegalovirus, Herpes simplex. During pregnancy ToRCH infectious agents present a high risk to the fetus. These microorganisms can cause miscarriage, premature labor, retarded growth and other developmental abnormalities.

Aim: The aim of this study was to identify and compare seroprevalence of antibodies against ToRCH infectious agents: *T. gondii*, CMV, Rubella virus, HSV1 and HSV2 in pregnant women in Korca, Albania.

Methods: 112 serum samples were collected from pregnant women at the first trimester of pregnancy. Women age ranged from 18 to 37 years old. The samples were tested for IgG antibodies to *T. gondii*, CMV, Rubella virus, HSV1 and HSV2 at a private clinic in Korca, Albania.

Results: From the samples tested, seroprevalence of IgG antibodies was 19.6% (22 samples) for *T. gondii*, 91.9% (103 samples) for CMV, 97.3% (109 samples) for Rubella virus, 86.6% (97 samples) for HSV1 and 5.3% (6 samples) for HSV2.

Conclusions: Rubella virus and CMV resulted with the highest seroprevalence of IgG antibodies respectively 97.3% and 91.9%. HSV2 resulted with the lowest prevalence of IgG antibodies 5.3%.

T132 - A real-life story: Drastic shift in leishmaniasis epidemiology in Turkey

Presenting Author - *Varol Tunali, Manisa Celal Bayar University, Turkey*

Author/s – *Ibrahim Cavus, Deniz Ozer, Yener Ozel, Cumhur Gunduz, Mehmet Harman, Ahmet Ozbilgin*

Abstract Content

Background: Turkey is an endemic country for Leishmaniasis and is located at the crossroads of Europe, Asia, the Middle East, and Africa and hosts the largest immigrant population in the world comprised mostly of Syrian refugees/migrants. In this retrospective study, we aimed to evaluate the clinical and genotypical alteration of leishmaniasis in Turkey.

Methods: All autochthonous cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL) patients diagnosed at Manisa Celal Bayar University after January 2010 were included in this study. Skin-scraping material from the lesion(s) and bone marrow aspirations were collected from CL and VL patients respectively. Touch preparations, culturing in NovyMacNealNicolle (NNN) and enriched NNN (E-NNN) medium, and PCR using primers designed specifically for ITS1 and cpb E/F regions were performed for each sample.

Results: A total of 450 autochthonous CL and 14 VL cases were identified. Diagnostic sensitivity for each diagnostic method was E-NNN culture, PCR, microscopy, and NNN culture respectively. Species distribution of CL patients was determined as *L. tropica*, *L. major*, *L. infantum*, *L. donovani*, *L. aethiopica*, and *L. infantum*/*donovani* hybrid. The VL species distribution was found to be *L. infantum*, *L. donovani*, *L. tropica*, and *L. infantum*/*donovani* hybrid.

Discussion: Immediately after the start of the Syrian civil war in 2011, there was a refugee influx to Turkey and recently from Afghanistan. Before 2010, no CL agents other than *L. tropica*, and VL agents other than *L. infantum* were reported in Turkey. Our study shows that all old-world CL agents and VL agents are present in Turkey.

T133 - Wastewater based epidemiology (WBE) approach to track SARS-CoV-2 dissemination: a longitudinal study in Bucharest, Romania

Presenting Author - *Elena Radu, Stefan S Nicolau Institute Of Virology, Romania*

Author/s – *Lilia Matei, Denisa Dragu, Carmen Cristina Diaconu, Mihai Nita-Lazar, Norbert Kreuzinger, Coralia Bleotu, Catalina Stoica and Dragos Radulescu*

Abstract Content

Background and Objectives: Wastewater-based epidemiology (WBE) has been shown to be a powerful tool to track SARS-CoV-2 dissemination in a defined catchment. The aim of our study is to implement a longitudinal campaign for SARS-CoV-2 surveillance in wastewater at city-level. A correlation of the wastewater SARS-CoV-2 genome copies with clinical data will provide a complete picture, as wastewater represents a pool of symptomatic, asymptomatic and pre-symptomatic patients who shed viral RNA via stool and urine. It is worth to mention that it represents the first study to implement SARS-CoV-2 WBE in Romania.

Methods: Urban untreated wastewater samples were collected twice a week, from the main sewer shed site of a hospital for infectious diseases (including Covid-19 patients) and the inflow of the main wastewater treatment plant. Viral particles concentration was carried-out using the PEG precipitation method. Total nucleic acid extraction was performed on Maxwell® device. SARS-CoV-2 RNA was quantified by RT-qPCR.

Results: SARS-CoV-2 viral RNA was detected in all wastewater samples. The genome copies present in wastewater were normalized to the population equivalent shedding in the respective catchment and compared with the epidemiological data provided by the health authorities. Total SARS-CoV-2 viral particles concentration detected in the untreated wastewater collected from the hospital was compared with the number of hospitalized patients and correlated also with patients' symptoms (eg. gastric manifestations).

Wastewater represents a suitable matrix to follow not only the dynamic of SARS-CoV-2 concentration but also the rise and fall of new variants.

T134 - Investigation of respiratory tract pathogens responsible for co-infection in COVID-19 patients

Presenting Author - *Arslan Ugur*

Author/s – *Sabiha Salar Gul, Nurullah Ciftci*

Abstract Content

Background: In December 2019, in Wuhan, the capital of China's Hubei province, an unknown betacoronavirus was isolated via metagenomic RNA sequencing from bronchoalveolar lavage fluid samples taken from pneumonia cases of unknown origin.

Objectives: Aim of the study is to determine coinfection rate in hospitalized patients with COVID-19. Furthermore, to investigate the etiological agents of coinfection, and to reveal the clinical characteristics of patients with coinfection.

Methods: In this study, 76 hospitalized patients samples with COVID-19 were included in the study. The patient's thoracic computed tomography findings were compatible with COVID-19 and biochemical test results supported coinfection. Other respiratory pathogens were studied in the respiratory tract samples of the patients by multiplex PCR using FTD 33 Respiratory pathogens kits.

Results: Coinfection with SARS-CoV-2 was detected by multiplex PCR in 54 of the 76 patients (71,1%) included in the study. A pathogen accompanying SARS-CoV-2 was detected in 35 patients, while more than one pathogen was detected in 19 patients. The most common pathogens were *S. aureus* in 24 (31,6%) patients, influenza viruses in 18 patients (23,7%), and *S. pneumoniae* in 15 (17,1%) patients. There was no significant difference among mortality rates, duration and site of hospitalization, laboratory test results (CRP, PCT, LDH, D-dimer, lymphocyte count, ferritin) of patients with and without coinfection. Although there was no significant difference in parameters of mortality rates and duration of hospitalization in patients with coinfection, we recommended that clinicians should consider the risk of coinfection, for these patients treatment and appropriate tests should be performed.

T135 - Cationic Streroid Antibiotics (CSA) Against *Pseudomonas aeruginosa* biofilms on soft contact lenses

Presenting Author - *Fatimanur Yilmaz, Istanbul University, Turkey*

Author/s – *Sibel Dosler, Mayram Hacioglu, Cagla Bozkurt Guzel, Paul B Savage, Ebru Haciosmanoglu Aldogan*

Abstract Content

Backgrounds: Contact lenses (CL) are important biomedical substances with not only the correction of some eye defects but also the cosmetic characteristics. Especially the formation of biofilms with adherent bacteria is very important for whole eye health. Especially *Pseudomonas aeruginosa* is the most common pathogen bacteria in eye infections. Ceragenins are a new group of cationic steroid antimicrobial (CSA) molecules, and promising agents for the treatment of infections caused by multi-drug resistant microorganisms, showing similar activity to antimicrobial peptides.

Objectives: This study was conducted with the aim of using CSAs as a new and alternative agent for combating the increasing number of biofilm originated CL-associated eye infections. We investigated the *in vitro* activities of CSA-44, CSA-131, and CSA-138 against *P. aeruginosa* (PAO1), biofilm on two kinds of new (PureVision and Softens-Toric), and one used (Pure Vision) soft CLs.

Methods: We determined the anti-biofilm activities of CSA molecules against the mature biofilms on CLs by counting the live bacterial cells. We also investigated the inhibition of bacterial attachment, and biofilm formation on CLs, at 4h, and 24h, respectively.

Results: Against the mature biofilms, CSA-44 and CSA-131 were the most active agents at 500µg/ml while only CSA-131 has -cidal activity at 100µg/ml concentration. Similarly, CSA-44 and CSA-131 could inhibit the attachment at 2.5xMIC (10µg/ml), while CSA-131 inhibits at MIC (4µg/ml). There was no significant inhibition of biofilm formation at 24h, with any CSA.

T137 - Peritoneal dialysis-associated peritonitis (PD-AP): spectrum and resistance of pathogens in a tertiary hospital

Presenting Author - *Ifigeneia Vasiliki Kontoteza, Laboratory Of Medical Microbiology, General Hospital Of Athens, Greece*

Author/s – *Anastasios Tsakalos, Dimitra Petropoulou, Eleni Petrou, Michael Ksifaras, Ekaterini Michelaki, Maria Orfanidou, Maria Kamperogianni, Stavroula Antonopoulou*

Abstract Content

Background: PD-AP is a major cause of morbidity and mortality for patients undergoing peritoneal dialysis.

Objectives: We studied the spectrum and resistance of PD-AP pathogens over an eight-year period in a tertiary hospital.

Methods: Retrospective records research for a total of 434 peritoneal dialysis fluid (PDF) cultures from 01/01/2014 to 31/10/2021.PDF samples with white cell count >100/μL and at least 50% neutrophils were considered representative of peritonitis. Bacterial identification was performed by VITEK 2 (bioMerieux) system and susceptibility testing by gradient concentration strip method and VITEK 2, according to EUCAST recommendations.

Results: A total of 152 (35%) PDF cultures were positive. There were 171 isolated strains: Gram-positive 71%, Gram-negative 26%, fungi 2% (*C. albicans* 1%), anaerobic bacteria 1%. The most frequent Gram-positive isolates were: *Staphylococcus* spp. 59% (Coagulase-Negative 71%, *S. aureus* 29%), *Streptococcus* spp. 26%, *Enterococcus* spp. 11% (*E. faecium* 54%, *E. faecalis* 46%). Among *S. aureus* isolates 14% were MRSA and among *E. faecium* isolates 57% were VRE. Resistance of streptococci to penicillin was 13%, but no resistance to third-generation cephalosporins was observed. The most common Gram-negative isolates were: *Pseudomonas* spp. 22%, *Klebsiella* spp. 20%, *Acinetobacter* spp. 18%, *S. marcescens* 13%, *E. coli* 9%, *Enterobacter* spp. 9%. Meropenem resistance was observed at 10%, 55% and 86% of *Pseudomonas*, *Klebsiella* and *Acinetobacter* isolates, respectively. Among *E. coli* isolates 25% produced ESBL and 14% of *Acinetobacter* isolates were colistin resistant.

Conclusions: The awareness of epidemiology and resistance of PD-AP pathogens is crucial to determine the appropriate empirical treatment.

T139 - The burden of COVID-19 in infants from at-risk, low-educated minority groups and educated, well-off families

Presenting Author - *Maria Pavlova, National Center Of Infectious And Parasitic Diseases, Bulgaria*

Author/s – *Zhivka Getsova, Metodi Popov, Yordan Kalchev, Valeri Velev*

Abstract Content

Background: Manifestation and severity of SARS-CoV-2 infection causing COVID-19 is still not well studied in infants, in contrast to adults. The present study aims to the burden of COVID-19 in infants from at-risk, low-educated minority groups and compare it to that from educated, well-off families.

Methods: This study is an analysis of the data collected from different infectious and pediatric medical care centres in Bulgaria. All the studied 233 RT-PCR COVID-19 confirmed infants 0 - 23 months of age admitted to hospitals were separated into two main groups: low-educated, minority groups and educated, well-off families. SARS-Cov-2 manifestations, severity and outcomes were assessed for the studied groups as well as parents' responsibility behaviour.

Results: Infants from risk groups are less affected by the SARS-Cov-2, their clinical manifestations are milder, and they recover faster than children from educated and well-income families. Parents of children from risk groups trust and cooperate with the physicians, unlike the other studied group. This affects as long as hospital stays, outcomes, as well as the severity of the infection. In conclusion, it should be emphasised that the social factor plays no less significant role in the recovery of COVID-19 in infants and young children.

T140 - Evaluation of the effects of CSA-131 - hydrogel coated silicone implants on the development of biofilm and capsular contracture

Presenting Author - *Cagla Bozkurt Guzel, Istanbul University, Turkey*

Author/s – *Ozlem Oyardi, Cengiz Ertekin, Muhammed Besir Ozturk, Zeynep Cagla Olgun, Tolga Aksan, Paul B. Savage*

Abstract Content

Background: Capsular contracture is one of the most common complications after aesthetic and reconstructive breast surgery. Today, although many theories have been proposed, the etiology of capsular contracture is not yet fully understood.

Objectives: In this study, it was aimed to reveal the effects of CSA-131, a subtype of antimicrobial ceragenin, on the development of biofilm and subclinical inflammation around the implant. Another purpose is to show CSA-131's effectiveness in preventing capsular contracture. The effects of bacterial contamination on biofilm formation were additionally investigated.

Methods: 34 rats were were divided into 5 different groups. Macroscopic evaluation was evaluated with Baker scores. Biofilm formations were evaluated. Capsule histology was evaluated by Hematoxylin Eosin staining, capsule thickness and Jansen staging.

Results: According to the data obtained, no colonies were detected in any sample of the second group, which includes sterile implants coated with hydrogel containing CSA-131. In comparison of Group 1 (just implant) and 2, Group 2 showed statistically significant activity against biofilm ($p<0.001$). Capsule thicknesses of Group 1 and 2 showed correlation with biofilm results. The capsules of the implants containing CSA-131 were found to be significantly thinner. Baker scores were evaluated and it was found that the prostheses in Group 2 had a significantly lower contracture grades.

As a result, the combination of hydrogel containing CSA-131 prevented biofilm formation and resulted in thinner capsule formation. The CSA-131 hydrogel is promising in the prevention of capsular contracture and further studies can support these data and lead to clinical trials.

T141 - Association between antibodies against SARS-CoV-2 and disease severity

Presenting Author - *Dhammika Atapattu, California University Of Science And Medicine, United States*

Author/s – *Priya Engel, Jade Bowmen, Sydney Cummings, Kaihong Su, Joseph Dhahbi, Dhammika Atapattu,*

Abstract Content

Introduction: The burden of disease associated with COVID-19 is highly variable, with symptomatology ranging from asymptomatic to acute respiratory distress syndrome and the predictability of severe disease remains low. While probe-based reverse transcription-quantitative polymerase chain reaction (RT q-PCR) remains the gold standard for the detection of SARS-CoV-2, other serological assays such as quantifiable IgM and IgG are used to assess host immunologic response and disease severity.

Objectives: In this study, we investigated the prevalence of specific SARS-CoV-2 Immunoglobulin M (IgM) and Immunoglobulin G (IgG) antibodies in a cohort of 191 covid-19 polymerase chain reaction (PCR) test-positive patients presenting to the Arrowhead Regional Medical Center (San Bernardino County, CA) between January 5, 2020, and July 20, 2021 to assess the association between patient comorbidities, specific antibodies, and the development of severe COVID-19.

Methods: Multivariable logistic regressions were performed utilizing STATA/BE 17.0 to evaluate the associations between IgG, IgM, patient demographics, and symptomology.

Results: Patients who were IgM positive were more likely to be male (58.5% vs 40.9%, $p<0.05$) (Table II) with Hispanic ethnicity (75.5% vs 54.1%, $p<0.05$), and were older (53.9 vs 44.1, 95% CI 49.4-58.9 vs 41.7-46.4) than patients who were IgM negative. Significantly more IgM-positive patients ($p<0.0001$) were likely to have moderate to critical disease burden compared to IgM-negative patients.

T142 - Development of a novel inhaled dry powder fluconazole formulation and investigation of the effectiveness in the eradication of candidiasis

Presenting Author - *Reham Aljalamdeh, Philadelphia University, Jordan*

Author/s – *Matthew Jones, Bernardo Dominguez, Albert Bolhuis*

Abstract Content

Background: *Candida albicans* is considered as an opportunistic pathogen that causes a wide range of fungal infections including invasive pulmonary candidiasis, particularly in immunocompromised patients. Treatment of such infections is challenging because of the ability of *C. albicans* to form biofilms that are more tolerant to treatment with antifungal drugs such as azoles.

Aims: The major purpose of this study was to develop a novel dry powder formulation of fluconazole suitable for inhalation and to test the effectiveness of this formulation on *C. albicans* biofilms.

Method: Firstly, electrospraying was used, which is an electrohydrodynamic technique to produce micronized respirable fluconazole particles. These particles were then tested on *C. albicans* biofilms using colony biofilm assay.

Results: Micronized fluconazole particles were made using electrospraying, resulting in particles with median size of D50 = 6.9µm (size range of 3.98-13.37µm). Powder X-ray diffraction suggested the presence of amorphous or nanocrystals. The activity of these particles on *C. albicans* biofilm demonstrated similar or better efficacy when compared to the activity of unprocessed crystalline fluconazole (median particle size D50 = 111.7µm; range 41.3- 339.13µm) against biofilms.

Conclusion: The results obtained suggested the potential use of inhaled fluconazole dry powder formulation in the treatment of pulmonary candidiasis or other fungal infections of the lung.

T143 - Low conservation of antibiotic interaction patterns within and between Gram-negative bacteria

Presenting Author - *Po-Cheng Tang, Uppsala University, Sweden*

Author/s – *Dione Sánchez-Hevia, Sanne Westhoff, Nikos Fatsis-Kavalopoulos, Dan Andersson*

Abstract Content

Background: Treatments with antibiotic combinations are becoming increasingly important, even though the supposedly clinical benefits of combinations are in many cases unclear.

Objectives: Here, we systematically examined how several clinically important antibiotics interact and influence the antimicrobial efficacy against five important Gram-negative pathogens.

Methods: A total of 232 bacterial isolates were tested against different pairwise combinations spanning five classes, and the ability of all combinations in inhibiting growth was quantified.

Results: Several important conclusions can be drawn from the 696 interaction patterns. Firstly, interaction profiles show low conservation between different Gram-negative species. Secondly, even within a species the interactions are often isolate-specific for a given combination, ranging from antagonistic to synergistic. Thirdly, additive and antagonistic interactions are the most common patterns observed across species and antibiotics, and 99.7 % of all isolate and antibiotic combinations belonged to either of these two types and only 0.3 % were synergistic.

Conclusion: These findings suggest that to achieve the highest precision and efficacy of combination therapy, isolate-specific interaction analysis ought to be routinely performed to avoid using drug combinations that show antagonistic interactions and an associated reduction in efficacy.

T155 - Seropositivity of selected pathogens and co-exposure among slaughtered livestock at Eastern Cape abattoirs, South Africa

Presenting Author - *Koketso Desiree Mazwi, University of Pretoria, South Africa*

Author/s – *Yusuf Ngoshe, Francis Kolo, Ayesha Hassim, Ishmael Jaja, Luis Neves, Henriette van Heerden,*

Abstract Content

A cross sectional study was conducted on slaughtered livestock at abattoirs in the Eastern Cape Province. The study aimed to detect *Brucella*, *Coxiella burnetti* and *Toxoplasma gondii* seropositivity and investigate the co-exposure. Slaughtered cattle (280), sheep (200) and pigs (85) were screened using Rose Bengal test and iELISA for *Brucella* antibodies followed by confirmation using the Compliment Fixation Test. Toxo-agent agglutination test and iELISA were used to detect antibodies against *T. gondii* and *C. burnetti*, respectively.

Brucella seropositivity of 5.4%, 2.0% and 1.2% was observed in cattle, sheep and pigs respectively using the three techniques. *T. gondii* seropositivity of 37,9%, 1.5% and 7.1% was observed in cattle, sheep and pigs respectively. *C. burnetti* seropositivity of 26.4%, 15% and 2.4% was observed in cattle, sheep and pigs, respectively. Co-exposure in cattle was detected for 40.54% *C. burnetti* and *T. gondii*, 4.05% for both *Brucella* and *T. gondii* as well as *Brucella* and *C. burnetti*. Co-exposure against all the pathogens (4.05%) was detected in cattle, while in sheep, 13.33% *Brucella* and *C. burnetti* was detected.

To our knowledge, this is the first study in South African livestock to investigate the co-exposure of these selected pathogens. The detection of antibodies against multiple zoonotic infections in livestock from abattoirs raises a major risk of exposure to abattoir workers, veterinary services and farmers. This study will raise awareness to the public on the presence of zoonotic pathogens from abattoirs and help develop effective strategic measures regarding the control of zoonotic infections in the country.

T156 - Isolation and characterisation of novel phages infecting *Listeria monocytogenes*

Presenting Author - *Hiba Shareefdeen, APC Microbiome Ireland, Ireland*

Author/s – *Colin Buttimer, Lorraine Draper, Paul Ross, Colin Hill*

Abstract Content

Background: *Listeria monocytogenes* is the causative agent of Listeriosis, a foodborne illness which poses danger to the elderly, pregnant women, and immunocompromised individuals. The commensal gut microbiota provides colonisation resistance against many orally acquired pathogens such as *L. monocytogenes*, and depletion of the microbiota by antibiotics could potentially reduce resistance to infection. While bacterial community composition has been studied in the context of colonisation resistance, it is also important to consider the impact that bacteriophages (phages), bacterial viruses that are also highly abundant in the human gut, can have on this phenomenon.

Objectives: To isolate and characterise novel bacteriophages against both *L. monocytogenes* and members of a defined 12 member synthetic gut bacterial community to develop a framework to study gut phage dynamics in the context of foodborne infection.

Methods: We isolated and purified novel phages that target *L. monocytogenes* as well as phages infecting members of the Oligo-Mouse-Microbiota (OMM12) synthetic bacterial community. We characterised these phages using electron microscopy, host-range assays, sequencing and bioinformatic approaches.

Results: Three novel phages that target *Listeria monocytogenes* from environmental samples were characterized and shown to have different host ranges and activity at a range of temperatures. Comparative genomics and phylogenetic analysis suggest that one of these phages represents a new genus. Additionally, three phages infecting members of the OMM12 bacterial community were isolated. Future work will focus on disrupting the OMM12 community using phages, to study how they may improve or impair colonization resistance in the gut during infection by a foodborne pathogen.

T157 - Antimicrobial resistance of some causative agents of healthcare-associated infections, including rare pathogens

Presenting Author - *Andrianna-Mariia Kis, Danylo Halytsky Lviv National Medical University, Ukraine*

Author/s – *Natalya Popovych, Yulian Konechnyi*

Abstract Content

Background: Antimicrobial resistance (AMR) is a significant public health problem. Monitoring AMR in healthcare-associated infections (HAIs) can assist in developing rational therapies.

Objectives: To screen for and identify current patterns of AMR in hospital-acquired microorganisms, including rare pathogens and those that produce ESBLs.

Methods: A total of 14 samples were collected from clinical samples of patients. Of the 14 samples, 4 were from faeces, 1 from blood, 1 from eye, 2 from navel, 3 from sputum, 2 from pharynx, 1 from trachea. Microbial identification (ID) and antibiotic susceptibility testing (AST) were performed using VITEK 2 system (bioMérieux). MIC was defined in µg/mL and interpreted as sensitive, intermediate, or resistant.

Results: Of the 14 cases identified as HAIs, 12 involved Gram-negative bacteria (including 6 strains of *K. pneumonia*, 3 of *Ps. luteola*, 1 of *Ps. aeruginosa*, 1 of *P. agglomerans*, 1 of *S. odorifera*) and 2 involved Gram-positive coccus *S. lentus*.

12 strains (85.7%) were MDR. 1 strain of *K. pneumonia* (16.7%) was ESBL-positive. 1 strain of *S. lentus* (50%) was Cefoxitin-positive.

K.pneumonia revealed 16.7% sensitivity to Amoxicillin/Clavulanic acid and Piperacillin/Tazobactam, 33.33% to Cefuroxime, Cefuroxime/Axetil, Cefotaxime, Ceftazidime, 50% to Cefepime, Imipenem, Meropenem, Amikacin, Gentamicin, Tobramycin, Trimethoprim/Sulfamethoxazole, 66.67% to Ciprofloxacin, and 100% to Tigecycline and Colistin. *Ps. luteola* was intermediate to Meropenem (50%) and Ciprofloxacin (100%), but sensitive to Colistin (100%). *Ps. aeruginosa* was only Colistin-sensitive. *P. agglomerans* was only Tigecycline-sensitive. *S. odorifera* was sensitive to Cefepime, Imipenem, Amikacin, Gentamicin, Tobramycin, Ciprofloxacin, Tigecycline, Colistin, Trimethoprim/Sulfamethoxazole. *S. lentus* was intermediate to Ciprofloxacin (100%) and Levofloxacin (100%), but sensitive to Tetracycline (50%) and Tigecycline (100%).

T158 - Enhancing the antimicrobial properties of silver nanoparticles against multi-drug resistant pathogens

Presenting Author - *Mateusz Wdowiak, Institute Of Physical Chemistry PAS, Poland*

Author/s – *Sada Raza, Mateusz Grotek, Bartłomiej Bończak, Jan Paczesny*

Abstract Content

The pandemic of COVID-19 brought general microbial awareness to society. As a consequence, the sale of antibiotics and antifungals skyrocketed since 2020. This drives us closer to a silent pandemic of antibiotic resistance. Which already resulted in the development of pathogens like ESKAPE bacteria (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.) and yeasts like *Candida auris*, there is a pressing need to develop efficient antimicrobial alternatives. Nanoparticles, especially silver nanoparticles (AgNPs), are believed to be promising candidates to supplement or even replace antibiotics in several applications. Here, propose a way to make silver nanoparticles more efficient at smaller concentrations. We synthesized AgNPs using tea extracts (black, green, and red) – TeaNPs - as both reductant and stabilizer. We tested their antifungal and antibacterial efficacies. We found that AgNPs synthesized with green tea (G-TeaNPs, 0.1mg/mL) were most effective in causing about at least 80% bacterial cell death in Gram-negative bacteria within just 3h. Tea extract silver nanoparticles also effectively eliminated *C. auris* cells, with G-TeaNPs being the most efficient.

T159 - Uropathogens recovered from CAUTIs in an Egyptian hospital show phenotypic and genotypic heterogeneity

Presenting Author - *Mohamed Eladawy, Nottingham Trent University, United Kingdom*

Author/s – *David Negus, Lesley Hoyles*

Abstract Content

Catheter-associated urinary tract infections (CAUTIs) represent one of the major healthcare-associated infections in Egypt. Little is known about uropathogens contributing to CAUTIs in Egyptian clinical settings. Between December 2020 and September 2021, 134 Gram-negative uropathogens were recovered from urinary catheters at the Urology and Nephrology Center, Mansoura, Egypt. To date, whole-genome sequences have been generated for 104 isolates. Analyses revealed 56 *Escherichia coli*, 16 *Pseudomonas* spp., 8 *Klebsiella pneumoniae*, 4 *Proteus mirabilis* and 20 other uropathogens, several of which represented novel species.

All isolates were characterized phenotypically for antimicrobial resistance (AMR) and biofilm formation (crystal violet assay), and genotypically for resistance (CARD) and virulence (VFDB) genes. AMR testing (EUCAST) showed the isolates were highly resistant (> 50 %) to quinolones and monobactams, and to a lesser extent cephalosporins and aminoglycosides. In terms of biofilm formation, 18.8 %, 17.8 %, 31.7 % and 31.7 % of isolates were strongly, moderately or weakly adherent or non-adherent, respectively.

Genotypic analyses revealed the isolates are predicted to encode an array of AMR genes conferring resistance to aminoglycosides, β-lactamases and fluoroquinolones, with new variants not previously reported in Egypt (OXA-114a, OXA-395, OXA-486, OXA-494, OXA-847, OXA-903, OXA-905, TEM-190, PDC-5, PDC-14, PDC-16, PDC-35, PDC-45, PDC-201 and VEB-6). Virulence genes related to biofilm formation are being analyzed.

The present study represents the first detailed analysis of different uropathogens contributing to CAUTIs in Egypt. Results will inform development of improved monitoring and treatment regimens for CAUTIs in the source hospital and potentially throughout Egypt.

T160 - The divisome but not the elongasome organizes capsule synthesis in *Streptococcus pneumoniae*

Presenting Author - *Rei Nakamoto, National University of Singapore, Singapore*

Author/s – *Sarp Bamyaci, Karin Blomqvist, Staffan Normark, Birgitta Henriques-Normark, Lok-To Sham*

Abstract Content

The bacterial cell envelope is a complex, multi-layered structure. Precise coordination of its synthesis is required to ensure every layer is faithfully produced. Many gram-positive bacteria conceal peptidoglycan (PG) and underlying antigens with capsular polysaccharides (CPS). Yet, how CPS synthesis integrates with PG synthesis remains unclear. In *Streptococcus pneumoniae*, the peripheral and septal PG is produced respectively by the elongasome and the divisome. We show that CPS synthesis initiates from the division septum and propagates along the long axis of the cell, organized by the bacterial tyrosine kinase system CpsCD. CpsC and the rest of the CPS complex are recruited to the septum by proteins associated with the divisome but not the elongasome. The CPS complex assembly starts with CpsCD, then CpsA and CpsH, the glycosyltransferases, and finally CpsJ. Remarkably, targeting CpsC to the cell pole is sufficient to reposition CPS synthesis, leading to diplococci that lack CPS at the septum. We propose that septal CPS synthesis is important for chain formation and complement evasion, thereby promoting survival inside the host.

T161 - Whole genome sequencing reveals scarce spreading of Beijing lineage of *Mycobacterium tuberculosis* in Bulgaria

Presenting Author - *Stanislava Yordanova, National Center For Infectious And Parasitic Diseases, Bulgaria*

Author/s – *Deyan Donchev, Ivan Stoikov, Ana Baykova, Yuliana Atanasova, Elizabeta Bachiyska*

Abstract Content

Background: The Beijing lineage of *M. tuberculosis* is highly transmissible and globally distributed. It is associated with multidrug tuberculosis (MDR-TB) outbreaks all over the world. Although the Beijing genotype represents about 30% of rifampicin-resistant strains in the European Union and European Economic Area, in Bulgaria, it was only 3% of MDR-TB cases between 2007 and 2019.

Objectives: The aim of this study was to reveal the presence of Beijing genotype among the drug resistant tuberculosis strains in Bulgaria from 2020 to 2022.

Methods: DNA was extracted from 43 resistant isolates *M. tuberculosis* in the stated time period with QIAamp®DNA Mini kit. Sequencing libraries were prepared with NEXTFLEX Rapid XP DNA-Seq Kit and sequenced with MiSeq V3 (2 x 300bp). Galaxy Sciensano, Mycobacterium pipeline 1.1 was used to process the raw data. According to the built-in quality control, genome assemblies were produced for n=39 (90.7) strains, which originated from 37 patients.

Results: The *M. tuberculosis* isolates were with different pattern of resistance: pre-extensively drug resistant n=7, MDR-TB n=15, rifampicin monoresistant n=2, isoniazid resistant n=13, others n=2. The Euro-American lineage was prevalent (n=36, 97.3%) and a single case was found to be caused by lineage 2.2.1 (East-Asian - Beijing) (n=1, 2.7%): male, age of 51, resident of a port city, with no data of traveling abroad.

T162 - Distribution of serotypes and antimicrobial resistance patterns among *Shigella flexneri* isolated from pediatric patients in Iran

Presenting Author - *Mehrzad Sadredinamin, Shahid Beheshti University of Medical Science, Islamic Republic of Iran*

Author/s – *Zohreh Ghalavand, Masoud Alebouyeh, Bahram Nikmanesh, Neda Yousefi Nojookambari, Nasim Almasian Tehrani*

Abstract Content

Background: *Shigella flexneri* continues to be one the most common species of *Shigella* causing diarrhea and dysentery in developing countries.

Objectives: Few data are available on the correlation between *S. flexneri* serotypes and antimicrobial resistance in Iran. So, we have made an attempt to understand this association among *S. flexneri* isolates.

Methods: *Shigella* isolates were recovered from stool of children with diarrhea between March 2016 and September 2018. Shigella isolates were identified using standard microbiological and serological tests. All *S. flexneri* isolates were subjected to molecular serotyping and phenotypic antibiotic resistance through multiplex-PCR and disk diffusion methods according to the Clinical Laboratory and Standards Institute (CLSI; 2018) guidelines, respectively.

Results: A total of 333 *Shigella* isolates, including *S. sonnei* (70.3%), *S. flexneri* (29.1%), *S. boydii* (0.6%), and no *S. dysenteriae*, were identified. The most prevalent *S. flexneri* serotype was 2b (35.05%), followed by 1b (33%), 2a (16.5%), 1c (8.24%), 4a (2.06%), 3a (1.03%), 3b (1.03%), 6 (1.03%), Y (1.03%) and X and/ or Xv (1.03%). A high level of multidrug resistance (MDR) was observed in serotype 2b (33.3%), followed by 1b (26.6%), 2a (20%), 3a (6.7%), 4a (6.7%) and X (6.7%). Overall, seven isolates were resistant to ciprofloxacin among which six isolates belonged to serotype 2a and a remaining isolate belonged to 3a serotype. The incidence of MDR serotypes of *S. flexneri* is a serious threat in diarrhea endemic regions. Thus, urgent strategies are required for its continuous monitoring and prevention.

T164 - Biofilm formation supports *S. epidermidis* survival in primary human and mouse macrophages

Presenting Author - *Patricia Bartsch, Universitätsklinikum Hamburg-Eppendorf, Germany*

Author/s – *Holger Rohde, Samira Weißelberg*

Abstract Content

Staphylococcus epidermidis is a leading pathogen in implant-associated infections. Biofilm formation protects *S. epidermidis* from innate immune effector mechanisms, and plays a key role for persistence in a hostile environment. Previous works from our lab have provided evidence that biofilm formation interferes with phagocytosis, bacterial killing and pro-inflammatory macrophage activation. So far, it is a common opinion that *S. epidermidis* is a strictly extracellular pathogen and lacks the ability to survive intracellularly.

Our aim was to determine the importance of bacterial aggregation and biofilm formation for *S. epidermidis* survival within human and mouse macrophages.

To study macrophage - biofilm interactions, *S. epidermidis* wildtype 1457 was employed in infection experiments. The up-take and fate of phagocytosed bacteria was followed during the very early phase of pathogen-macrophage contact (0 - 20 h) using imaging and FACS approaches. To test the specific role of biofilm matrix production, isogenic biofilm-negative mutant 1457-M10 was analyzed in parallel. Our data confirmed that biofilm formation is critical for interference of *S. epidermidis* with phagocytic up-take. We demonstrated that the ability to form biofilms also supports the intracellular survival of bacteria after uptake into the macrophage. In fact, *S. epidermidis* was able to survive within primary human and mouse macrophages. While isogenic biofilm-negative mutant 1457-M10 was readily degraded and almost completely killed with 5 hours after uptake, the biofilm forming *S. epidermidis* wild type strain was able to persist within macrophages for up to 18 hours.

T165 - Mechanism of high-level quinolone resistance in *Haemophilus* spp. revealed through quinolone resistance transfer assay

Presenting Author - *Takeaki Wajima, Meijo University, Japan*

Author/s – *Emi Tanaka, Kei-ichi Uchiya*

Abstract Content

Background and Objectives: High-level quinolone-resistant *Haemophilus haemolyticus* was isolated from a paediatric patient in 2019. In this study, we aimed to identify the mechanism underlying this high-level quinolone resistance by performing a resistance transfer assay with *Haemophilus influenzae*.

Methods: A resistance transfer assay to *H. influenzae* was performed using genomic DNA or PCR-amplified quinolone-targeting genes from the high-level quinolone-resistant *H. haemolyticus* 2019-19 strain. The amino acids responsible for conferring quinolone resistance were identified through site-directed mutagenesis.

Results: By adding the genomic DNA of *H. haemolyticus* 2019-19, resistant colonies were obtained, which showed the same level of resistance as the 2019-19 strain. Sequencing analysis showed that gyrA, parC, and parE of the resulting *H. influenzae* colonies were replaced by those present in *H. haemolyticus*. When the quinolone-targeting gene fragments were added sequentially, the addition of parE, as well as gyrA and parC, contributed to high-level resistance. In particular, amino acid substitutions at both the 439 th and 502 nd residues of ParE were associated with high-level resistance. These findings indicate that amino acid substitutions at the 439 th and 502 nd residues of ParE, in addition to amino acid substitutions in both GyrA and ParC, contribute to high-level quinolone resistance.

T166 - Evaluation of gargle lavage sampling for the detection of SARS-CoV-2 using rRT-PCR or antigen assay

Presenting Author - *Hana Jaworek, Institute of Infectious Disease and Molecular Medicine, Czech Republic*

Author/s – *Ondrej Bouska, Vladimira Koudelakova, Rastislav Slavkovsky, Petr Pavlis, Jana Vrbkova, Marian Hajduch,*

Abstract Content

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused considerable disruption worldwide. Despite ongoing vaccination throughout the world, rapid detection and isolation of infected individuals play a crucial role in stopping the further spread of SARS-CoV-2. For SARS-CoV-2 detection, new methods for rapid, non-invasive sampling are needed.

Objectives: We aimed to evaluate the agreement between clinician-obtained nasopharyngeal swabs (NPS) and self-sampled gargle lavage (GL) for SARS-CoV-2 detection using rRT-PCR. The second aim of this study was to evaluate the accuracy of antigen diagnostic tests in self-sampled GL.

Methods: In total, 494 paired GL and NPS samples were obtained in April 2021. SARS-CoV-2 detection in paired samples was performed by one rRT-PCR test (real-time reverse transcription polymerase chain reaction) and two antigen diagnostic tests.

Results: SARS-CoV-2 was detected in 55.7% of NPS and 55.7% of GL samples using rRT-PCR, with an overall agreement of 91.9%. The positive percent agreement (PPA) of rRT-PCR in GL samples was 92.7% and the negative percent agreement (NPA) was 90.9% compared to NPS samples. The PPA of rRT-PCR in NPS and GL samples was 93.2% when all positive tests were used as the reference standard. Both antigen detection assays showed poor sensitivity compared to rRT-PCR (33.2% and 36.0%). rRT-PCR SARS-CoV-2 detection in self-collected GL samples had a similar PPA and NPA to that of NPSs. GL self-sampling offers a suitable and more comfortable alternative for SARS-CoV-2 detection.

T167 - Alternative quinolone-resistance pathway caused by horizontal gene transfer in *Haemophilus influenzae*

Presenting Author - *Emi Tanaka, Meijo University, Japan*

Author/s – *Takeaki Wajima, Hidemasa Nakaminami, Kei-ichi Uchiya*

Abstract Content

Background: In general, quinolone resistance is caused by mutations in the target genes gyrA and parC. However, we previously reported resistant isolates with highly similar gyrA sequences despite a different genetic background, suggesting that this gene could be transferred horizontally.

Objectives: The aim of this study was to clarify quinolone resistance mediated by horizontal gene transfer.

Methods: Quinolone susceptible *H. influenzae* isolates were co-cultured with culture supernatant, PCR-amplified quinolone-targeting genes or genomic DNA from a quinolone resistant *H. influenzae*, 2018-Y40 strain, and the emergence of resistant colonies was evaluated using quinolone-containing agar. Horizontal gene transfer was confirmed via Sanger sequencing. The genomic sequence of the obtained resistant colonies was also analysed using DNB Seq-G400.

Results: Through mixing with the supernatant or a gyrA fragment from 2018-Y40, quinolone-resistant isolates harbouring gyrA of 2018-Y40 were obtained. When these isolates were mixed with the parC fragment, more resistant isolates harbouring both gyrA and parC of 2018-Y40 were acquired. These horizontal transfers required the existence of uptake signal sequences on the fragments. Furthermore, using genomic DNA instead of PCR-amplified fragments, more resistant isolates harbouring both gyrA and parC of 2018-Y40 were obtained, indicating the simultaneous transfer of more than two genes. Whole genome analysis of the acquired isolate showed recombination events throughout the genome. Our findings suggest that quinolone resistance in *H. influenzae* can be transferred horizontally. In addition, they suggest the presence of an alternative pathway of quinolone resistance for the simultaneous horizontal transfer of multiple genes.

T168 - Fecal bacterial composition of goat infected with *Mycobacterium avium subsp. paratuberculosis*

Presenting Author - *Han Gyu Lee, National Institute Of Animal Science, Republic of Korea*

Author/s – *Sudu Hakuruge Madusha Pramud Wimalasena, Sang-Ik Oh, Eunju Kim, Eun-Yeong Bok, Tai-Young Hur, Young-Hun Jung*

Abstract Content

Background and Objective: Paratuberculosis is a chronic intestinal infectious disease of ruminants, caused by infection with *Mycobacterium avium subsp. paratuberculosis* (MAP). The stage of paratuberculosis classified as silent, subclinical, clinical and advanced. Each of the stage has specific pathologic changes. However, there is limited information about microbiota depending on stage. The purpose of this study was to investigate the diversity patterns of fecal bacterial populations in goats infected with MAP depending on stage, and also compared to those of uninfected controls.

Materials and Methods: In this study, total 26 goat fecal samples were used in silent (silent group, n = 7) and clinical stage animals (clinical group, n = 9), negative goats (negative group, n = 10) also used as control. Samples were collected from same farm, which regularly quarantine ELISA positive animals. Fecal microbiome was analyzed by using 16S rRNA metagenomics after total genomic DNA extraction and library construction.

Result: Compare species richness, they were significantly lower in the order of clinical, silent and negative group. Clinical group significantly lower than silent and negative group in species evenness. Among 40 genera occupying over 1.0% of the reads Lentimicrobiaceae was most significantly highest in silent group, and eight genera including Fibrobacteres, Oscillospiraceae, and Treponemataceae were most significantly lowest in clinical group.

Conclusion: The present results demonstrate, as the disease state getting worsens, so does the alpha diversity score and beneficial bacterial population. This study will serve as the basis for the microbiota and paratuberculosis.

T169 - Biofilm characterization of *Clostridioides difficile* strains isolated from Equidae

Presenting Author - *Anais Lemaire, Inrae, France*

Author/s – *Ludovic Bridoux, Sandrine Petry, Frédéric Barbut, Isabelle Poquet*

Abstract Content

Clostridioides difficile, a Gram-positive spore-forming strict anaerobe, is a One Health player. It is can infect both humans and animals and spread spores widely in the environment, finally allowing inter-species and zoonotic transmission. *C. difficile* is an opportunistic enteropathogen that can emerge after antibiotic treatment leading to microbiota dysbiosis. It is the primary agent of nosocomial diarrhoea worldwide and community-acquired infections are increasing, probably due to inter-species transmission. Biofilms could play a role in *C. difficile* infectious cycle. Our project is based on a collection of strains previously isolated from Equidae in our laboratories. The strains have been characterised at the molecular and phenotypic levels. The aim of our research is to characterise and compare strain ability to form biofilms. Biofilms are being analysed by direct observation, crystal violet staining and confocal scanning laser microscopy to measure their biomass and determine their morphology and structure. Preliminary results show few quantitative differences between strains according to biofilm biomass and some qualitative differences in biofilm morphology and structure. Our study contributes to a better understanding of biofilm formation in *C. difficile* according to strain diversity.

T170 - Probiotic *Limosilactobacillus reuteri* KUB-AC5 reduced gut dysbiosis in *Salmonella*-infected mice

Presenting Author - *Songphon Buddhasiri, Chiang Mai University, Thailand*

Abstract Content

Background: Gut microbiota plays an important role in several infectious diseases by enhancing host colonization resistance, immunity, and homeostasis. Probiotic *Limosilactobacillus reuteri* KUB-AC5 (AC5) reduced *Salmonella* enterica Typhimurium (STM), the causative agent of acute non-typhoidal salmonellosis (NTS), proliferation, and STM-induced gut inflammation in mice. However, the role of AC5 on gut microbiota in acute NTS is still unknown.

Objectives: To explore the therapeutic effect of short-term AC5 consumption on gut microbiota composition in a mouse colitis model for acute NTS.

Methods: STM-infected mice were divided into two groups (1) treatment group and (2) control group (n = 6-7 per group). For the treatment group, mice were orally fed with AC5 (1x109 cfu/mouse) consecutively for four days. Mouse stool bacterial DNA was collected from feces or colon content. Then, the bacterial 16S rRNA genes V4 region was amplified using 515F and 806R primers and sequenced on Illumina NovaSeq. Sequence reads were analyzed using QIIME2 pipeline and SILVA database for microbiota composition, biodiversity analysis, and differential abundance analysis.

Results: The AC5-treated group showed increased alpha diversity of gut microbiota and the abundance of short-chain fatty acid (SCFA)-producing bacteria (*Akkermansia*, Muribaculaceae, and *Coprococcus*) compared to the control group. Moreover, the AC5 decreased Firmicutes/Bacteroidota (F/B) in STM-infected mice. However, no alteration in the gut microbiota beta diversity between both groups. In conclusion, short-term of AC-5 consumption after STM infection could reduce STM-induced gut dysbiosis in some parameters.

T172 - Novel antimicrobial peptides against methicillin-resistant *Staphylococcus aureus* derived from *Achatina fulica* hemocyanin

Presenting Author - *Andrés Esteban Pereira Patiño, Universidad Industrial De Santander, Colombia*

Author/s – *Libardo Andrés Suárez Largo, William Fernando Hidalgo Bucheli*

Abstract Content

Background: Methicillin-Resistant *Staphylococcus aureus* is an important cause of skin infections leading to significant mortality and morbidity in patients with wounds. Mucus secretion from the Giant African snail *Achatina fulica* is a potential source of biologically active substances that might be an important source for new drugs to treat resistant bacteria such as *S. aureus* (Suárez et al., 2021).

Objetives: To design, synthesize, characterize and evaluate new synthetic peptides derived from *A. fulica* hemocyanin against MRSA.

Methods and Results: From hemocyanin peptide sequences obtained from the mucus secretion of *A. fulica*, analogous peptides were designed by *in silico* rational design strategy. Solid-phase chemical synthesis was performed and *in vitro* antimicrobial activity was evaluated against *S. aureus* CMPUJ 015, *S. aureus* ATCC 25933 and *S. aureus* ATCC 43300. Minimum Inhibitory Concentration (MIC) was determined by broth microdilution method according to the method established in CLSI-M07-A11 (Patel et al., 2015). Haemolytic activity was determined according to Cruz et al., (2014). Cytotoxic activity on Vero and HaCat cells was evaluated using the MTT (3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazola bromide) method (Mosmann, 1983).

The results showed that the 15-residue synthetic peptides, alpha-helical and cationic, were those with the highest biological activity against *S. aureus* CMPUJ 015 and *S. aureus* ATCC 25933 strains, with Minimum Inhibitory Concentrations (MIC) in the range of 20 to 30 µM. The positive selectivity index suggests a higher selectivity mainly on the microorganisms evaluated and not on eukaryotic cells.

T174 - Results of study of synergistic action of meropenem and new synthetic compounds against nosocomial pathogens

Presenting Author - *Elizaveta Rogacheva, St. Petersburg Pasteur Institute, Russian Federation*

Author/s – *Stanislav Kalinin, Liudmila Kraeva, Mikhail Krasavin*

Abstract Content

Background: The situation of spreading multidrug-resistant strains amidst critically delayed development of new antimicrobials has been recognized as a global challenge in WHO and many national government statements. In recent years, resistance has developed particularly rapidly among Gram-negative bacteria, especially those in the ESKAPE group. This leads to forced therapy of patients with maximum doses of antimicrobials which entails a number of adverse consequences.

Objectives: Our aim was to study the ability of β-carboanhydrase inhibitors to potentiate the effect of known antibacterial drugs when used in combination to treat infections caused by Gram-negative microorganisms of the ESKAPE.

Methods: We analyzed PAZ-056 against multidrug-resistant bacteria of ESKAPE group isolated from ambulatory and in-patients in Saint-Petersburg was studied by means of determination of minimal inhibitory concentration of MIC according to the Standard Operating Procedure EUCAST. PAZ-056 was investigated at concentrations of: 3 µg/ml, 1.5 µg/ml, 0.75 µg/ml, 0.375 µg/ml, 0.1875 µg/ml, and 0.09 µg/ml. Solutions of compounds made up in DMSO (1 mg/10mL) were prepared and diluted to a total volume of 1 mL with deionized water. At the same time, sensitivity of the bacteria to meropenem was determined by serial dilutions in a plate. Also meropenem was used in these assays as a positive control. All measurements were done in triplicate.

Results: The antibacterial synergistic effect against *Klebsiella pneumoniae* was noted with the following combinations: 1.5 µg/ml meropenem and 0.75 µg/ml PAZ-056, for *Acinetobacter baumannii*: 0.75 µg/ml and 1.5 µg/ml respectively, for *Enterobacter cloacae*: 1.5 µg/ml and 0.75 µg/ml respectively.

T175 - Effect of salinity on the differential innate immune response in *Salmo salar* by challenge with two strains of *Piscirickettsia salmonis*

Presenting Author - *Denise Haussmann, Universidad Andres Bello, Chile*

Author/s – *Alex Romero, Jaime Figueroa*

Abstract Content – The innate immune response in infection by *Piscirickettsia salmonis* has been scarcely evaluated *in vivo* with different salinities. For this reason, data from fish with natural infections and with two salinities were analyzed with the strains, Psal-075, (LF89-Like) and Psal-025, (EM90-like) regarding mortality, and levels of TLRs and interleukins transcripts.

In a challenge by cohabitation with Atlantic salmon (~90g) in two salinities (5-20‰), accumulated mortality and transcripts were evaluated at 5, 10, 15, 20, 25, 37, 44 and 48 days-post-infection. (dpi) in anterior kidney.

Psal-025 at 5‰ salinity generated higher mortality (90% at 48dpi), while Psal-075 generated lower mortality (~60%). Indicating that Psal-025 produces greater susceptibility to infection in low salinity due to better adaptation, since it was isolated from the estuary. The qPCRs showed a differential response in both salinities. For TLR1, a peak in the first 10 dpi of both strains at 5‰ salinity. In 20‰ salinity, overexpression is maintained during 48dpi, slightly higher for Psal-025. Regarding IL-1β, at low salinity, the fish generate overexpression, while at 20‰ salinity, they show early mortality (~10dpi), with fewer transcripts for the rest of the assay. For IL-12 between strains, they are also similar at baseline up to day 20 (6 times over control, except 10 dpi with 20‰ salt). As mortality increases, the levels of transcripts increase (5-25dpi, both salinities), and the rest of the assay differentiates mortalities between salinity, and strains and transcript levels and different from IL-1β, does not reflect significant changes for both experimental conditions.

T176 - Air microbial quality in healthcare units and its role as pool for pathogens and multidrug resistance

Presenting Author - *Catarina Santos-Marques, Polytechnic Of Leiria, Portugal*

Author/s – *Camila Teixeira, Wolfram Manuel Brück, Sónia Gonçalves Pereira*

Abstract Content

Transmission of pathogens through the air is responsible for millions of deaths. However, little is known about the role that air may have as a pool for pathogens and multidrug resistance (MDR) in healthcare settings.

To address this, we screened the air of a central hospital (CH) and a long-term healthcare unit (LTHU).

During 2021/2022, samples were collected fortnightly in plate count agar plates using an air sampler. Antimicrobial susceptibility testing was performed and bacterial identification through MALDI-TOF is currently ongoing.

At the moment, 58 isolates were identified. The majority were *Staphylococcus* (n=41; 23 from LTHU, 18 from CH) including highly concerning pathogens: *S. hominis* (n=11), *S. haemolyticus* (n=9), *S. saprophyticus* (n=5), *S. epidermidis* (n=3), *S. capitis* (n=3), *S. cohnii* (n=3), *S. aureus* (n=2), *S. succinus* (n=2), *S. warneri* (n=2), *S. caprae* (n=1). Regarding CH isolates, 4 presented an MDR profile: 1 being resistant to 3 different antimicrobial classes, and 3 resistant to 4 different classes. Overall resistance in CH *Staphylococcus* were: cefotaxime (n=7), amoxicillin/clavulanic acid (AUG) (n=6), cefoxitin (n=6), imipenem (n=3). Regarding LTHU *Staphylococcus* isolates, 8 were classified as MDR: 5 resistant to 3 different antimicrobial classes, and 3 resistant to 4 different classes. Overall resistance in LTHU *Staphylococcus* were: ciprofloxacin (n=13), AUG (n=11), cefotaxime (n=8), cefoxitin (n=7).

Although still preliminary, results suggest the presence of MDR pathogens in the air of CH and LTHU, also that LTHU may actually have a higher burden of pathogens and MDR in the air than the hospital setting.

T177 - A systematic review to determine the prevalence of antibiotic resistance genes in the oral cavity

Presenting Author - *Laura Brooks, Eastman Dental Institute, United Kingdom*

Author/s – *Unnati Narvekar, Ailbhe McDonald, Peter Mullany*

Abstract Content

Background: UK dentists are responsible for 10% of all antimicrobial prescriptions (Marra et al., 2016; Sweeney et al., 2004) plus the oral cavity is an important reservoir of antibiotic resistance genes (ARGs) (Xu & Gunsolley, 2014). Understanding the role these factors have in selecting for the spread of antibiotic resistance is essential for developing optimum prescribing practices.

Objectives: determine the prevalence of ARGs in the oral cavity and what mobile genetic elements (MGEs) are important in disseminating them.

Methods: Studies describing the prevalence of ARGs in the oral cavity and methods of spread of antimicrobial resistance were identified in Embase, Medline and the Cochrane Library using 'free text' and 'MeSH' terms from January 2000-November 2020. Primary and secondary screening was completing using inclusion and exclusion criteria.

Results: From 1509 articles, 44 met the selection criteria and the key findings are that the most prevalent ARG was tet(M), the mode of birth and a child's environment in early life influenced which ARG are present, countries with higher consumption of antibiotics have more ARGs and *E. faecalis* is a reservoir of resistance especially in root canals. The most common MGE that transfers ARGs is the conjugative transposon Tn916.

Conclusion: 50% of the studies in the review were low quality. Recommendations for future work include: use of larger sample sizes, investigation of a broader range of ARGs, improved methodologies and reporting to improve quality of genetic testing in microbiology, randomization of subject selection.

T178 - Comparing the performance of three resazurin dyes in the *Bacillus anthracis* rapid antimicrobial susceptibility test

Presenting Author - *Julia Bugrysheva, United States Centers For Disease Control And Prevention, United States*

Author/s –*Pierre Michel, Heather P McLaughlin, Nazia Kamal, Laurel T Jenkins, Jennifer D Thomas, Julie Villanueva, David Sue*

Abstract Content

Background: Conventional antimicrobial susceptibility testing (AST) methods for *Bacillus anthracis* require at least 16 hours of incubation before results are ready, but a new resazurin dye-based rapid method developed by our group shortens the time to about 4 hours by quickly detecting cell viability using a spectrophotometer. During an emergency involving anthrax, rapid AST results can reduce morbidity and mortality by informing decisions about treatment and post-exposure prophylaxis.

Objective: To compare resazurin dye-based *B. anthracis* susceptibility test performance for ciprofloxacin, penicillin, and amoxicillin using three commercially available resazurin dyes: PrestoBlue, PrestoBlue HS, and TOX8 (*In vitro* Toxicology Assay kit).

Methods: Fifteen *B. anthracis* strains with known antimicrobial susceptibility profiles were prepared and inoculated following Clinical and Laboratory Standards Institute guidelines into microtiter panels with wells containing two-fold dilutions of ciprofloxacin, penicillin, or amoxicillin. After incubating each panel for 3.5 hours, cell suspensions were transferred to microtiter plates with aliquots of one of three resazurin dyes. After 30 minutes of incubation, fluorescence was measured. To evaluate the test performance, categorical agreement was determined by comparing the rapid AST results with broth microdilution results, and discrepancies were identified.

Results: Rapid AST results from PrestoBlue and PrestoBlue HS were equivalent to the broth microdilution results. Categorical agreement was 100% for all antimicrobials (CIP, PEN, AMX) and no discrepancy was observed. For rapid AST experiments using TOX8 reagent, bacterial cells for most strains yielded insufficient fluorescence. PrestoBlue and PrestoBlue HS dyes, but not TOX8, performed acceptably in the resazurin dye-based *B. anthracis* rapid AST.

T179 – N-acetylcysteine inhibits *Proteus mirabilis* urease activity and prevents catheter blockage in catheter associated UTIs

Presenting Author - *Arthika Manoharan, The University Of Sydney, Australia*

Author/s – *Arthika Manoharan, Theerthankar Das Ashishkumar, Greg Whiteley, Jim Manos*

Abstract Content

Background: P.mirabilis catheter-associated urinary tract infections (CA-UTIs) accounts for >40% of all nosocomial UTIs. *P. mirabilis* urease activity causes the formation of crystalline biofilms that block indwelling catheters. This prevents urinary drainage, causing severe infection and septicaemia. With a lack of urease-inhibitors and inefficiency of antibiotic treatment, alternative treatments are necessary to target P.mirabilis. We investigated the efficacy of N-acetyl cysteine (NAC) in inhibiting urease activity and catheter blockage in *P. mirabilis* CA-UTIs.

Objectives: To establish the anti-urease effect of NAC, its downstream effects on *P. mirabilis* catheter biofilm formation and host response to infection.

Methods: Urease activity was quantified in five clinical *P. mirabilis* isolates using the Berthelot's method. Enzyme kinetics of urease was quantified by spectrometry. Catheter encrustation was investigated using an *in vitro* glass bladder model, where a 2-way Foley catheter was "infected" with *P. mirabilis*. Artificial urine with NAC was pumped through and "infection" was run till complete blockage. Mass spectrometry was performed on catheter sections to describe biofilm elemental compositions. ELISAs were used to quantify inflammatory responses in bladder epithelial cells (BECs).

Results: 0.5mM NAC suppressed *P. mirabilis* urease activity by >3-fold. Kinetics studies predicted strong competitive binding of NAC to urease. Moreover, catheter blockage was significantly delayed ($p<0.05$) to >120hrs by NAC, and bacteria reduced by >3log10 CFU/mL in catheter biofilms. Ca^{2+} and Mg^{2+} concentrations were 3 times lower in treated catheters due to lack of urease activity. Interestingly, NAC was non-toxic to BECs and muted IL-6 and IL-8 responses to infection by >4-fold.

T180 - Exploring the potential wastewater-based epidemiology beyond SARS-CoV-2: Influenza and the respiratory syncytial virus

Presenting Author - *Nina Lackner, University Of Applied Sciences Kärnten, Austria*

Author/s – *Rudolf Markt, Gunther Vogl, Astrid Paulitsch-Fuchs*

Abstract Content

During the SARS-CoV-2 pandemic, wastewater-based epidemiology (WBE) proofed to be a valuable tool to track viral pathogens. WBE allows monitoring large populations with few samples and is independent of symptoms, test strategies, and test compliance of the population. As the threat from SARS-CoV-2 is declining, the existing infrastructure should be used to expand the pathogen panel and exploit the total potential of WBE. Thereby, costs for additional pathogens are low, as the nucleic acid extraction step has to be done only once. With expiring SARS-CoV-2 related measures, infections with other respiratory viruses are becoming a relevant health risk again.

Therefore, we re-investigated archived RNA-extracts from a national SARS-CoV-2 monitoring with a diagnostic multiplex qPCR assay targeting influenza and respiratory syncytial virus as well as SARS-CoV-2. We analyzed over 500 samples, covering eleven wastewater treatment plants once a week throughout 2022. SARS-CoV-2 copy numbers showed multiple waves, which corresponded well to the already existing data from the national monitoring. The respiratory syncytial virus and influenza showed weak waves in January/February and March/April, respectively. During the following months, no viral RNA was detected until a sharp increase for both viruses in November. Comparing the data to patient-based monitoring systems for both viruses, showed a high agreement.

Our study shows that the wastewater-based approach to track viruses can be easily expand to a broader pathogen spectrum, delivering high quality data. Further, we highly recommend archiving RNA extracts from wastewater for upcoming research projects or future pandemics.

T181 - Antibiotic resistance and metallo-beta-lactamase positivity in carbapenem-resistant Gram negative bacteria

Presenting Author - *Ayşe Seher Birteksöz Tan, Istanbul University, Turkey*

Author/s – *Mayram Hacioglu, Fatima Nur Yilmaz, Ozlem Oyardi, Nese Inan*

Abstract Content

Background: Gram negative bacteria are common agents for community-acquired, nosocomial and opportunistic infections and antimicrobial resistance is a growing crisis in clinical medicine.

Objectives: The aim of the study is to evaluate the resistance profile and metallobetalactamase (MBL) production of total one hundred and seven Gram negative strains including 37 *Acinetobacter baumannii*, 34 Klebsiella pneumonia, 16 *Pseudomonas aeruginosa*, 13 *Escherichia coli* and 7 *Enterobacter* sp. isolates from various specimens submitted to the Synevo Laboratories Ankara Central Laboratory in Turkey (2020-2021).

Methods: Antimicrobial susceptibility was tested by the disk diffusion method according to the recommendations of the Clinical and Laboratory Standards Institute. Imipenem (10 µg), meropenem (10 µg), gentamicin (10 µg), tobramycin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), ceftazidime (30 µg), cefepime (30 µg), piperacillin (100 µg) and aztreonam (30 µg) disks were used (Bioanalyse, Turkey). Isolates resistant to imipenem and/or meropenem were tested for MBL production by combined disk diffusion method.

Results: Most of the isolates were found resistant to carbapenems (89% n=96) and more than 90% of *A. baumannii*, *K. pneumoniae*, *E. coli* and *Enterobacter* spp. isolates were found resistant to ceftazidime and piperacillin. Most of *P. aeruginosa* isolates (75% of them) were found resistant to imipenem and aztreonam. According to combined disk diffusion method, 54% (n= 52) of carbapenem resistant isolates were found MBL positive. 83%, 54% and 30% of *A. baumannii*, *K. pneumoniae* and *P. aeruginosa* isolates were found positive for MBL production, respectively.

T182 - Inhibition of biofilm formation in murine wounds model by dressings containing silver oxynitrate

Presenting Author - *Sajwa Baqader, University of Manchester, United Kingdom*

Author/s – *Helen Thomason, Gavin Humphreys, Andrew McBain*

Abstract Content

Non-healing chronic wounds biofilm are a considerable worldwide healthcare burden. We have developed a murine excisional wound biofilm model to study bacterial biofilm tolerance and assess novel wound dressing antimicrobial effectiveness. We evaluated the efficacy of silver oxynitrate dressings, which produce highly reactive Ag2+ and Ag3+ ions, in murine wounds infected with preformed *Staphylococcus aureus* biofilms compared to non-antimicrobial control and to uninoculated wounds. Wound biofilm samples were harvested at 1, 3 and 7 days. Healing was characterised by quantification of wound width, area and re-epithelialisation from histological sections, and neutrophils/macrophage marker immunohistochemistry. Biofilms were characterised by tissue Gram stains, bacterial enumeration, and scanning electron microscopy (SEM). Over 7 days, uninoculated murine wounds healed whereas wounds infected with preformed biofilm and treated with non-antimicrobial dressing showed delayed healing. Treatment with Silver Oxynitrate reduced wound width, area, and inflammation level, and increased reepithelialisation in biofilm-infected wounds compared to infected wounds treated with non-antimicrobial dressing. Histological analysis and SEM images revealed fewer visible bacteria cells on the wound surface that were treated with Silver Oxynitrate compared to wounds treated with non-antimicrobial control dressings. Silver Oxynitrate exhibited a greater efficacy in reducing *S. aureus* viable counts in a wound infected with biofilm compared to infected wounds treated with non-antimicrobial dressing. These data show the effectiveness of Silver Oxynitrate in disrupting *S. aureus* wound biofilms and promoting the healing of biofilm-infected murine excisional wounds. This model can be used to evaluate the effectiveness of other antimicrobial wound dressings to inform decisions on biofilm management.

T183 - The potent effect of camel's urine as an antibacterial agent against multi-drug resistant bacteria in clinical specimens

Presenting Author - *Amina Ressmi, Sultan Moulay Slimane University Beni Mellal, Morocco*

Author/s – *Abouddihaj Barguigua, Nabila Soraa, Raqraq Habiba*

Abstract Content

Nowadays, the prevalence of antimicrobial resistance is responsible for more than 7 million deaths per year worldwide and it will reach around 10 million deaths in 2050. In fact, to cope with the austerity of this public health problem namely the emergence of MDR bacteria as well as the pitfalls caused by uncontrolled and indiscriminate antibiotic usage, it is necessary to seek alternative agents or therapeutic approaches in order to overcome multidrug resistance problems, particularly by using natural products such as camel's urine. By using the well diffusion mean, eight multi-drug resistant bacteria especially isolated from clinical samples were tested with sterilized camel's urine collected from both male and female camels. All strains were initially identified phenotypically by culturing on specific media and also by using MALDI-TOF Mass-Spectrometry as well as BD Phoenix. Our study recorded that the robust effect was shown by female camel's urine more than male one. In light of these findings, we proved the presence of largest inhibition zones on Mueller Hinton agar media by measuring the following diameters: 32 mm for NDM-1 producing *Enterobacter cloacae*, 30 mm for *Klebsiella oxytoca* strain producing NDM-1, 29 mm for *Acinetobacter baumannii* and *Citrobacter freundii*, 25 mm for *Pseudomonas aeruginosa* and *Escherichia coli* producing OXA-48 and 22 mm for Klebsiella pneumonia. Despite of the absence of inhibition zones on inoculated plates by Methicillin-resistant *Staphylococcus aureus*, we revealed a synergistic effect of sterilized camel urine in combination with cefoxitin (by measuring a diameter of 29 mm) against this pathogen.

T185 - The impact of the interleukin-27 receptor-alpha subunit on regulatory T cells in Tuberculosis

Presenting Author - *Kristina Ritter, Research Center Borstel, Germany*

Author/s – *Jasmin Rousseau, Alexandra Hölscher, Booki Min, Christoph Hölscher*

Abstract Content

During *Mycobacterium tuberculosis* (Mtb) infection, mice with a global deficiency in IL-27Rα (IL-27Rα-/-) exhibit lower bacterial burdens in comparison to wild type mice. The protective effect of IL-27Rα deficiency is dependent on the increased expression of the Th17 cytokine IL-17A, which is accompanied by the IL-17A-dependent formation of highly stratified protective granulomas in the lungs of IL-27Rα-/- mice consisting of aggregates of Mtb-containing macrophages surrounded by a distinctive rim of lymphocytes. Moreover, the enhanced IL-17A expression in IL-27Rα-/- mice supports the accumulation of IFN-γ, IL-2 and TNF co-expressing multifunctional CD4 T-cells in the lung parenchyma but is also associated with a decrease in Foxp3+- T regulatory cells (Treg). In the presence of IL-27Rα-signaling, Treg accumulate in the lungs and pulmonary lymph nodes during experimental TB and are immunosuppressive by slowing the onset of the post-infection adaptive immune response. Based on these results, we assumed that IL-27-induced Treg may be involved in undermining the protective immune response in experimental TB. By use of Treg-specific IL-27Rα-deficient mice, we were able to show that the absence of IL-27Rα on Treg results in lower bacterial burdens, although to a lesser extent than observable for global IL-27Rα-/- mice. Concurrently, the specific lack of IL-27Rα on Treg also leads to the formation of protective stratified lung granulomas. Future experiments will be performed to examine the magnitude and quality of protective immune response in the Treg-specific IL-27Rα-deficient mice as well as the direct effect of IL-27 on the development and function of Treg during experimental TB.

T186 - The role of SIRT6 during *Mycobacterium tuberculosis* infection

Presenting Author - *Soumya Mal, Bose Institute, Kolkata, India*

Author/s – *Pankaj Jankiram Birari, Arun Kumar Mal, Kuladip Jana, Zhumur Ghosh, Joyoti Basu, Manikuntala Kundu, Arun Kumar Sharma, Thurbu Tshering Lepcha*

Abstract Content

Background: The balance between the host immune response and the ability of *Mycobacterium tuberculosis* (Mtb) to persist within its intracellular niche, dictates the outcome of infection. Development of effective host-directed therapeutic strategies require a detailed understanding of the immune response to infection.

Objective: Our aim was to uncover the role of Sirtuin 6 (SIRT6) in the immune response of macrophages during Mtb infection.

Methods: Macrophages were infected with Mtb. qRT-PCR and Western blots were done to check expression of genes and proteins. Seahorse Flux analyser was used for glycolysis analysis. ELISA was performed to determine cytokine levels. Glucose uptake was assayed using NBD-glucose.

Results: Downregulation of miR-26a was associated with the upregulation of SIRT6 during Mtb infection in macrophages. Depletion of SIRT6 reduced mycobacterial survival in macrophages. SIRT6 repressed several HIF-1α regulated glycolytic genes through H3K9 deacetylation, and reduced glucose uptake. Knock down of SIRT6 increased succinate production and concomitantly increased the levels of HIF-1α (probably by inhibiting prolyl hydroxylase) and augmented IL-1β production. Knockdown of SIRT6 also augmented NF-κB dependent IL-6 production. SIRT6 showed a positive correlation with arginase-1 expression and negative correlation with inducible nitric oxide expression during Mtb infection, thus favoring M2 polarization.

Conclusions: This work throws light on how SIRT6 plays a regulatory role during Mtb infection, influencing host immune metabolism and inflammatory pathways. It opens up an avenue for further exploration of the SIRT6 network as a target for host-directed therapy.

T187 - Uncovering the virulence potential of the gut bacterium *Bilophila wadsworthia*

Presenting Author - *Andreia I. Pimenta, ITQB NOVA, Oeiras, Portugal*

Author/s – *Dalila Mil-Homens, Inês A. C. Pereira,*

Abstract Content

Background: The human gut microbiota is widely recognized to play key roles in human physiology. However, dysbiosis of the gut flora is also associated with disease. Bacteria that produce sulfide (H2S), called sulfidogenic bacteria (SB) have been associated with inflammatory bowel diseases and colorectal cancer. SB, like *Bilophila wadsworthia*, use sulfur compounds for anaerobic respiration, forming H2S in large amounts. H2S can be highly cytotoxic and can interfere with the integrity of the colonic epithelium and mucus barrier, inducing inflammation. *B. wadsworthia* can also adhere to intestinal cells and promote an inflammatory state. However, the mechanisms used to cause disease are still poorly understood.

Objectives: This study aims to establish *Galleria mellonella* larvae as an infection model to study *B. wadsworthia* pathogenicity.

Methods: *G. mellonella* was used as experimental organism and different biomarkers of infection were studied. Bacterial suspensions were used to induce a systemic infection, and larval health status and survival were analyzed. The viable bacterial load was assessed in the larvae hemolymph, hemocytes were extracted and counted, and hemolymph melanization levels were determined.

Results: This work reveals that *B. wadsworthia* can induce a symptomatic infection in *G. mellonella*, measured by deficits in mobility and cocoon formation and increased melanization. Bilophila was able to grow and proliferate inside *G. mellonella* resulting in larval death. Larvae's immune system is impacted by bacterial infection. Overall, our findings show that *G. mellonella* is a valuable model for investigating *B. wadsworthia* pathogenicity, providing new insights into its virulence mechanisms.

T188 - Two-year specter of bloodstream infections: influence of COVID-19 pandemic

Presenting Author - *Emma Keuleyan, Bulgarian Society for Microbiology, Bulgaria*

Author/s – *Georgi Todorov, Deniz Hamidov*

Abstract Content

Background: COVID-19 pandemic influenced human health in many aspects.

Objectives: To evaluate its contribution to the hospital bacteremia specter and antibiotic susceptibility.

Methods: Blood-culture diagnostics in 2021 and 2022-year was analyzed. Patients with SARS-CoV-2 infections were admitted from June 2021 to April 2022. Microbiological diagnosis was performed on Bactec, BD, and BactAlert, BioMerieux, followed by Gram-stain, culture method, MALDI-tof, Brucker identification and susceptibility testing by EUCAST.

Results: A total of 1375 blood-culture sets were investigated and for 194 patients were positive. Majority of cases were hospital-acquired and represented COVID-19 super-infections. Their specter included a high relative rate of Gram-negative non-fermenters: *Acinetobacter baumannii*–25, *Stenotrophomonas malthophilia*–7, *Pseudomonas aeruginosa*–6, *Cupriavidus metallidurans*-4, *Achromobacter xylosoxidans*–2, all were multiple drug resistant organisms (MDRO), incl. to carbapenems; resistant *Enterobacter*ales: carbapenem-resistant *Klebsiella pneumoniae*–10, *Escherichia coli* with ESBL–4; *Enterococcus faecium*–28 and *Candida*–36. The specter of infections before COVID-19 period was presented by single isolates of *Staphylococcus aureus*, *Enterococcus faecalis*, *Enterobacter*ales, and that of the post-COVID-19 – by single isolates of *S. aureus*, *E. faecalis*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *A. baumannii* and *S. malthophilia*. The atypical specter of bacteremia in COVID-19 period and the high rate of MDRO, which management was challenging, are obviously related to the high usage of antibiotics in Bulgaria in that time and some gaps in Infection control. In conclusion, the changed specter of bacteremia with prevalence of MDRO during the COVID-19 pandemic should be regarded as a lesson not to be repeated.

T189 - *Gardnerella vaginalis* induced vaginosis in a mouse model comparable with human bacterial vaginosis

Presenting Author - *Sheena Kim, Dankook University, Republic of Korea*

Author/s – *Sumin Ryu, Gi Beom Keum, Eun Sol Kim, Jinok Kwak, Sriniwas Pandey, Hyeun Bum Kim*

Abstract Content

Background: A common imbalance of the vaginal microbiota by overgrowth of diverse Actinobacteria and Firmicutes causes the human bacterial vaginosis (HBV). Moreover, *Gardnerella vaginalis* (*G. vaginalis*) has been known to induce HBV. Women with HBV have the increased risk of reproductive tract infections, however, there are no suitable animal models to study HBV.

Objectives: The objective of this study was to develop the standardized HBV mouse model infected with *G. vaginalis*.

Methods: To establish the HBV model, 7-week-old female ICR mice were intraperitoneally injected with 1mg/kg of estradiol benzoate for 3 days before the vaginal inoculation with 1X107 CFU/ml of *G. vaginalis*. At 1 and 4 days post inoculation (dpi), vaginal lavage fluid (VLF) was collected by gently flushing the vagina with 100µl of sterile PBS. The collected VLF was inoculated on Columbia blood agar plate containing supplements to confirm *G. vaginalis* infection in mice. The mice were humanly sacrificed at 7 dpi, then the mouse vaginas and serum were collected from all mice. The collected mouse vaginas were used for H&E staining and MPO assay, and the serum was used for ELISA.

Results: Histological examination of vagina infection with *G. vaginalis* showed that stratum corneum of the vagina was dropped out. In addition, the mice infected with *G. vaginalis* had increased MPO activity. The mice infected with *G. vaginalis* produced more pro-inflammatory cytokine (IL-1ß, TNF-α) and less anti-inflammatory cytokine (IL-10) than non-infected mice. In summary, the *G. vaginalis* induced vaginosis in a mouse model was comparable with HBV.

T190 - Clinical mastitis-related *Prototheca* infections among dairy herds in Brazil: a large-scale study

Presenting Author - *Angelika Proskurnicka, University Of Warsaw, Poland*

Author/s – *Marcelo Fagali Arabe Filho, Simony Trevizan Guerra, Sâmea Fernandes Joaquim, Felipe Freitas Guimarães, Luisa Fernanda García Sánchez, Helio Langoni, José Carlos de Figueiredo Pantoja2, Vera Lúcia Mores Rall, Rodrigo Tavanelli Hernandes, Simone Baldini Lucheis, Márcio Garcia Ribeiro, Tomasz Jagielski*

Abstract Content

Background: *Prototheca* spp. are unicellular, non-photosynthetic algae (Chlorophyta, Trebouxiophyceae), known to cause opportunistic infections in vertebrates (protothecosis). The most common and emerging form of Protothecal disease in animals is bovine mastitis, which incurs heavy economic losses to the global dairy industry, due premature culling and early replacement of infected cows.

The purpose of this large-scale study was to investigate the occurrence of *Prototheca* mastitis among 4,275 cows on ten dairy herds located in Sao Paulo and Minas Gerais states, in the southeast region of Brazil, where dairy farming is extensively practiced.

Methodology: The animals were from medium-scale farms (20–200 hectares), with different average sizes of herds and breeding with similar nutrition, management, technical level, and sanitary conditions, including mandatory vaccinations. All cows from eligible farms that experienced clinical mastitis between 2017-2019 were sampled. A total of 4,275 quarter milk samples from cows with clinical signs of mastitis were collected aseptically and cultured on sheep blood agar for the isolation of pathogens. The *Prototheca*-like colonies were subcultured and subjected to species identification by CYTB gene-based sequencing.

Results: Among all cows sampled, 44 (1.02%) were phenotypically compatible with *Prototheca* species, isolated among three (30%) studied farms. All cultured isolates were confirmed as *P. bovis*, upon molecular identification. Overall, the study demonstrates the importance of bovine mastitis due to *Prototheca* algae in Brazil, pointing to *P. bovis* as the major etiological agent.

T191 – A lesson from measles outbreak in healthcare workers in South Korea: the importance of knowing the prevalence of susceptibility

Presenting Author - *Sungim Choi, Republic of Korea*

Author/s – *Seong Yeon Park*

Abstract Content

Background: Despite high vaccination coverage rate, in-hospital transmission of measles continued to occur in South Korea. We present a measles outbreak that involved two healthcare workers (HCWs) with presumptive evidence of measles immunity after contact with index patient and evaluated measles seroprevalence in HCWs in a university-affiliated hospital in South Korea.

Methods: In 2018, after an outbreak of measles occurred in our hospital, all HCWs underwent measles Immunoglobulin (Ig) G tests for point-prevalence surveillance. Additionally, we have routinely performed measles IgG test for new HCWs since 2019.

Results: A total of 2,310 HCWs underwent measles IgG test from 2018 to 2022 and seroprevalence data stratified by birth year are shown in Figure 1. The mean age at the time of test was 32.6 years, the overall seropositivity of measles was 88.9 % (95% confidence interval, 87.5 - 90.1). and seropositivity tended to increase with age.

HCWs born before and 1968 had 99.3% seropositivity, and therefore may be considered as having full herd immunity against measles. However, However, among the 195 seronegative cases, 175 (89.3%) HCWs were born after 1985, although the birth cohorts between 1985 and 1994 were presumed to have received the measles-rubella (MR) catch-up vaccination in 2001. In particular, our data show that the birth cohort between 1994 and 1996 have substantially low seropositivity for measles.

Conclusion: This observation prompt us that identification the prevalence of susceptibility to measles of newly employed HCWs regardless of age is important for policy-making on hospital-wide vaccinations to prevent vaccine-preventable diseases.

T192 - Core-shell gold nanoparticles functionalized with ceragenin CSA-13 display strong antibacterial activity against of multidrug-resistant *Klebsiella pneumoniae*

Presenting Author - *Karol Sklodowski, Medical University Of Bialystok, Poland*

Author/s – *Sylwia Joanna Chmielewska-Deptuła, Łukasz Suprewicz, Joanna Depciuch, Magdalena Parlińska-Wojtan, Paul B. Savage, Robert Bucki*

Abstract Content

Background: The rapid emergence of resistant bacteria is occurring worldwide, threatening the effectiveness of currently use antibiotics. For this reason, there is increasing experimental efforts and interest in new technologies (especially nanotechnology) that might alleviate antimicrobial resistance.

Objectives: The study aimed to determine the *in vitro* antibacterial activity of ceragenin- coated non-spherical gold nanoparticles against *Klebsiella pneumoniae* strains.

Methods: The antimicrobial activity of rod- (AuR), peanut- (AuP) and star- (AuS) shaped gold nanoparticles (Au NPs) coated with CSA-13 (a cationic steroidal antimicrobial agent) was evaluated against a clinical strain of *K. pneumoniae* resistant to β-lactams (production of NDM-1 and ESBL), aminoglycosides and fluoroquinolones. Minimal inhibitory and bactericidal concentrations, killing assays and kinetic growth with resazurin were used to determine bactericidal efficacy. The biocompatibility of the tested compounds and the ability of pathogens to adhere and internalize against lung-derived cells (A549) were also determined.

Results: AuR NPs@CSA-13, AuP NPs@CSA-13 and AuS NPs@CSA-13 showed high antimicrobial activity against the multidrug-resistant *K. pneumoniae* (a member of bacteria included in ESCAPE group). The tested compounds also showed a high ability to inhibit internalization of tested strain into A549 cell line. It is noteworthy that the tested compounds at bactericidal concentrations were characterized by high biocompatibility.

T193 - Tracing the kynurenine pathway in COVID-19 progression

Presenting Author - *Mona Dehhaghi, Macquarie University, Australia*

Author/s – *Lucette A. Cysique, Sophia Bracken, Yasmin Allen-Davidian, David Darley, Anthony Byrne, Anthony Kelleher, Gregory Dore, Gail Matthews, Benjamin Heng*

Abstract Content

Background: Coronavirus disease 2019 (COVID-19) is a major public concern. In addition to the acute phase symptoms, some people develop chronic health complications (long COVID) causing inability to concentrate, brain fog, fatigue, etc.

Objectives: The knowledge on COVID-19 impacts on the kynurenine pathway (KP), the main route of tryptophan metabolism, is limited. The present study aims to fill this gap by comprehensively exploring the alterations in the level of KP metabolites during and after COVID acute phase.

Methods: The study cohort of COVID-19 patients (n=152, serum collecting timepoints: 0, 2, 4, 6, >6 months) was recruited as part of the ADAPT study from St. Vincent Hospital, Sydney. The control cohort consisted of 100 healthy individuals. The levels of KP metabolites (tryptophan, kynurenine, 3-hydroxylkynurenine, 3-hydroxyanthranilic acid, anthranilic acid, picolinic acid, and quinolinic acid) were quantified using ultra-high performance liquid chromatography (uHPLC) and gas chromatography mass spectrometry (GC-MS).

Results: Compared to the controls, the levels of kynurenine, 3-hydroxylkynurenine, anthranilic acid, and kynurenine/tryptophan ratio were significantly increased. Conversely, the levels of picolinic acid, a neuroprotective metabolite, significantly decreased in COVID-19 patients. A sustained increase in kynurenine, 3-hydroxykynurenine, quinolinic acid, anthranilic acid, and kynurenine/tryptophan ratio was observed and peaked at 3 months post-acute infection and then started to drop. Sustained elevated levels of KP metabolites can be attributed to chronic activation of the immune system and long COVID symptoms. The outcome of such studies will allow the development of KP-based biomarkers for COVID progression.

T194 - Recombinant endolysin effectively disrupts biofilm formed by resistant uropathogenic *Escherichia coli*

Presenting Author - *Ritam Das, Techinvention Lifecare Private Limited, Mumbai, India*

Author/s – *Sonia Nain, Vaishali Gupta, Sakshi Dhar, Utkarsh Gaharwar, Sarmad Hanif, Urmi Bajpai, Bhakti Chavan, Deepa Sikriwal, Aasiya Choudhary*

Abstract Content

Background: Antibiotic misuse has led to multi-drug resistance (MDR) in uropathogenic *Escherichia coli* (UPEC) that impacts clinical outcomes in urinary tract infections (UTIs). Hence, we are exploring phage encoded-lysins as plausible alternatives.

Objectives: Our study involved an *in-silico* strategy for the discovery and characterization of lysin sequences (seq) targeting *E. coli* cell wall and evaluating bactericidal activity of these recombinant lysins using in-vitro assays.

Methods: Novel lysin sequences were searched by BLAST homology and by screening *E. coli* prophages in the database (using PHASTER). Lysozyme-like domain was observed in 9/16 lysins. Their characterization depicted modular or globular structure. Based on physicochemical properties, 7/16 were selected for cloning, expression, and purification as recombinant proteins for evaluating bactericidal activity.

Results: Lysin seq 5 demonstrated highest activity by in-vitro assays. Using static biofilm assay, lysin seq 5 [180µg] showed efficient reduction (>50%) in the biofilm formation by resistant ATCC UPEC 700928 strain. Turbidity reduction method with lysin seq 5 [50µg] showed a drop of 43% in OD600nm on UPEC 700928 strain after 2.5 hrs of incubation at 37°C. The drop in OD600nm was higher [75%] on BL21 DE3 cells treated with lysin seq 5 [45µg] after 3 hrs of incubation at 37°C. Log killing assay displayed 4 log10 reduction on BL21 DE3 lysin treated cells. Lysozyme assay also corroborated *in-silico* analysis and showed comparable lysozyme activity with the positive control. Lysin seq 5 exhibited highest activity against UPEC 700928 strain. Screening UPEC clinical isolates is underway.

T195 - Plasmid-mediated antibiotic resistance gene dissemination between dogs and their owners

Presenting Author - *Tatsuya Unno, Chungbuk National University, Republic of Korea*

Author/s – *Hokyung Song*

Abstract Content

Antibiotic resistance (AMR) has become one of the major health concerns since the incidence of infections caused by antibiotic resistant bacteria increased every year. In this study, we investigated antibiotic resistance gene transfer between dogs and their owners through analysis of whole genome sequence (WGS) data obtained from ESBL producing *E. coli* isolates. WGS data was obtained using MinION and Illumina sequencing and these data were assembled using MicroPIPE. Annotation of antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) were done using CARD and mobile element finder, respectively. Plasmid was predicted using plasClass and their mobility was analyzed using mob suite. Prophage was also predicted using DBSCA-SWA. Our results showed close similarity in TEM-1 sequence, gene associations with TEM-1, and prophage-derived gene sequence between dog isolate and human isolate, suggesting that transduction may play a major role in horizontal gene transfer between humans and dogs. However, we also found these prophages are on plasmids, thus conjugation is likely to play the major role. Transductomics likely indicated origin of ARGs, not the route of ARG dissemination. As dogs and humans have physically close relationship, our results suggest the exchange of antibiotic resistant bacteria between dogs and humans.

T196 - Whole genome based characterization of *Escherichia albertii* strains isolated from paediatric diarrheal cases in Kolkata, India

Presenting Author - *Goutam Chowdhury, ICMR-National Institute of Cholera and Enteric Diseases, India*

Author/s – *Yuki Hoshiko, Kei Kitahara, Shin-ichi Miyoshi, Yoshitoshi Ogura, Shanta Dutta, Asish K Mukhopadhyay*

Abstract Content

Background: *Escherichia albertii* is Gram-negative facultative anaerobic emerging enteropathogenic bacteria causing diarrhea and gastroenteritis in humans.

Objectives: The present study shows isolation and genome based characterization *E. albertii* strains isolated from hospitalized paediatric diarrheal cases in Kolkata, India.

Methods: *E. albertii* strains were isolated by standard biochemical procedures and Eal-CDT PCR. Susceptibility to antimicrobials was tested by disc diffusion method. Genome of the *E. albertii* was sequenced to identify (i) genes encoding for virulence (ii) antibiotic resistance genes with MGEs (iii) core gene-based phylogenetic tree and pan-genomic analysis.

Results: A total of 10 (1.2%) *E. albertii* isolates were recovered from 833 paediatric diarrheal samples processed and 6 (60%) were isolated as the sole pathogen. *E. albertii* infected patients presented cholera-like diarrhea, i.e., with watery stool (60%), some dehydration (100%) and abdominal pain (20%). The antimicrobial susceptibility testing showed that most of the strains were resistance to erythromycin (80%), tetracycline (50%), ampicillin (40%), doxycycline (30%) and ceftriaxone (20%). In WGS analysis of the *E. albertii* strains revealed several genes associated with virulence like the intimin encoding gene (eae), Cytolethal distending toxin type II subunit A (cdt-IIA), adhesion (paa), non-LEE-encoded effector A (nleA) and antimicrobial resistance gene i.e. tetracycline (tetA; tetR), sulfonamides (sul2), fluoroquinolones (qnrS) and Beta-lactamases (blaCTX-M; blaTEM). The single nucleotide polymorphism based phylogenetic analysis of *E. albertii* isolates did not reveal a similar clustering pattern based on the biological source and place of isolation but the genome of these isolates were closely related to isolates from China, and United Kingdom.

T199 - Proteomic profile of bovine primary microglia and monocyte-derived macrophages (MDM) responses to *Listeria monocytogenes*

Presenting Author - *Margherita Polidori, University Of Bern, Switzerland*

Author/s – *Camille Monney, Anne-Christine Uldry, Manfred Heller, Anna Oevermann*

Abstract Content

Listeriosis is food-borne disease caused by *Listeria monocytogenes* (Lm), an opportunistic but life-threatening Gram-positive bacterium that affects ruminants and vulnerable individuals. Listeriosis may present as a self-limiting gastroenteritis or, after bacterial dissemination, septicemia, abortion, perinatal infection and neuroinfection (neurolisteriosis). Resident microglia and monocyte-derived macrophages (MDM) contribution to bovine neurolisteriosis remains to be defined. Our group has shown that Lm can access the cytosol of microglia producing listeriolysin-O (LLO), where Lm efficiently replicate. In contrast, MDM confine Lm to acidic vacuoles. In this study, we used liquid chromatography-tandem mass spectrometry (LC-MS/MS) to investigate intracellular responses to Lm infection in bovine primary microglia and MDM. Differences in protein expression are mostly related to cell type, rather than infection according to protein measures of abundance. Both phagocytic populations are involved in the attraction of inflammatory cells and the expansion of inflammation, since they start to express markers and regulatory molecules of inflammation after infection, including CXCL2, CXCL3, IL1A, coagulation factor III (F3) and zinc finger protein 36 (ZFP36). Among others, infected microglia start to express CXCL6 and growth-regulated protein homolog alpha compared to uninfected cells, while infected MDM: CCL5, CCL8, tumor necrosis factor alpha-induced protein 3 (TNFAIP3) and tumor necrosis factor superfamily member 15 (TNFSF15). Strikingly, infected and uninfected MDM have significantly higher levels of lysozyme, an intravacuolar enzyme with anti-bacterial activity, compared to microglia. This study provides the basis for further investigation of the mechanisms involved in the differences in intracellular lifestyle of Lm in MDM and microglia.

T201 - *Salmonella* enhances osteogenic differentiation in adipose-derived mesenchymal stem cells

Presenting Author - *Bart Weimer, University of California, Davis, United States*

Author/s – *Claire Shaw, Matthias Hess*

Abstract Content

The potential of mesenchymal stem cells (MSCs) for tissue repair and regeneration has garnered great attention for regenerative medicine. These cells are also intimately involved in immune regulation and inflammatory responses as well. While MSCs are likely to interact with microbes at sites of tissue damage and inflammation, like in the inflamed gastrointestinal system, the consequences of pathogenic association on MSC activities have yet to be elucidated. This study investigated the effects of pathogenic interaction on MSC trilineage differentiation paths and mechanisms using model intracellular pathogen *Salmonella enterica ssp enterica* serotype Typhimurium. Electron microscopy and gentamicin protection assays were used to enumerate infection of MSCs. A combination of qRT-PCR, GeneChip expression analyses, and RNAseq was used to probe stem cells changes at the genetic level. The examination of key markers of differentiation, apoptosis, and immunomodulation demonstrated that *Salmonella* altered osteogenic and chondrogenic differentiation pathways in human and goat adipose-derived MSCs without inducing cell death. Anti-apoptotic and pro-proliferative responses were also significantly induced ($P < 0.05$) in MSCs during *Salmonella* association. Together, these results indicate that *Salmonella*, and potentially other pathogenic bacteria, can induce pathways that influence both apoptotic response and functional differentiation trajectories in MSCs, highlighting that microbes have a potentially significant role as influencers of MSC physiology and immune activity.

T202 - Antimicrobial effect of hydrogel matrix based on gum karaya resin supplemented by the phage preparation on methicillin-resistant

Presenting Author - *Lukáš Vacek, Masaryk University, Czech Republic*

Author/s – *Dominika Kleknerová, Břetislav Lipový, Roman Pantůček, Lucy Vojtová, Filip Růžička, Eva Čern, Edita Jeklová*

Abstract Content

Background: Gum karaya (GK) is a natural polysaccharide with great potential in the wound management of complicated deep skin and soft tissue infections (SSTIs). The polysaccharide-based hydrogels keep a moist environment and stimulate faster moist wound healing. Its antimicrobial potential against methicillin-resistant *Staphylococcus aureus* (MRSA) strains has been previously established.

Objectives: We sought to increase the potential of GK by supplementing this material with phage preparation and testing its antimicrobial potential in in vivo porcine model of complicated deep SSTI.

Methods: Four female pigs, each with twenty 5 x 5 cm skin defects, were used. The MRSA strain NRL/Atb 5921 was applied after the surgical excisions. On Days 4, 7, 10, and 14, an imprint from each wound was made for semi-quantitative microbiological evaluation. Further, tissue samples of the wound base from each pig were then taken. After tissue sampling, GK hydrogels and films supplemented with staphylococcus phage JK2/phi 812 at concentrations 10^8 and 10^9 PFU/mL were applied on the wound surface.

Results: The evaluation showed a statistically significant decrease from the starting numbers of bacteria over a two-week treatment. Greater than 10^5 CFU/gram of tissue are widely used as a key indicator associated with delayed wound healing. In the case of the imprint technique, the reduction was from >1000 CFU/25cm2 to 42 CFU/25cm2. The decrease in bacterial counts in the tissue samples was from 6.8 log CFU/g of tissue to 4.28 ± 0.32 log CFU/g (2.54 log CFU/g difference). These numbers indicate successful treatment of the infection.

T204 - Approaching microbial literacy through the exploration of extreme environments

Presenting Author - *Kenneth Timmis, Technical University Braunschweig, Germany*

Author/s – *Cecilia Flocco*

Abstract Content

Background: Microbial activities are intrinsically bound to our personal lives, socio-economic and sustainable development, and global crises (as the current COVID-19 pandemic exemplifies). In order to be able to take evidence-based decisions in many spheres at all levels, an understanding of relevant aspects of microbiology is essential1.

Objectives: The International Microbiology Literacy Initiative (IMiLI) aims to educate and expand societal perspectives on microbiology through the creation of experience-centric resources tailored to children, educators and audiences not familiar with microbiology.

Methods: Through the exploration of extreme environments, in this case Antarctic terrestrial environments, and tapping into children´s natural curiosity, societally-relevant microbiology topics and activities are treated. Given the unique features of the remote continent, microbiology concepts are interlinked to geography, history, physics, natural sciences, human and environmental health, sustainability and governance of the global commons.

Results: Antarctica is often described as a cold desert since the prevailing environmental conditions preclude the existence of most forms of life -except for microorganisms adapted to those conditions. Thus, their key contributions to geochemical cycles and ecosystem functions emerge. Overall, the unique features and metabolic capacities of microorganisms adapted to extreme environments (caves, hydrothermal vents, hypersaline lakes and other) render them a unique source of biomolecules of biotechnological interest, linking them to everyday issues to which children can relate. IMiLI´s curriculum and activities are designed with a focus on inclusivity, participation and creativity, encouraging the exploration of other extreme environments -remotely located or hiding in plain sight- that may spark children’s curiosity.

T205 - Supporting the microbiology community with access to data, analysis services, standards and training (NFDI4Micriobiota)

Presenting Author - *Cordula Hege, Helmholtz Centre for Infection Research (HZI), Germany*

Author/s – *Carmen Paulmann, Barbara Götz, Konrad Förstner, Alice McHardy*

Abstract Content

NFDI4Microbiota aims to support the microbial community by providing access to data, analysis services, data/metadata standards and training. It belongs to the National Research data Infrastructure (NFDI), which aims to develop a comprehensive research data management. Different consortia ensure a broad coverage from cultural sciences, engineering to life sciences and natural science. NFDI4Microbiota intends to facilitate the digital transformation in the microbiological community (bacteriology, virology, mycology and parasitology).

The German microbial research network will be supported through training and community building activities, and by creating a cloud-based system that will make the storage, integration and analysis of microbial data, especially omics data, consistent, reproducible, and accessible. Thereby, NFDI4Microbiota will promote the FAIR (Findable, Accessible, Interoperable and Re-usable) principles and Open Science.

Central for the NFDI4Microbiota consortium is the development and provision of the computational infrastructure and analytical workflows required to store, access, process, and interpret various microbiome-related data types. Here, NFDI4Microbiota will work on developing and implementing software and standardized workflows for users to analyse their own data.

Several workshops and training events for the community happened already and further will take place in future. Moreover, the consortium launched an ambassador program to connect with the participants, thereby helping to identify the needs of their local community. Technical solutions are developed, tested and refined in several use cases from different fields of microbiology. All relevant information and specific services are available via the web portal.

T206 - Strain variations in a bacterial adhesin lead to different binding partners in the pathogen *Yersinia enterocolitica*

Presenting Author - *Dirk Linke, University of Oslo, Norway*

Author/s – *Ina Meuskens, Per Eugen Kristiansen, Reidar Lund, Kristian Prydz, Benjamin Bardiaux, Nadia Izadi-Pruneyre, Vladimir Rosenov Koynarev, Daniel Hatlem*

Abstract Content

Background: Many bacterial adhesins in pathogenic species interact with the extracellular matrix of host cells to initiate contact. This triggers further processes that ultimately can lead to cell or tissue invasion. It is usually assumed that a specific adhesin from a given bacterial species will bind to a specific receptor or matrix molecule.

Objectives: Our objective is to better understand the relevance of strain-specific variations in bacterial adhesins, and how these might contribute to different clinical outcomes between different isolates of the same species.

Methods: To better understand the binding interactions between our adhesin variants and the extracellular matrix, we use biophysical methods such as surface plasmon resonance, small angle x-ray scattering, ELISA-based binding assays, NMR, and computational modelling. Most of our assays use both purified adhesins or whole bacterial cells.

Results: Here, we describe how a short insertion of 30 amino acids in the head domain of the bacterial adhesin YadA from enteropathogenic Yersiniae changes the binding mode of the adhesin, switching from collagen binding to binding negatively charged glycans, including heparin. We demonstrate how the insertion forms a polyproline II helix upon binding. We discuss the physiological and evolutionary relevance of the binding partners, and speculate how the strain-specific difference in binding might lead to different clinical outcomes.

T207 - Anaerobic phosphite oxidation, a highly efficient kind of energy conservation

Presenting Author - *Bernhard Schink, University of Konstanz, Germany*

Author/s – *Zhuqing Mao, Nicolai Mueller*

Abstract Content

Oxidation of phosphite (HPO32-) to phosphate (HPO42-) releases electrons at a very low redox potential (E0'= -690 mV) which renders phosphite an excellent electron donor for microbial energy metabolism. Two pure cultures of strictly anaerobic bacteria have been isolated so far which run their energy metabolism on the basis of phosphite oxidation, the Gram-negative *Desulfotignum phosphitoxidans* and the Gram-positive *Phosphitispora fastidiosa*. In *P. fastidiosa*, a cytoplasmic enzyme catalyzes phosphite oxidation in the presence of adenosine monophosphate (AMP) to form adenosine diphosphate (ADP), with concomitant reduction of NAD+ to NADH. This enzyme was heterologously expressed in *Escherichia coli*. It is highly oxygen-sensitive, has a molecular mass of 35.2 kDa and a high affinity for phosphite and NAD+. A similar enzyme was found in *D. phosphitoxidans* but this enzyme was partially membrane associated and could not be heterologously expressed. According to gene sequence and functional analysis, both enzymes were found to be closely related to a nucleosidediphosphate sugar epimerase of *E. coli*. These phosphite-oxidizing enzymes represent a novel type of substrate-level phosphorylation that allows highly efficient energy conservation with concomitant nearly complete electron assimilation into cell material. Microbial phosphite oxidation might be a remnant of early metabolic evolution when reduced phosphorus compounds were more common than today.

T209 - Structural basis of CO biosynthesis for the active site in NiFe-hydrogenase

Presenting Author - *Shigetoshi Aono, National Institutes of Natural Sciences, Japan*

Author/s – *Norifumi Muraki*

Abstract Content

Hydrogenases are metalloenzymes that catalyze the oxidation of gaseous hydrogen into electrons and protons and the reduction of protons into hydrogen reversibly. The active site in [NiFe]-hydrogenase consists of a dinuclear NiFe cluster, in which carbon monoxide (CO) is bound to the Fe atom as an intrinsic ligand. Though it is reported that CO is biosynthesized by a novel enzyme HypX and assembled into the metal cluster in hydrogenase, the molecular mechanisms of biosynthesis of CO remain unknown. We have determined the crystal structure of HypX at a resolution of 1.8 Å. HypX binds CoA constitutively as a prosthetic group in the continuous cavity connecting the N- and C-terminal domains. We have also solved the structure of tetrahydrofolate (THF)-bound HypX at a resolution of 2.1 Å, in which THF is accommodated in the cavity in the N-terminal domain. Based on these crystal structures and MD simulations, we propose the molecular mechanism of CO biosynthesis by HypX as follows.

The reaction starts with binding N10-formyl-THF as a substrate in the N-terminal domain of HypX. The formyl-group transfer takes place from N10-formyl-THF to CoA to form formyl-CoA in the N-terminal domain. In this formyl-group transfer reaction, His74, Asp80, and Asp109 act as the catalytic triad. The resulting formyl-CoA is converted into CO and CoA by decarbonylation of the formyl group, which is catalyzed by Tyr416 and/or Glu426 in the C-terminal domain of HypX.

T210 - Effects of Pta and AckA on *Klebsiella pneumoniae* pathogenesis

Presenting Author - *Ching-Ting Lin, China Medical University, Taiwan*

Author/s – *Tien-Huang Lin, Chien-Chen Wu, Yi-Min Hong*

Abstract Content

Background: Protein lysine acetylation is a post-translational modification to regulate bacterial virulence in many bacteria. Acetyl-phosphate (AcP), is an intermediate product in acetate metabolism, and could be directly produced by phosphate acetyltransferase (Pta), while is hydrolyzed by acetate kinase (AckA). In many bacteria, AcP is related to global regulation by phosphorylating or acetylating proteins and other molecules to further affect many cellular processes and virulence factors expression. However, the role of Pta and AckA in *Klebsiella pneumoniae* (Kp), a common opportunistic hospital-associated pathogen, still remains unknown.

Objectives: To demonstrate the role of Pta and AckA in regulation of virulence factors in Kp.

Methods: The deletion mutants of pta and ackA were generated in Kp CG43S3 (a K2 serotype) to detect the effects on ATP and AcP amount, the global acetylated protein profiles, and the virulence factor expressions. In addition, the complemented experiments were also performed to confirm the effects of Pta and AckA in Kp.

Results: In Kp CG43S3, we first demonstrated the role of Pta-AckA pathway in mediating the concentration of ATP and AcP. In addition, the global protein acetylation, CPS amount, and serum resistance are positively corrected with the concentration of intracellular AcP via Pta-AckA pathway. However, Pta-AckA pathway is also involved in type 3 fimbriae production and bacterial resistance to oxidative stress (hydroxyurea), but the effects are independent of the intracellular AcP amount. Taken together, we considered that Pta-AckA pathway is the critical post-translational mechanism to affect the Kp pathogenesis via pleiotropic regulation.

T211 - Role of EmaSR and iron-containing alcohol dehydrogenase in Ethanol Metabolism by *Acinetobacter baumannii*

Presenting Author - *Guang-Huey Lin, Tzu Chi University, Taiwan*

Abstract Content

Acinetobacter baumannii is a nosocomial pathogen that can survive in different environments including antibiotic and alcohol toxicity through the use of intricate networks to regulate gene expression. Two-component systems (TCS) form an important part of such regulatory networks, we describe the identification and characterization of a novel EmaSR TCS in *A. baumannii*. Meanwhile, most bacteria possess alcohol dehydrogenase (ADH) genes to alleviate alcohol toxicity, but these genes have functions more than alcohol degradation. We also identified 7 alcohol dehydrogenases (NAD+-ADHs) from *A. baumannii* ATCC 19606, and characterized the roles of 3 iron-containing ADHs, ADH3, ADH4, and ADH6. Markerless mutation was used to generate emaS, emaR, Adh3, Adh4, and Adh6 respective mutants. Disrupted adh4 mutants failed to grow in ethanol-, 1-butanol-, or 1-propanol-containing mediums, and recombinant ADH4 exhibited strongest activity against ethanol. We also found that emaS/emaR single-mutants and double-mutants were unable to replicate in M9 medium with 1% ethanol as the single carbon source and lost biofilm forming ability. Stress resistance assays with inorganic or organic hydroperoxides showed that Adh3 and Adh6 were key to oxidative stress resistance. Furthermore, virulence against *Galleria mellonella* was diminished in emaS, emaR, adh3 and adh6 mutants. Finally, RNA-seq results revealed that this novel EmaSR TCS is involved in the regulation of *A. baumannii* ethanol metabolism and acetate metabolism, with important implications on motility, virulence, and biofilm formation if mutated.

T213 - Acetoin assimilation by *Pseudomonas putida* is controlled by global regulatory elements that respond to nutritional cues

Presenting Author - *Fernando Rojo, Centro Nacional de Biotecnología (CNB-CSIC), Spain*

Author/s – *Renata Moreno, Luis Yuste*

Abstract Content

Background: The trophic cooperation seen when a compound excreted by one microorganism is used as a carbon source by another, is key in the establishment and maintenance of microbial ecosystems. Many microorganisms produce and excrete acetoin when growing in environments that contain glucose or other fermentable carbon sources. This excreted compound can then be assimilated by other bacterial species such as pseudomonads.

Objectives: We analysed whether the expression of the *Pseudomonas putida* KT2440 genes required for acetoin assimilation is inhibited by the presence of other potential carbon sources, and the mechanisms involved.

Methods: Expression of the acetoin degradation genes was analysed when cells were provided with acetoin alone or in combination with other compounds. The effect of several global regulatory systems that modulate bacterial metabolism was investigated.

Results: When succinate, glucose or components of the LB medium were also present, induction of the acetoin degradation genes by this compound was found to be down-modulated by the Crc/Hfq, Cyo and PTSNtr global regulatory elements, with the impact of each of these varying according to the carbon source present in addition to acetoin. Pyruvate, a poor carbon source for *P. putida*, did not repress acetoin assimilation. Indeed, the presence of acetoin significantly improved growth on pyruvate, revealing these compounds to have a synergistic effect. This would provide a competitive advantage to *P. putida* when growing in environments in which the preferred carbon sources have been depleted and pyruvate and acetoin remain as leftovers from the fermentation of sugars by other microorganisms.

T214 - Characterisation of the gut microbiomes of Australian marsupials using shotgun metagenomics and metabolomics

Presenting Author - *Rochelle Soo, The University of Queensland, Australia*

Author/s – *Elizabeth Jackobsen Neilson, Mette Sørenson, Disan Gunbileg, Mark Morrison, Birger Lindberg Møller, Philip Hugenholtz*

Abstract Content

Background: The gut microbiome plays a critical role in animal health and well-being. However, relatively little is known about the gut microbiomes of iconic Australian marsupials, including those capable of digesting toxic eucalyptus leaves.

Objective: To investigate the gut microbiomes of a wide range of Australian marsupials belonging to the family Diprotodontia using culture-independent methods, providing the first such data for many of these species.

Methods: We collected 94 faecal samples from 25 different Australian marsupial species representing 17 genera and analysed them using shotgun metagenomics and metabolomics. From these datasets, we obtained 1,096 medium quality metagenome-assembled genomes (MAGs) (dereplicated at 95% ANI) and 333 distinct metabolites.

Results: Marsupial hosts have characteristic gut microbiomes and metabolite profiles that largely reflect host phylogeny. Adaptation to a eucalyptus diet in koala, greater glider, and some possum species appears to have arisen independently via convergent evolution. We found no gut species exclusively present in eucalyptus feeders, suggesting that adaptations to this specialised diet did not occur due to horizontal transfer of eucalyptus-degrading gut species. Our study provides a baseline of molecular data for the study of marsupial gut microbiomes.

T216 - Production of novel lantibiotics using oral Streptococci

Presenting Author - *Saswati Biswas, Kansas State University, United States*

Abstract Content

The recent surge of genomics/metagenomics research boosted the availability of numerous novel lantibiotic sequences in public databases. A vast majority of them are uncharacterized regarding their target and potency. The predominant reason for the lack of characterization is that many sequences are derived from a metagenomic study of the microbial community. Thus, isolated strains of lantibiotic producers are not available. Furthermore, the lantibiotic producer strains are occasionally non-culturable. Additionally, many times, the lantibiotic genes are encoded as a part of a silent gene cluster. Thus, for novel lantibiotic screening, we tested a lantibiotic mutacin II producer strain *Streptococcus mutans* T8 as the production host. This organism is genetically easy to manipulate and is naturally a prolific producer of lantibiotics. Although a lantibiotic pre-peptide is only ~60 residues comprised of leader and core regions, the biosynthesis gene cluster of a lantibiotic is organized in a large operon of ~10 kb in size that includes the structural gene of the lantibiotic and the genes encoding the modification enzyme, exporter, immunity protein and occasionally the regulator. Therefore, we decided to use the host's biosynthesis system and replace only the core sequence of mutacin II in the T8 chromosome with its homologs. Since the lantibiotic biosynthetic apparatus use the leader peptide for activation, we did not alter this sequence. For screening of novel lantibiotics, we used a target panel comprised of multiple bacterial strains. We further modified the host to include a few other targets which enhanced the screening success of novel lantibiotics.

T217 - Identification of promoter activity in gene-less cassettes from Vibrionaceae superintegrons

Presenting Author - *Paula Blanco, Complutense University of Madrid, Spain*

Author/s – *Cristina Ortega, Filipa Trigo da Roza, Alberto Hipólito, José Antonio Escudero*

Abstract Content

Background: Integrons are genetic platforms that enable bacteria to capture and incorporate new genes encoded in mobile genetic elements known as integron cassettes (ICs). Typically, ICs encode a single open reading frame (ORF) that is promoterless, and whose expression is granted by the PC promoter within the platform. This means that cassettes that are far from the Pc can be silent. We have observed that some cassettes are very small and do not likely contain protein-encoding genes. Therefore, we hypothesize that they may have a regulatory function.

Objectives: We aim to study the potential regulatory role of gene-less cassettes in driving the transcription of adjacent ICs in long cassettes arrays.

Methods: We have identified and synthesized 29 gene-less cassettes from various Vibrionaceae species, where large sedentary chromosomal integrons are prevalent. We have evaluated their potential activity as promoters by cloning these fragments into the plasmid pDProm, a bidirectional transcription detection system based on the expression of two fluorescent proteins.

Results: Our results indicate that gene-less cassettes commonly have promoter activity and that is typically biased towards promoting the expression of downstream cassettes. We also found that, when located upstream a silent antibiotic resistance IC (dfrB9), these promoter-containing cassettes can lead to an increase in trimethoprim resistance of over 2000-fold. These findings reveal that one of the functions of gene-less cassettes may be the transcriptional regulation of adjacent cassettes, and highlight their importance in clinical settings.

T218 - Characterization of *Escherichia coli* harboring no two component system genes

Presenting Author - *Kaneyoshi Yamamoto, Hosei University, Japan*

Author/s – *Yukari Miyake, Genki Hirano, Shingo Sugawara, Yu Yagasaki, Hikari Yoshitane, Miho Yoshimura*

Abstract Content

Bacteria cell survives in sophisticated environment using stress response system. Two component signal transduction system (TCS), conserved in prokaryote, consists of a pair of sensor kinase (SK) and response regulator (RR). Several TCSs are known to require for cell growth under laboratory condition. The genome sequence of *Escherichia coli* K-12 predicts 30 TCSs, which form signal transduction network. Bacteria responding to environmental stress could proliferate via adaption, termed adaptive growth. Our objective is whether growth of *E. coli* is require for SK and/or RR.

The developed genome edit technology, HoSeI method (1,2), isolated all 30 SK gene- or 34 RR gene-deprived strains. The resulted mutants were subject to growth and omics analyses. Both mutants showed no significant proliferation difference in LB. Phenotypic microarray analysis showed similar growth ability profiles of both parent and ΔSK strains but growth range of ΔRR was more narrow. Omics data were evaluated by PCA. Transcriptomic and proteomic PCAs showed different profiles among parent, ΔSK, and ΔRR strains. Metabolomic PCA indicated that profiles of ΔSK was more different than parent strain but ΔRR strains not. ΔSK significantly increase the level of amino acids and dipeptides. Protein profiles of ΔSK and ΔRR were mapped in metabolic pathway, resulting that ΔSK increased the level of enzymes for branch chain amino acid biosynthesis and ΔRR increased the level of enzymes for fatty acid degradation. Taken all results together, TCS is potentially no essential for *E. coli* proliferation whereas non-TCS *E. coli* could change metabolic flux for growth in LB.

T219 - Microtubule maintenance factor Alf1 genetically interacts with RAD51 in response to genome instability and mutagenesis in yeast

Presenting Author - *Ji Eun Choi, Duksung Women's University, Republic of Korea Korea*

Author/s – *Youn Young Heo, Woo-Hyun Chung,*

Abstract Content

Microtubules are heterodimeric polymer composed of alpha- and beta-tubulin playing pivotal roles in multiple cellular processes such as transport, cytoskeleton and cell division. Specific cofactors required for the folding of alpha- and beta-tubulin have been found in many organisms including *Saccharomyces cerevisiae*. Alf1, the cofactor B homolog, is functionally and physically related with alpha-tubulin. Rad51 plays central roles in homologous recombination (HR) pathway. Rad51 catalyzes a homology search and DNA strand invasion. In this study, we investigate genetic interactions between DNA double-strand break (DSB) repair pathway and microtubule metabolism in *S. cerevisiae*. The rad51 alf1 double mutant displays slow growth and synergistic sensitivity to caffeine and DSB-inducing drugs. Nuclear Rad52 foci representing DSB damages are accumulated more in rad51 alf1 mutant than each single mutant. However, we observed that lack of Alf1 ameliorates highly-mutated phenotype of rad51 mutants. Overexpression of Alf1 rescued the impaired growth of alf1 mutants. Our data suggest specific genetic interactions between genomic stability and the cytoskeletal maintenance.

T220 - The residues N210 and D182 in VIM-2 metallo beta-lactamase influence on substrate specificity and stability of the enzyme

Presenting Author - *Diamond Jain, Indian Institute Of Technology Kharagpur, India*

Author/s – *Anindya S. Ghosh*

Abstract Content

Background: Verona integron metallo beta-lactamase (VIM-2) is one of the most widespread class B beta-lactamase responsible for beta-lactam resistance. Although active-site residues help in metal binding, the residues nearing the active-site possess functional importance and are crucial for comprehending the mechanism of its action.

Objective: Deciphering the role of non-active site residues in the activity and stability of VIM-2 metallo beta-lactamase.

Methods: The full length and truncated blaVIM-2 was cloned in pBAD18cm and pET28a, respectively. The residues (D182, N210, D213, S207) were selected using *in silico* methods and substituted with alanine using site-directed mutagenesis. Antibiotic susceptibility testing and effect of zinc depletion on *E. coli* host expressing these proteins were estimated to understand the involvement of these substitutions on VIM-2 performance. Further, proteins were purified using affinity chromatography and zinc was estimated using PAR assay, enzyme kinetic parameters were determined spectroscopically and thermal stability was assayed using differential scanning fluorimetry.

Results: The expression of N210A substituted VIM-2 in the host displayed ~4-8 fold enhanced susceptibility towards tested penicillins and cephalosporins. D182A substitution had detrimental effect on VIM-2 activity. The host expressing VIM-2_N210A was susceptible to cefotaxime in zinc depleting conditions. Both the mutants displayed reduced zinc binding and thermal stability in homogenously purified proteins as compared to VIM-2. *In vitro* catalytic efficiency of VIM-2_N210A was reduced against penicillins and cephalosporins by >4 fold while VIM-2_D182A retrieved activity in presence of zinc. Therefore, N210A affects substrate specificity and stability while D182A affect the stability of VIM-2.

T221 - Deletion of conjugation genes repressors of the IncM plasmid pCTX-M3 affects global gene expression in the host cell

Presenting Author - *Izabela Kern-Zdanowicz, Institute Of Biochemistry And Biophysics PAS, Poland*

Author/s – *Jan Gawor, Karolina Żuchniewicz, Robert Gromadka*

Abstract Content

Conjugative plasmids are main players in horizontal gene transfer in Gram-negative bacteria. They contribute to rapid dissemination of antibiotic resistance genes causing the crisis of antibiotic-resistant bacteria which urgently requires new solutions. The IncM plasmids are one of major families of resistance plasmid of clinically relevant Enterobacterales worldwide. The range of recipients in their conjugative tranfer is broad and comprises Alpha-, Beta-, and Gammaproteobacteria as well as representatives of Firmicutes, what was shown for pCTX-M3, the IncM representative.

Here, we show that deletion of orf35- and orf36-encoded regulators of conjugation genes deeply affects expression of other plasmid genes as well as chromosomal genes of *Escherichia coli*.

T222 - The expression of aminoglycoside resistance genes in integron cassettes is not controlled by riboswitches

Presenting Author - *Alberto Hipólito, Complutense University of Madrid, Spain*

Author/s – *Lucía García-Pastor, Paula Blanco, Filipa Trigo da Roza, Nicolas Kieffer, Ester Vergara, Thomas Jove, Julio Álvarez, José Antonio Escudero*

Abstract Content

Background: The success of antimicrobial resistance genes is largely determined by their cost. Inducible expression mechanisms can alleviate this cost in the absence of antibiotics. It has been reported that a conserved riboswitch controls the expression of aminoglycoside (Ag) resistance genes. However, the presence of such a riboswitch is counterintuitive because this sequence matches the integration site of an integron, a genetic platform that recruits genes of unrelated functions.

Objective: In this study we seek to provide experimental evidence of the existence of such Ag-sensing riboswitch in integrons.

Methods: We have retrieved all (64) integron cassettes conferring resistance to aminoglycosides from the INTEGRALL database. We have fused each 5'-untranslated regions (UTRs) to a GFP reporter-gene and inserted them in first position of a mobile integron. We have then measured their expression levels in the presence of antibiotics by flow cytometry and Western blot.

Results: in the absence of antibiotics, the 64 5'-UTRs show a large range of fluorescence levels, a phenomenon incompatible with the expected off state of riboswitches. In the presence of multiple aminoglycosides, we did not observe biologically-relevant levels of induction for any gene. Instead, unrelated antibiotics caused higher (although still mild) increases in expression. We proved that these effects were non-specific uncoupling the presence of an antibiotic from its activity. This suggests that pleiotropic effects of antibiotics might have been misinterpreted as an inducible riboswitch. Our data, rules out the existence of an Ag-sensing riboswitch regulating aminoglycoside resistance genes in integron cassettes.

T223 - The flavoprotein MftG is a mycofactocin dehydrogenase connected to respiration in *Mycolicibacterium smegmatis*

Presenting Author - *Patricia Graça, Leibniz Institute for Natural Product Research and Infection Biology, Germany*

Author/s – *Vadim Nikitushkin, Mark Ellerhorst, Gerald Lackner*

Abstract Content

Mycofactocin (MFT) is a redox cofactor of Mycobacteria like *M. tuberculosis*, the causative agent of tuberculosis, involved in the metabolism of alcohols. Interferences of the mycofactocin system with the growth of *M. tuberculosis* in infection models reinforced the importance of the cofactor. Studies on the MFT gene cluster (mftA-G) exposed a redox-active core in either oxidized or reduced form. While MftA-E synthesize the redox core, the glycosyltransferase MftF decorates the coenzyme with a α-1,4-glucan chain. However, the function of the mftG gene, encoding a flavoenzyme belonging to the glucose-methanol-choline oxidoreductase (GMC) superfamily, remained elusive.

To investigate its function, a deletion mutant (ΔmftG) was generated in *Mycolicibacterium smegmatis*. Like other MFT deletion strains, the mutant was hampered in its ability to grow on ethanol as the sole carbon source, while the complement mutant grew to high density. Profiling of the MFT pool using LC-MS showed depletion of oxidized MFTs and accumulation of reduced MFTs, indicating a potential bottleneck in MFT re-oxidation. Using protein fractions of *M. smegmatis*, the MFT dehydrogenase activity of MftG could be confirmed. Transcriptomics analysis further suggested a role of MFT in respiration. Indeed, the respiration rate measured in membrane fractions of *M. smegmatis* was boosted by the addition of reduced MFT. The respiration assays further revealed decreased respiration of the ΔmftG mutant compared to WT. This study unveils the function of MftG as MFT dehydrogenase and encourages further elucidation of the link between the MFT system and mycobacterial respiration.

T224 - Molecular detection of chicken mycoplasma iners and *Mycoplasma gallinarum* in backyard flocks using the 16S-23S ribosomal RNA intergenic spacer gene

Presenting Author - *Rizgar Sulaiman, University of Sulaimani, Iraq*

Abstract Content

Mycoplasma infections are common in poultry breeds and result in considerable economic losses due to reduced productivity and increased mortality. Mycoplasma iners and *Mycoplasma gallinarum* are two important species of Mycoplasma that can cause respiratory and reproductive problems in poultry. This study aimed to identify and analyse the prevalence and diversity of *M. iners* and *M. gallinarum* in backyard brethren using the 16S-23S intergenic spacer gene. From March to October 2021, samples were collected from various fields of Sulaymaniyah/Iraq, and DNA was extracted. PCR and sequenced amplified the 16S-23S ribosomal RNA intergenic spacer gene. The 16S/23S IGS sequence of a non-cultivated chicken Mycoplasma species was submitted to GenBank and received its accession numbers (OM200338.1-OP617735.1, OP617736.1 and OP617737.1). Phylogenetic analysis was performed to determine the position of *M. iners* and *M. gallinarum* in different Mycoplasma trees. Our results showed that *M. iners* and *M. gallinarum* were present in backyard flocks at 40% and 35% respectively. With the help of the online software blast/MCBI, the sequences (Suly2-iners OP617735) with a high similarity of 99.39% of the *M. iners* PG30 USA strain isolate the accession number. (JN935870), and comparison strains (Suly-gallinarum OP617736) showed that VRLCU5 was more similar to *M. gallinarum*, with a relative number of 99.39 % (KF895038) in Egypt.

T225 - A Novel Flux Regulation of Lysine Acetyltransferase(PatZ) in *Escherichia coli*

Presenting Author – *Gwanwoo Lee, Seoul National University, Republic of Korea*

Author/s – *Yeong-Jae Seok*

Abstract Content

Nε-Lysine acetylation is a post-translational modification that occurs in diverse bacterial species. It modulates several biological processes, such as RNA metabolism, enzymatic activity, motility, cell shape, and bacterial pathogenesis. However, most lysine acetylation is non-enzymatic and done by acetyl phosphate (AcP). The enzymatic acetylation of lysine is not well understood. In non-enzymatic reactions, AcP directly donates its acetyl group to the deprotonated lysine amino group. In enzymatic reactions, lysine acetyltransferase (KAT) catalyzes the transfer of the acetyl group from acetyl-CoA. This enzymatic acetylation has high site specificity and acts as a key regulatory point specific to metabolism. In *Escherichia coli*, the only well-known regulatory role of acetyltransferase (Patz, also known as YfiO and Pka) is the regulation of acetate metabolism. We found that the growth rate of the K-12 MG1655 patZ deletion mutant was significantly faster than wild type on galactose but not on glucose and fructose. In addition, we observed differences in the flux of central metabolism through transcriptome analysis of the wild type and the patZ deletion mutant.

T226 - Identification and characterization of the cell wall channels of *Rhodococcus corynebacteroides* and *R. ruber*

Presenting Author - *Claudio Piselli, Jacobs University, Germany*

Abstract Content

The cell walls of Gram-positive bacteria from the class Actinomycetia are surrounded by a supplementary waxy layer of various lipid-like molecules, decorated by pore forming proteins for the solute exchange. These outer membranes show the characteristics of a molecular sieve and play a role not only in the antibiotic resistance, but also in the interaction with the host's immune system (i.e. *M. tuberculosis*). The cell walls from the genus *Rhodococcus* contain channels that belong to the MspA-superfamily. We studied one polypeptide of *R. corynebacteroides* (MspARc) and two from *R. ruber* (MspARr and MspBRr). Their ORFs were cloned without signal peptide into pET vectors and separately expressed in the porin-deficient BL21Gold(de3)ΔABCF strain of *E. coli*. The channels were purified to homogeneity and studied via their reconstitution into artificial lipid bilayers. The activity of MspARc in reconstition experiments showed the same 2 maxima, which overlapped to those of the protein isolated from the original host. Only exception being that the main peak of the distribution shifted from about 4.5 nS for the native cell wall channel to about 3 nS for the recombinantly expressed one suggesting some post-translational modifications *in vivo*. The MspARr and MspBRr could create alone channels in artificial bilayers, despite both subunits are encoded *in vivo* into the same operon and may form cell wall channels together also. The characterization of MspA-superfamily of cell wall channels, together with the α-hemolysins, provide the biotechnological tools behind slow DNA translocation though a nanopore and high resolution single channel sequencing.

T227 - Evaluation of virulence and antibiotic resistance profile of *Escherichia coli* causing Infective endocarditis and its potential unitary origin

Presenting Author - *Nathália Andrade, Rio De Janeiro State University - UERJ, Brazil*

Author/s – *Ana carolina Campos, Andrea Maria Cabral, Ana Claudia Rosa, Paulo Damasco*

Abstract Content

Background: Infective endocarditis (IE) is the fourth life-threatening infectious syndrome after urosepsis, pneumonia, and intra-abdominal sepsis in medical units in developed countries. IE by *Enterobacter*iaceae is rare, especially the ones caused by *Escherichia coli* (*E. coli*), nonetheless, the number of cases has increased in Brazil.

Objectives: Our project aims to unveil which virulence mechanisms are associated with IE with urinary origin caused by *E. coli* and identified their antibiotic-resistance profiles.

Methods: The whole genomes of those isolates were sequenced and compared with genomes of other *E. coli* isolates from a bigger public database to identify virulence genes associated with IE with urinary origin.

Results: Eight genes were identified in the IE infections isolates, however, only four of those genes were consistently found in *E. coli* isolates from urinary infections (chuA, fyuA, irp2 and sitA). Those genes are associated with iron uptake system, an important step in the pathogenicity of urinary pathogens. Iron uptake systems particularly irp2 in ST69 isolates that when deleted had demonstrated attenuation of virulence in the sepsis models. Interestingly, other common virulence genes present in urinary pathogens were absent from IE isolates, which raises questions about the virulence mechanism and other genes associated. Resistance genes strA, strB, aadA5, blaTEM-1B, sul1, sul2 and dfrA17 were also identified in IE isolates. In addition, the IE isolates belonging to ST69 are commonly associated with urinary infections. Our preliminary results indicate similarities in the antibiotic resistance and virulence profiles from *E. coli* isolates from urine and IE with urinary origin.

T228 - Genome-encoded ABCF factors implicated in Clostridial intrinsic antibiotic resistance

Presenting Author - *Nozomu Obana, University of Tsukuba, Japan*

Author/s – *Hiraku Takada, Nobuhiko Nomura, Gemma Atkinson, Vasili Hauryliuk*

Abstract Content

Bacterial antimicrobial resistance is a growing threat to human health. The F subfamily of ABC ATPases includes ribosome-associated antibiotic resistance (ARE) determinants, ARE-ABCF. We discover that diverse clostridial bacteria possess an ARE-ABCF resistance factor (cplR: clostridial pleuromutilin lincosamide resistance), encoded in chromosomes of a clinically important human pathogen *Clostridioides difficile*, a causative agent of food poisoning *Clostridium perfringens*, as well as a commensal gut bacterium *Clostridium sporogenes*. MIC tests using the cplR mutant strains of these bacteria show that CplR contributes to intrinsic pleuromutilin, lincosamide, and streptogramin A resistance in Clostridia. We also demonstrate that *C. difficile* CplR synergizes with the transposon-encoded 23S ribosomal RNA methyltransferase ErmB to confer high antibiotic resistance to *C. difficile*. Furthermore, we find that antibiotic treatment induces the cplR gene expression in *C. difficile*, which depends on the 5' leader region of the cplR gene. The mutational analysis in the 5' leader region indicates that the upstream open reading frame and RNA secondary structure are indispensable for regulating cplR expression upon an antibiotic challenge. We demonstrate that the translation attenuation mechanism controls the induction of cplR expression. Given that clindamycin treatment is associated with *C. difficile* infection, the cplR-mediated lincosamide resistance mechanism in *C. difficile* demonstrated in this study may be of clinical importance.

T229 - Decoding the synergistic interaction between trimethoprim and nitrofurantoin in *Escherichia coli*

Presenting Author - *Dione L. Sánchez-Hevia, Uppsala University, Sweden*

Author/s – *Sanne Westhoff, Po-Cheng Tang, Dan I. Andersson*

Abstract Content

Antibiotic combination therapies are commonly used clinically; however, the efficacy of these combinations varies, partly depending on whether the used drug combination shows an increased (synergy), equal (additivity) or reduced (antagonism) effect on efficacy compared to that predicted from the individual antibiotic's effect combined.

Our laboratory previously identified a synergy interaction between trimethoprim and nitrofurantoin in *Escherichia coli* clinical isolates. These two antibiotics are often used to treat urinary infections, although typically not in combination. Recently, we expanded the analysis to 245 isolates and demonstrated that this combination shows synergy in the majority of the isolates (79%).

In order to analyse the underlying molecular mechanism causing synergy, we performed a genetic selection using different concentrations of trimethoprim together with nitrofurantoin, to select for spontaneous mutants that had lost the synergistic interaction. We excluded mutants that became resistant to either or both of the two antibiotics since we were interested in understanding the mechanism(s) causing the loss of the synergy and not the resistance mechanisms to individual antibiotics.

Though the minimal inhibitory concentrations (MIC) of both antibiotics were, in almost all the cases, identical in the parental strains and the mutants, we observed that 53 out the 61 mutants obtained had lost the trimethoprim-nitrofurantoin synergy when exposed to subinhibitory antibiotic concentrations. Whole genome sequencing of these mutants has been performed, and it is currently under analysis. Preliminary data indicate a possible role of the ribosomal proteins.

T230 - High-throughput characterization of interspecies interactions in oral implant-associated biofilms

Presenting Author - *Rumjhum Mukherjee, Medizinische Hochschule Hannover, Germany*

Author/s – *Meike Stiesch, Szymon P. Szafranski*

Abstract Content

Background: During polymicrobial biofilm formation on implant surfaces, the involved species of oral microorganisms interact on different levels. In this regard, exploitation of metabolites released by other species can be an important survival strategy that contributes to biofilm fitness and leads to disease development and progression. Identification and characterization of these hallmark interactions are therefore imperative to detect early pathology and prevent implant-associated diseases.

Objectives: Characterization of synergistic metabolic interactions in implant-associated biofilms and tracing of chemical compounds that are actively involved in these inter-species metabolite flows are the primary goals of this study. The knowledge gained should contribute to the decoding of these interaction networks in complex clinical biofilms.

Methods: Interspecies metabolic interactions were characterized using diverse agar diffusion assays. Clinical oral biofilm samples, more than one hundred reference strains of diverse species, various metabolites and their corresponding combinations were studied. Liquid biofilm cultures were used to verify selected interactions.

Results: Porphyromonas gingivalis showed satellite growth exclusively around colonies of other species from classes such as Actinobacteria, Bacteroidia, Negativicutes and Gammaproteobacteria, thereby indicating metabolite exploitation under experimental conditions. Chemical complementation experiments identified menaquinones and their related precursors as major drivers of these interactions.

Conclusion: We were able to characterize exploitation interactions involving the key oral pathogen Porphyromonas gingivalis. Targeting these interactions may support the development of innovative preventive and therapeutic strategies for implant-associated infections.

T231 - Signal transduction of DJ41_1407/DJ41_1408 and GacS/A in *Acinetobacter baumannii* ATCC19606

Presenting Author - *Yee Huan Toh, Tzu Chi University, Taiwan*

Author/s – *Guang Huey Lin*

Abstract Content

Acinetobacter baumannii often cause nosocomial infection because of derived multiple drug resistance. The common signal transduction system in bacteria, two-component system (TCS) was composed of a sensor that self-phosphorylated upon trigger and a regulator that regulate downstream gene expression upon sensor phosphorylation. Previous study showed that GacA in GacSA TCS will be phosphorylated by DJ41_1407. Another transcriptional regulator DJ41_1408 was found to be in the same operon with GacA and DJ41_1407 suggested that there may be crosstalk between TCSs. Upon studying GacS/A and DJ41_1407/DJ41_1408 relationship, Phos-tagTM results revealed that DJ41_1407/DJ41_1408 was a TCS and GacS have a more important role in GacA phosphorylation compared to DJ41_1407. RNA-seq results showed that DJ41_1408 may play roles in carbon metabolism, amino acids metabolism, benzoate degradation. A gene cluster (DJ41_358-DJ41_366) function in tryptophan dependent indole-3-acetic acid (IAA) biosynthesis was found to be regulated by DJ41_1408. Immunoblot results showed expression of DJ41_1408 only in IAA or tryptophan as single carbon source. MEME analysis showed that binding box of GacA and DJ41_408 located on promoter region DJ41_358-DJ41_266 gene cluster and DJ41_1407. The minimal inhibitory concentration (MIC) of kanamycin, apramycin and gentamicin to *A. baumannii* decreased when GacS/A was mutated. Survival of *G. mellonella* infected by *A. baumannii* decreased when DJ41_1407/DJ41_1408 was mutated and increased when GacS/A was mutated. In conclusion, GacSA, DJ41_1407/GacA, DJ41_1407/1408 was TCS and GacS have more important role in GacA phosphorylation. GacSA enhanced antibiotics resistance and virulence, while DJ41_1407/DJ41_1408 inhibits virulence of *A. baumannii* ATCC19606.

T232 - Identification of the pyruvate-binding site of the BtsS/BtsR signaling cascade in *Escherichia coli*

Presenting Author - *Jin Qiu, LMU München, Germany*

Author/s – *Kirsten Jung, Nathalie Sisattana, Ana Gasperotti*

Abstract Content

The histidine kinase/response regulator system BtsS/BtsR is one of the pyruvate-sensing systems in *E. coli*. BtsS is a high-affinity sensor for pyruvate and, together with the response regulator BtsR, activates the expression of btsT, which encodes a high-affinity pyruvate transporter. However, the molecular mechanism of how pyruvate binding triggers a response is still unclear. Here, we experimentally show that BtsS consists of seven transmembrane helices, with the N-terminus exposed on the periplasmic side. Screening by site-directed mutagenesis identified Arg72, Arg99, Cys110, and Ser113, all located on the periplasmic side, as crucial for binding of pyruvate to BtsS. In addition, autophosphorylation and dimerization of wild-type BtsS were demonstrated for the first time, and individual replacement of the four amino acids affected both processes and consequently btsT expression. Replacement of Arg192 also prevented btsT expression, but due to a weaker interaction with BtsR. This study demonstrates how binding of a metabolite to the membrane-integrated sensor domain triggers signaling in the cytoplasm. Our results can serve as a starting point to convert BtsS into a sensor for other ligands, such as lactate, that can be used as biosensor in biomedicine.

T233 - Reintroduction of single genes encoding c-di-GMP modulating enzymes into a *P. aeruginosa* PA14 mutant lacking all 32 genes encoding diguanylate cyclases (DGCs)

Presenting Author - *Melisa Gür, Twincore GmbH (MHH/HZI), Germany*

Author/s – *Tim Rick, Jelena Erdmann, Susanne Häußler, Oliver Hartmann*

Abstract Content

The perception of environmental stimuli and the mediation of the switch in lifestyle between planktonic and biofilm growth is mainly regulated by the second messenger c-di-GMP (cdG). This important signaling molecule can be produced or degraded by many different proteins, present in varying numbers in different bacterial species. Diguanylate cyclases (DGC), which synthesize cdG from 2 molecules of GTP, typically contain a GGD(E)EF domain in their catalytic center. For many of the 32 GGD(E)EF domain proteins present in the PA14 *P. aeruginosa* type strain neither their enzymatic activity nor their activating conditions or downstream effects have been studied. While most previous studies have focused on the analysis of deletion mutants of single DGCs, we took a different approach and used a cdG null mutant in which each individual gene encoding for GGDEF-domain proteins was functionally knocked out by STOP codon insertions (D. Volke, P. Nikel lab DTU, Copenhagen). We re-introduced all 32 genes encoding for (putative) diguanylate cyclases and comprehensively characterized their phenotypes, including cdG levels and transcriptomes. Our data show that the majority of the cdG dependent phenotypes, such as swimming motility or biofilm formation, were influenced by a combination of several cdG-modulating enzymes.

T234 - K fragment, a PCR-based vector for antibiotic resistance gene hunting

Presenting Author - *Hanife Salih Doğan, Aydin Adnan Menderes University, Turkey*

Author/s – *Erman Oryaşın, Bülent Bozdoğan*

Abstract Content

Antimicrobial resistance (AMR) is a significant health issue in the world. It limits treatment options for infectious diseases and causes higher mortality, and costs because of a more extended stay at a hospital. To evaluate and predict resistance problems, the resistance genes, both known and unknown genes that are responsible for AMR should be identified and characterized. Known genes can be detected by common methods like PCR however unknown genes are difficult to detect. In this study, we aimed to develop a method to detect both known and unknown resistance genes by cloning. We constructed a template to generate amplicons without a resistance marker for cloning purposes. Multiple cloning site (MCS) and the ampicillin resistance gene were removed from pUC19 and a newly designed Prom-RBS sequence was added. The generated vector, plasmid of K fragment (pKF) (accession number: OM304286), was used as a template for amplification of K fragment with modified primers with restriction enzyme recognition sites. K fragment is composed of RBS and promoters at both directions and plasmid origin of replication. The useableness of amplified K fragment as a cloning vector was tested by successful cloning of antibiotic resistance genes from amplicon (cat) and total DNA of *Staphylococcus aureus* (ermC). K fragment is a PCR-based and antibiotic resistance marker-free cloning vector. It allows the cloning of antibiotic resistance genes at all orientations just by changing restriction enzyme sites at primers and fragmenting extracted DNA with the same enzyme.

T235 - Uncovering the mechanism of autoinhibition by *P. aeruginosa* aminopeptidase aids development of a potent antibiofilm inhibitor

Presenting Author - *Christopher Harding, University of St Andrews, United Kingdom*

Author/s – *Clarissa Czekster, Marcus Bischoff, Megan Bergkessel*

Abstract Content

Pseudomonas aeruginosa is an opportunistic pathogen that causes serious illness, especially in immunocompromised individuals, including those with cystic fibrosis. *P. aeruginosa* is well known for forming biofilms, which significantly contributes to its growth and persistence in a wide range of environments. In addition, its success can be attributed to several secreted proteases. Here, we investigated the aminopeptidase, PaAP, from *P. aeruginosa*, which is one of the most abundant extracellular proteins in the biofilm matrix. Increasing evidence suggests this quorum-sensing-regulated peptidase, is associated with biofilm development, and contributes to nutrient recycling by complementing the activity of other secreted proteases. We confirmed that post translational processing was required for activation, as a C-terminal truncation mutant possessed ~100-fold higher activity than the full-length enzyme. We determined that PaAP is a promiscuous aminopeptidase acting on unstructured regions of peptides and proteins. High-resolution crystal structures of wild type enzyme and mutants revealed the mechanism of autoinhibition, whereby the C-terminal pro-peptide locks the protease-associated (PA) domain and the catalytic peptidase domain, into a closed, inhibited conformation. Inspired by this self-regulatory mechanism and guided by structural data, we designed a highly potent small cyclic-peptide inhibitor (Ki in the nM range). This inhibitor recapitulates the deleterious phenotype observed with a PaaP deletion mutant in liquid cultures and biofilm assays, and suggests a path towards targeting secreted products in a biofilm context.

T236 - Non-active site residues D192 and S217 influence the metallo beta-lactamase activity of NDM-4

Presenting Author - *Anindya Ghosh, Indian Institute Of Technology Kharagpur, India*

Author/s – *Jyoti Verma, Diamond Jain*

Abstract Content

Background: New Delhi Metallo beta-lactamase (NDM) producing bacteria impose a significant threat to the antimicrobial treatment of bacterial infections due to the ability of NDMs to hydrolyse nearly all the existing beta-lactam antibiotics. The activity of Metallo beta-lactamases is affected by the active site residues as well as residues near the active site.

Objectives: To identify the specific amino acids located around the active site of NDM-4 that influences its function.

Methods: Eight substitution mutations namely, S191A, D192A, S213A, K216A, S217A, D223A and D225A were generated through site-directed mutagenesis. The effect of these mutations on NDM-4 activity was determined by assessing the change in susceptibility of *E. coli* against beta-lactam antibiotics. The mutants showing effect were purified using Ni/NTA affinity chromatography and assessed for the kinetic parameters of enzymes *in vitro*. Also, the thermal stability was assessed using differential scanning fluorimetry. In addition, the zinc content of purified proteins was estimated using PAR assay.

Results: Expression of NDM-4_D192A and NDM-4_S217A in *Escherichia coli* cells had substantially increased the beta-lactam susceptibility as compared to wild-type NDM-4 by >8 fold. These substitutions decrease the catalytic efficiency of NDM-4 towards beta-lactam substrates while do not affect the thermal stability in the presence of zinc, suggesting that the difference in activity is mostly due to catalytic changes and not due to structural variations. However, the D192A substitution alters the zinc content of NDM-4.

Conclusion: We infer that the non-active site residues D192 and S217 of NDM-4 modulate the performance of this beta-lactamase.

T237 - Endolysin as an alternative to antibiotics to treat *Clostridioides difficile* infection

Presenting Author - *Younjin Cho, Ajou University, Republic of Korea*

Author/s – *Jina Seo, Daechan Park, Hyunjin Yoon*

Abstract Content

Clostridioides difficile is a Gram-positive and spore-forming anaerobe that can cause toxin-mediated colitis. The main contributing factor to *C. difficile* infection (CDI) has been identified as gut microbial dysbiosis which is associated with the abuse of antibiotics such as clindamycin and ampicillin. We established a CDI model *in vitro*, where repetitive clindamycin treatments reduced the diversity of fecal microbiota and altered the composition of bacterial abundance. In order to circumvent the drawbacks of antibiotics, we explored the potential of endolysin as a therapeutic drug against CDI. Endolysin is a peptidoglycan hydrolase that is encoded by a phage and has a wider host-range activity than the cognate phage. Based on a plate screening approach, six endolysins were found to selectively decompose the cell-wall of *C. difficile*. Among these six endolysins, CD27 was modified to remove its cell-wall binding domain. The modified endolysin, CD27_EAD, showed a greater killing activity on *C. difficile* than the intact form. Fecal microbiota was treated with CD27-EAD and bacterial composition was analyzed by 16S rRNA sequencing. The abundance of *C. difficile* was significantly decreased from 20% to 0.8% and the other bacterial species were not influenced by CD27-EAD. This result suggests that endolysins can be a promising alternative to antibiotics for CDI treatments.

T238 - LeuO's plays a news role in *Salmonella*'s virulence regulation within intra-macrophage environment

Presenting Author - *Eunsuk Kim, Ajou University, Republic of Korea*

Author/s – *Hyunjin Yoon*

Abstract Content – *Salmonella* has become one of the most studied bacterial pathogens due to its exclusive nature of intracellular survival, eventually leading to fatal outcomes. Even though extensive studies have had success in linking the network of *Salmonella* virulence factors, the regulatory elements are an ongoing area of interest. The primary transcriptome of *Salmonella* inside host macrophages was acquired at 9 hours post-infection in order to unveil the comprehensive transcriptional regulatory circuits during intra-macrophage survival. RNA-Seq analysis revealed 1,013 differentially expressed genes (DEGs) with a threshold of at least 3-fold change. Among the DEGs, genes enrolled in transcriptional regulation were further investigated in consideration of their potential to orchestrate the transcription of multiple genes associated with the intracellular survival. *Salmonella* mutant strains lacking transcriptional regulators, incuding ΔSTM14_0016, ΔnhaR, ΔleuO, ΔydhB, and ΔyneJ, showed increases in survival inside macrophages, which is indicative of their critical roles in modulating intracellular survival. When the deleted genes were complemented in trans, only ΔleuO strain recovered its altered intracellular survival. Interestingly, the overexpression of LeuO repressed the transcription of *Salmonella* pathogenicity island (SPI)-2 genes, which are essential for *Salmonella* virulence. ChIP-Seq analysis employing MAST module predicted the binding motifs of LeuO in the region of ssrAB, the primary regulatory loci of SPI-2. Binding of LeuO to the presumable sites of ssrAB genes was validated by electrophretic mobility shift assays. These results suggest a new role of LeuO in downregulation of SPI-2 and help understanding the regulatory network of *Salmonella* virulence factors during survival inside macrophages

T239 - Stress adaptation to Pi starvation leads to polymyxin resistance in *E. coli* through a Mg-Fe-BasSR signal transduction circuit

Presenting Author - *Guangming Zhang, University of Hong Kong, China*

Author/s – *Ziqing Deng, Minji Wang, Aixin Yan*

Abstract Content

Phosphorus (P) is an essential element for building blocks of biomolecules and plays important role in vary biological processes. In bacteria, P is acquired mainly as inorganic orthophosphate (Pi), shortage of Pi can serve as a strong stimulus. Despite numerous works focused on how bacteria respond to Pi starvation, the whole process has not been fully understood. We now report that bacteria presented enhanced resistance to polymyxin during Pi starvation. We establish that the BasSR TCS were activated by Fe3+ signal and led to up-regulation of downstream arn operon when *E. coli* MG1655 experiences Pi starvation. Such response led to LPS modification and enhanced resistance to polymyxin. Further study proved that Mg2+ were disassociated from outer membrane (OM) during Pi starvation, which caused OM perturbation and charge imbalance. Charge driven iron to associate with OM even with low concentration, and iron together with iron induced modification stabilized OM. Our results proposed a novel environment cue which could activate BasSR TCS and led to enhanced polymyxin resistance. Furthermore, we provided evidence of the connection between Pi, Mg and Fe pools.

T240 - Target recognition nicety of Type III CRISPR-Cas systems

Presenting Author - *Karyna Karneyeva, Skolkovo Institute of Science and Technology, Russian Federation*

Author/s – *Daria Artamonova, Matvey Kolesnik, Konstantin Severinov*

Abstract Content

CRISPR-Cas is prokaryotic immunity that provides a target-specific response against mobile genetic elements (MGE). Six types of CRISPR-Cas systems have been described. Type III system is notable for its sophisticated and multilayer defense organization. The key challenge for its understanding is revealing the principles of target recognition by the effector complex for further MGE destruction *in vivo*. An accurate pattern of crRNA-target complementarity needed for efficient immunity response has not been characterized yet. The main obstacle is three interference activities performed by the Cas10 subunit of the Type III effector. A model organism *Thermus Thermophilus* bearing Type III-A and III-B system subtypes represents an attractive species for CRISPR research. However, adapted and convenient genome editing tools are not available for these thermophilic bacteria. Here, we implemented efficient modifications of known genome editing techniques for *T. Thermophilus*. We obtained a set of mutants with inactivated Cas10 domains of Thermus Type III effectors and describe their roles in CRISPR immunity. We investigated the influence of various mismatches in crRNA-target duplex for Type III interference activities. Along with similarity to the *in vitro* data obtained so far, our results revealed intriguing contradictions. We found that both III-A and III-B systems can tolerate a different number of mismatches at the proposed Cas10-activating regions. Moreover, the activation of both systems highly depends on the level of target production. Additionally, we characterized the ability of *T. Thermophilus* to escape its CRISPR immunity in the case of benefits borne by MGE.

T241 - A pathway from beta-alanine to 3-hydroxypropionate in *A. baumannii*

Presenting Author - *You-Ching Chang, Tzu Chi University, Taiwan*

Author/s – *Guang-Huey Lin*

Abstract Content

Although 3-hydroxypropionate (3-HP) wasn't a common metabolite in most organisms, 3-HP can be used in the production of bioplastics. We found 2-oxogltarate aminotransferase (PydD1) and malonic semialdehyde reductase (PydE) involved in a pathway could metabolize beta-alanine to 3-HP in *A. baumannii*. Additionally, the identity of PydD1 and PydE between *E. coli* and *A. baumannii* were 59.9% and 57.7%, respectively. The aim of this study is to understand the function of the pathway from beta-alanine to 3-HP. As PydE belonged to short chain dehydrogenase (SDR), it increased the biofilm formation and environmental stress which had been reported by previous studies. Results revealed pydE deletion mutants decreased biofilm formation and the survival rate after treated by tert-butyl hydroperoxide. Furthermore, we determined the virulence of PydE by *G. mellonella* killing assay. Results revealed pydE deletion mutants decreased the virulence because malonic semialdehyde accumulated in bacteria. In addition to PydD1, pyruvate aminotransferase (PydD2) could metabolize beta-alanine to malonic semialdehyde with pyruvate as substrate. Coupled enzymatic activity showed PydD1/PydE or PydD2/PydE involved in the pathway could metabolize beta-alanine to 3-HP. The kinetic parameters of PydE showed KM value was 5.3 mM. Compared to previous study, the KM value was 46 mM in *E. coli*. It meant that PydE had high affinity in *A. baumannii*. Finally, PydE agreed with the structure of SDR because they were usually aggregated into polymer. In conclusion, we found a pathway from beta-alanine to 3-HP in *A. baumannii*, and also proved PydE played the role of biofilm formation, stress resistant and virulence.

T242 - A pathway from beta-alanine to acetyl-CoA in *Acinetobacter baumannii*

Presenting Author - *En Chen Liu, Tzu Chi University, Taiwan*

Author/s – *You Cheng Chang, Guang Huey Lin*

Abstract Content

D-alanine was an essential amino acid which played a key role in bacterial cell wall synthesis. According to previous study, there was a beta-alanine metabolism pathway in Bacillales bacteria, which coverted beta-alanine to acetyl-CoA. The by product L-alanine could be converted to D-alanine by racemase. Two enzyme, pyruvate aminotransferase (PydD2) and malonic semialdehyde dehydrogenase (MSDH) were involved in the pathway. Additionally, the identity of PydD2 and MSDH between *Pseudomonas* and *A. baumannii* was 75.1% and 66%, respectively. In our study, we tried to find out the main function of PydD2 and MSDH in *A. baumannii*. Result showed that PydD2 deletion mutant caused cell wall frangible through hydrophobic assay. We also confirmed PydD2 didn't play a role to virulence to *G. mellonella*. Furthermore, the result showed that enzymatic activity of MSDH was decreased in PydD2 deletion mutant by coupled enzymatic assay. The kinetics parameters of PydD2 showed KM value was 9.182 mM and kcat/KM ratio was 389.2 (s-1M-1). Compared to previous study, the KM value and kcat/KM ratio of PydD2 in *Bacillus megaterium* was 13.7 mM and 109 (s-1M-1). This meant that PydD2 had high affinity with beta-alanine and well catalytic efficiency in *A. baumannii*. In conclusion, we ensured that PydD2 and MSDH were involved in beta-alanine metabolism pathway in *A. baumannii*, In addition, this pathway was related to steady of cell wall but didn't connect with virulence. In the future, the MSDH related mutants will be constructed to study the function of beta-alanine metabolism pathway in *A. baumannii* in detail.

T243 - Physiological and Functional roles of Clp protease for re-growth of *Escherichia coli*

Presenting Author - *Miho Yoshimura, Hosei University, Japan*

Author/s – *Hiroyuki Horino, Hikari Yoshitane, Kaneyoshi Yamamoto,*

Abstract Content –

Background: *Escherichia coli* cell in stationary phase stops vigorous growth but proliferates in fresh medium after lag phase, called re-growth. However, it has been unknown how *E. coli* achieves re-growth in the transition from stationary to lag phase. The AAA+ protease is known as key player of the post-translational regulation system of cellular functions. *E. coli* has two AAA+ proteases, Clp and Hsl, consist of two distinct proteins, which might change the protein profile for re-growth.

Objectives: The aim of this study is to reveal how AAA+ proteases are involved in re-growth of *E. coli*.

Methods: We isolated triple-, double- and single-gene mutants of *E. coli* K-12 MG1655 using the developed genome editing technology, Homologous Sequence Integration (HoSeI) method (Miyake & Yamamoto, 2020). The resultant mutants were subject to the growth, the differential proteomics, and fluorescent microscopic analyses.

Results: The lag phase of ΔClp mutant was prolonged whereas ΔHsl started the growth as well as the parent strain in re-growth. Proteome data indicated that deficient of Clp caused the delay of chromosomal segregation and the unstable supply of amino acids and nucleotides in lag phase. Fluorescent microscopic observations revealed that ClpP-GFP localized at a pole in early log phase, diffuse in log phase and localized at pole in stationary phase again. Putative target proteins co-localized with ClpP-GFP at a pole in stationary phase. Taken these results together, cellular localization of Clp was functional and could change profile for re-growth of *E. coli*.

T244 - Role of zinc-containing alcohol dehydrogenase in *Acinetobacter baumannii*

Presenting Author - *Meng Hua Lin, Tzu Chi University, Taiwan*

Author/s – *Guang Huey Lin*

Abstract Content

Alcohol dehydrogenase (Adh) is an essential enzyme present in almost all organisms, and *Acinetobacter baumannii* is no exception. Seven alcohol dehydrogenases (Adh1-Adh7) from *A. baumannii* 19606 were identified in the previous study. There are three zinc-containing Adhs (Adh1, Adh2, and Adh7), three iron-containing Adhs (Adh3, Adh4, and Adh6), and a short-chain Adh (Adh5). Although they are all annotated as adh genes, they have functions beyond metabolizing alcohol. As mentioned in the previous study on iron-containing Adhs, Adh4 is mainly involved in the metabolism of alcohol. Adh3 and Adh6 are involved in environmental stress responses. This study focused on the functional analysis of three zinc-containing alcohol dehydrogenases in *A. baumannii*. Western blotting results showed that among all the Adhs of *A. baumannii* except for Adh5, only iron-containing Adhs and Adh7 can be induced in an environment that contained alcohol. The results of zinc-containing Adhs expressed under different stresses revealed that osmotic stress induced Adh1 and Adh7. Oxidative and heat stress induced Adh7. But Adh2 was not induced by any stress. However, Adh2 was expressed in an environment that contained benzaldehyde and cinnamaldehyde. Analyzing the cell lysate by using thin-layer chromatography (TLC) didn't show that Adh2 can metabolize cinnamaldehyde to cinnamyl alcohol. These results showed that more investigations have to be carried out to reveal the role of Adh1 in *A. baumannii*. Adh2 may be a cinnamyl alcohol dehydrogenase (CAD) in *A. baumannii*, and Adh7 is involved in alcohol metabolism.

T245 - The ISPpu9 insertion sequence of *Pseudomonas putida* KT2440: relevance of the conserved flanking sequences

Presenting Author - *Elena Parés-Guillén, Centro Nacional de Biotecnología (CNB-CSIC), Spain*

Author/s – *Luis Yuste, Fernando Rojo, Renata Moreno*

Abstract Content

Background: Insertion sequences (ISs) have important roles in the evolution of bacterial genomes. Most ISs have inverted repeated sequences flanking the 5' and 3' ends, and insert at sites with little or no sequence specificity. *Pseudomonas putida* KT2440 genome contains seven copies of an IS named ISPpu9, which has no flanking inverted repeats. All seven copies are inserted at sequences named REP, which are highly conserved inverted repeats found mostly in non-coding regions, and present in high numbers in many bacterial genomes.

Objectives: Our aim was unravelling the relevance of the ISPpu9 flanking sequences for target selection and DNA transposition, as well as to characterize the insertion sites when ISPpu9 was introduced into *P. putida* F1, a strain that lacks this IS but has REP sequences similar to those of strain KT2440.

Methods: We first made *In silico* study of the insertion sites, and of the transposon ends, of ISs highly similar to ISPpu9 present at other annotated *Pseudomonas* genomes. The insertion sites of ISPpu9 when introduced into *P. putida* F1 were also characterized.

Results: The *In silico* analysis showed that ISPpu9-like ISs are always inserted at REP-like sequences, and that all of them include highly conserved sequences at their 5' and 3' ends, named A and B boxes, similar to those of ISPpu9. In strain F1, ISPpu9 also inserted at REP sequences. ISPpu9 was observed to generate circles that arise from the cutting and ligation of A and B boxes, and which could be possible transposition intermediates.

T246 - Gene expression of EmaSR regulon

Presenting Author - *Yu-Wen Huang, Tzu Chi University, Taiwan*

Author/s – *Guang-Huey Lin*

Abstract Content

Acinetobacter baumannii is a common pathogen in hospital that regulate several pathways via the two-component regulatory systems (TCSs). In previous study, EmaSR are known to be involved in the ethanol and acetate metabolism in *A. baumannii* ATCC 19606. The RNA-seq showed EmaSR were upregulated DJ41_566-571, DJ41_2796, DJ41_3173-3174, DJ41_3218 and DJ41_3568, which the genes function in carbon metabolism. EmaR can binding with consensus sequence by AAnCTTATnCnnAnnnTTnnCn which analyzed by MEME (Multiple Em for Motif Elicitation). In this study, the genes promoter region with green florescence gene was constructed in an *A. baumannii-E. coli* shuttle vector, pWH1266, to know whether EmaSR regulated these genes. Moreover, DJ41_2796 had higher expression ratio (log2= 6.20) regulated by EmaSR in transcriptome. DJ41_2796 was annotated with the function as acetate: succinate CoA transferase (ASCT), which the substrates of acetate and succinyl-CoA may converted to acetyl-CoA and succinate. To know whether DJ41_2796 that function as ASCT, DJ41_2796 showed the Michaelis–Menten constant (KM) was 39 mM for potassium acetate, which was lower than the homologous enzyme in *Acetobacter aceti*. The result suggested DJ41_2796 was function as ASCT, also to confirm DJ41_2796 have optimum activity in pH 8.0 phosphate buffer and the temperature around 55℃. In conclusion, we demonstrated the DJ41_2796 involved in ethanol metabolism transfer acetate to acetyl-CoA.

T247 - Establishing CRISPR-Cas9 for site specific mutagenesis in *Lactococcus lactis*

Presenting Author - *Melina Piesch, Universität Hamburg, Germany*

Author/s – *Agnes Weiß*

Abstract Content

Background: Clustered, Regularly-Interspaced Short Palindromic Repeats (CRISPR) and their associated enzyme (Cas9) are a widely used tool for mutagenesis like insertion, deletion and point mutations. In recent years the implementation of CRISPR-Cas9 systems in procaryotes has increased. It is an excellent tool for genome editing of chromosomal DNA, but only little research focuses on editing plasmid DNA which encodes auxotroph functions in prokaryotes.

Objectives: In this study aims at establishing CRISPR-Cas9 in *Lactococcus lactis* for site specific mutagenesis of the catalytic triad of a cell enveloped proteinase gene (prtP) of *L. lactis* as an example for an plasmid-encoded gene conveying proteolytic function.

Methods: *In silico*, through biochemical analyses and CRISPR-Cas programs suitable sites for the guide-RNA and the PAM-sequences were identified to establish CRISPR-Cas9 in *L. lactis*. Subsequently, a two-plasmid system with one plasmid carrying a recombinase gene and a another carrying CRISPR-Cas9 was established. Additionally, a donor template was designed to make a three-point-mutation in a single CRISPR-Cas9 event at the catalytic triad of the prtP gene.

Results: One of the major challenges in this study is the target in form of a (mega)plasmid instead of chromosomal DNA due to high sequence variation and varying copy numbers. There are several PAM sites of different qualities in the vicinity of the sequences encoding the active site of prtP which were calculated by CRISPR programs. There need to be more genes integrated in databases of CRISPR programs to evaluate the efficiency of targeting plasmids instead of chromosomal DNA.

T248 - Unequal shiga toxin subunit gene expression in enterohemorrhagic *Escherichia coli* O26:H11 strain HUSEC018

Presenting Author - *Katrin Neudek, University of Hohenheim, Germany*

Author/s – *Prof. Dr. Herbert Schmidt*

Abstract Content

Shiga toxins (Stx) of enterohemorrhagic *Escherichia coli* (EHEC) are AB5 type protein toxins consisting of one enzymatically active A-subunit and a pentamer of non-covalently linked B-subunits. The genes encoding the Stx2 subunits, stxA2 and stxB2, are located in the late-regulated phage region within the genome of lambdoid prophages, downstream of the antiterminator gene Q and upstream of the genes encoding for the phage lysis cassette.

The aim of this study was to quantitatively analyze the relation of the transcription of stxA2 and stxB2. Due to the operon structure of the stx genes, we hypothesized either a 1:1 transcription ratio, or due to the AB5 structure of the toxin, a 1:5 ratio.

To perform quantitative transcriptional analysis, total RNA was isolated and purified from *E. coli* O26:H11 strain HUSEC018. Subsequently, qRT-PCR was performed to determine the transcript levels for stxA2, stxB2, and the housekeeping gene rrsB, which was used as endogenous control.

Surprisingly, stxA2 was expressed approximately two times stronger than stxB2 in *E. coli* strain HUSEC018. The analyzed genes were not expressed in the expected ratios, indicating that free A-subunits might circulate in the bacterial environment which do not find a B-pentamer for holotoxin formation. Further analysis on translational and protein level should help to clarify this phenomenon.

T249 - The SLT domain of the gp15 protein of phage BFK20 has the ability to degrade peptidoglycan

Presenting Author - *Kristína Pápayová, Comenius University Bratislava, Slovakia*

Author/s – *Kristína Pápayová, Gabriela Bukovská, Lucia Bocánová*

Abstract Content

Background: The phage tail functions as a connector during infection of bacterial cell, to provide phage-host connection essential for transfer of phage DNA. Bacteriophages use hydrolytic activity of tail proteins to cross the peptidoglycan layer. Minor tail protein of phage BFK20 – gp15 contains SLT (soluble lytic transglycosylase) domain. SLT proteins are mostly known as bacterial enzymes used in cell wall metabolism and cell division.

Objectives: The aim of this study is to examine the lytic activity of recombinant proteins derived from gp15.

Methods: Overall, 6 different SLT proteins were produced, each containing a region with the SLT domain and different length of regions adjacent to the SLT domain. SLT proteins were expressed using vector pET28a+, in *E. coli* BL21(DE3) cells and were purified by IMAC affinity chromatography. We tested the lytic activities of proteins SLT01, SLT02 and SLT05 using Lysozyme activity assay kit (abcam). The thermal stability was estimated for proteins SLT02 and SLT05 by nanoDSF Prometheus (NanoTemper).

Results: The individual recombinant proteins were expressed with variable level of expression. The proteins SLT01, SLT02 and SLT05 were expressed in soluble form and with high yield. The proteins, SLT03, SLT04 and SLT06 were expressed weakly with non-sufficient purity. The protein SLT05 had the highest lytic activity, corresponding with lytic activity of lysozyme. The SLT02 and SLT05 proteins were stable in all measured pH conditions (pH 5.0 – 8.4). Highest inflection points were 47.6°C for SLT02 and 45.9°C for SLT05.

T250 - Intestinal colonization of carbapenem-resistant Enterobacteriaceae causes endogenous Bacteremia in immunocompromised children

Presenting Author - *Nasim Almasian Tehrani, University Of Tehran, Iran, Islamic Republic of*

Author/s – *Masoud Alebouyeh, Leila Azimi, Mehrzad Sadredinamin, Kianoush Khashayar, Neda Soleimani, Shahnaz Armin, Somayeh Delfani*

Abstract Content

Background: Carbapenem-resistant Enterobacteriaceae (CRE) infection is life-threatening, especially for immunocompromised children. Source tracking of CRE could prevent bacteremia during hospitalization.

Objective: In this study, intestinal colonization of CRE and their translocation were source tracked in immunocompromised children.

Methods: Stool of immunocompromised pediatrics was collected after admission, secondary stool and blood were collected in case of fever. After CRE phonotypic detection, OXA-48, NDM-1, VIM, IMP, and KPC genes were detected by PCR. ERIC-PCR was used to determine phylogenic relatedness of the blood and fecal isolates.

Results: Bacteremia was recorded in 71.4% of the patients. Enterobacteriaceae spp. were recorded in 100% of the stool and 31% of the blood samples. Antimicrobial susceptibility testing confirmed them as CRE. The correlation between LOS, day of fever, chemotherapy regimens, and death rate between patients with and without CRE bacteremia was significant ($P \leq 0.05$). OXA-48 was present in all the primary, secondary stools, and the blood CR E isolates. According to phylogenetic data, 58.33% of the patients had identical blood and stool isolates. The death rate was 24.4% in children with CRE bacteremia.

Conclusion: Primary intestinal colonization of CRE in immunocompromised pediatrics and their translocation to blood have been established in this study for the first time. CRE bacteremia had an endogenous source in 85.71% of these patients. Owing to the increased risk of mortality and dominance of CRE in the intestine of immunocompromised children, attention to infection prevention and control policies should be paid in the chemotherapeutic and transplantation units.

T251 - RofA-family transcriptional regulator, GadR, controls expression of the glutamate decarboxylase system in *Listeria monocytogenes*

Presenting Author - *Jialun Wu, University Of Galway, Ireland*

Author/s – *Jialun Wu, Olivia McAuliffe, Conor O'Byrne*

Abstract Content

The highly conserved GadT2D2 glutamate decarboxylase system, which comprises the decarboxylase GadD2 and the Glu/GABA antiporter GadT2, is a key determinant of acid resistance in food-borne pathogen *Listeria monocytogenes*, although earlier studies showed that it plays a more significant role in some strains than in others. In this study, we identified a truncation in a gene encoding RofA-like transcription regulator in an acid sensitive food isolate 1381. Survival, transcriptional, and biochemical experiments carried out in strain 1381, reference strain 10403S and their derivatives (e.g. ΔgadR, ΔgadT2D2, and ΔgadT2D2R) suggested that this gene controls the expression of its adjacent operon gadT2D2 and acid resistance and therefore, this gene was designated gadR. Bioinformatic analysis revealed that gadT2D2R as gene cluster is conserved in Listeria sensu strictu spp. but not in Listeria sensu lato spp., which suggested that gadT2D2R might play a role in colonisation of the gastrointestinal tract since only the sensu strictu species are associated with this niche. The presence of a premature stop codon in the gadR gene of the commonly studied EGD-e strain was also shown to explain its comparatively acid-sensitive phenotype. Acid stress adaption experiments demonstrated that GadR mediated gadT2D2 transcription is rapidly and continuously induced by acid stress (peaking at pH 5). Adaption at pH 5.0 also results in a strong GadR mediated adaptive acid tolerance response, which is SigB-independent. Taken together, we report a previously undocumented regulatory mechanism that is of primary importance to the adaptive acid tolerance response of the deadly food-borne pathogen *L. monocytogenes*.

T252 - Recovered persister cells broadcast regrowth initiation to surrounding kin

Presenting Author - *Sofie Louwagie, KU Leuven, Belgium*

Author/s – *Sofie Louwagie, Dorien Wilmaerts, Natalie Verstraeten, Jan Michiels*

Abstract Content

Effective treatment of bacterial infections is often hampered by the presence of a fraction of cells that temporarily display reduced antibiotic susceptibility. Termed persisters, these cells are genetically identical to their antibiotic-sensitive kin comprising the majority of the population. Bacterial cells can switch back and forth between these phenotypic states. The switch from persister to antibiotic-sensitive cell, called persister recovery, is accompanied by regrowth. Recovery after discontinuation of an antibiotic treatment allows persister cells to re-establish a bacterial population, resulting in a relapse of infection. Triggering persister recovery during treatment represents a promising approach to eliminate persister cells and, consequently, prevent reinfection. Two main processes are known to contribute to persister recovery: repair of damage caused by the antibiotic and reversal of the physiological changes induced by the effectors of persister formation. Unfortunately, intensive research on the mechanisms involved is still wanting.

Using single-cell time-lapse microscopy, we have identified communication between persister cells of *Escherichia coli* as a third factor contributing to persister recovery. Our data show that the distance between persister cells is correlated with regrowth lag. In addition, the variation in regrowth lag correlates with the fraction of persister cells. These results suggest that persisters communicate with each other through a diffusible signal. Indeed, treating with spent-medium results in an increased number of persister cells and a decrease in distance-dependent recovery and lag times. Identifying the signal and mechanisms involved in communication between persister cells can signify an important step in the search for persister recovery drugs.

T253 - VapC-1 toxin from the leptospiral vapbc-1 toxin-antitoxin module displays ribonuclease activity and affects cell viability

Presenting Author - *Deborah Kohn Damiano, University Of Sao Paulo, Brazil*

Author/s – *Bruna Oliveira Pigatto Azevedo, Alexandre Paulo Yague Lopes*

Abstract Content

Background: Human leptospirosis in Brazil is mainly caused by *Leptospira interrogans* serovar Copenhageni. Toxin-Antitoxin (TA) systems code for a toxin and an antitoxin and are considered an important survival mechanism during stress. In regular environmental conditions, the antitoxin blocks the toxin, however, during imbalanced conditions antitoxin concentration decreases, resulting in cell exposure to a range of toxic events, with growth arrest being the most common, therefore TA are generally described as active as a function of bacterial growth kinetics. VapBC is a type II TA system, in which VapC is predicted to display ribonuclease activity. Using TADB database we designated four TA modules*L. interrogans* serovar Copenhageni.

Objectives: To biochemically and functionally characterize the VapBC-1 module and evaluate VapC-1 activity.

Methods: Proteins were obtained by conventional methods. Toxin and antitoxin interaction was tested by pull-down assay. *E. coli* growth kinetics, CFU count and ribonuclease activity assays were used to test activity.

Results: The affinity between toxin and antitoxin was demonstrated by co-purification of VapB-1 and VapC-1. Expression of the toxin did not decrease the culture's optical density, however, interestingly, cells viability studies via CFU count showed a decrease of more than 100-fold in viable cells 2h after expression, comparing to complexed toxin-antitoxin. We are currently working on preliminary indications of bacterial morphological changes. RNAse activity assays showed that VapC-1 cleaves MS2 RNA. Together, our results indicate that the VapBC-1 module is functional, and the attribution of functionality to TA modules cannot be defined by the inhibition of bacterial growth.

T254 - VapBC-4 Toxin-Antitoxin is a new active module of *Leptospira interrogans* serovar Copenhageni

Presenting Author - *Bruna Oliveira Pigatto Azevedo, University Of Sao Paulo, Brazil*

Author/s – *Deborah Kohn Damiano, Alexandre Paulo Yague Lopes*

Abstract Content

Background: *Leptospira interrogans* serovar Copenhageni is accountable for the majority of leptospirosis cases in humans in Brazil. Toxin-antitoxin (TA) systems are spread in bacterial genomes and are considered as adaptation modules to unfavorable conditions. The VapBC family is the predominant among type II TA, encoding two proteins, the stable toxin (VapC) and the unstable antitoxin (VapB). The toxin is grouped due to the homology of a PIN domain of the toxin that acts as endoribonuclease. Based on *In silico* analyzes our group designated four VapBC modules coded in *L. interrogans* serovar Copenhageni genome.

Objectives: To evaluate functionality and to characterize the VapBC-4 TA module.

Methods: The systems' components were cloned into pET28a and expressed in *E. coli* BL21(DE3). Recombinant proteins were renatured from inclusion bodies and purified by immobilized metal affinity chromatography. VapB and VapC interaction was tested by pull-down assay, dot blot and ELISA. Activity was tested by *E. coli* growth kinetics and by ribonuclease assay.

Results: The toxic effect of VapC-4 was confirmed by inhibition of bacterial growth, which is restored by the expression of the antitoxin VapB-4. The affinity between the toxin and the antitoxin was demonstrated *in vivo* and *in vitro*. VapC-4 showed activity towards MS2 RNA substrate. Presently, we are working on possible morphological changes of cells expressing the toxin in contrast of antitoxin and the complex. VapC-4 3D structure resembles VapC from *Shigella flexneri*. Studies on the functionality of new TA systems are important for understanding TA's functions in bacteria.

T255 - Characterization of PA14_RS04555 gene in *Pseudomonas aeruginosa* associated with persistent cystic fibrosis lung infections

Presenting Author - *Valerio Baldelli, University Of Milano, Italy*

Author/s – *Valerio Baldelli, Stacy Julisa Carrasco Aliaga, Aditi Shenoy, Helle Krogh Johansen, Søren Molin, Arne Elofsson, Moira Paroni*

Abstract Content

Background: *Pseudomonas aeruginosa* infections are difficult to treat primarily due to the ability of this bacterium to adapt to the host environment and withstand antimicrobial treatments. Hence, there is a strong need to identify new *P. aeruginosa* persistence mechanisms that can be exploited for novel treatment development.

By analysing *P. aeruginosa* gene expression of chronically infected patients, we observed that *P. aeruginosa* populations share a common transcriptional program in the lungs. Several identified genes are poorly characterized and might represent persistence determinants, thus potential targets for novel antimicrobial strategies.

Objectives: The aim of this study is to characterize one of these genes, PA14_RS04555, which is homologous to the *Salmonella enterica* sirB gene in which controls virulence.

Methods: *In silico* promoter analysis, molecular genetics and biochemical approaches were used to deciphering the PA14_RS04555 gene regulation. The generation of *P. aeruginosa* PA14 knockout mutants allowed to investigate the role of PA14_RS04555 on phenotypes known to be essential for *P. aeruginosa* persistence (i.e. biofilm formation, *in vitro* and *in vivo* virulence assays).

Results: The *P. aeruginosa* virulence regulator Vfr was identified as the PA14_RS04555 transcriptional repressor. The lack of PA14_RS04555 leads to an increase of both *P. aeruginosa* virulence and biofilm. These results, combined with the increased production of the second messenger c-di-GMP in the PA14_RS04555 knockout mutant, suggest that PA14_RS04555 globally modulates *P. aeruginosa* pathogenicity altering the c-di-GMP signalling pathway. Experiments in different media and conditions are in progress to better deciphering the stimuli and the pathways involved in PA14_RS04555 functionality.

T256 - Gastroprotective effects of *Lactiplantibacillus plantarum* LB 1020 via PI3K/Akt/mTOR expression

Presenting Author - *Kiyoung Kim, Chung-Ang University, Republic of Korea*

Author/s – *Jong-Hwa Kim, Wonyong Kim*

Abstract Content

Background: *Helicobacter pylori* (*H. pylori*) activates signaling factors associated with inflammation and this may lead to gastric mucosa damage. Among these, PI3K/Akt/mTOR signaling is critical role in inflammation and carcinogenesis.

Objectives: We evaluate gastroprotective effects of *Lactiplantibacillus plantarum* LB 1020 (*Lb. plantarum* LB 1020) using AGS cells.

Methods: AGS cells were treated with *Lb. plantarum* LB 1020 for confirmed whether *Lb. plantarum* LB 1020 inhibit PI3K/Akt/mTOR signaling expression. Anti-bacterial activity of *Lb. plantarum* LB 1020 was evaluated using minimum inhibitory concentrations test. AGS cells were treated with *H. pylori* and *Lb. plantarum* LB 1020 or only *H. pylori*. Expression of inflammatory and carcinogenesis genes was quantified by real-time PCR.

Results: Treatment of *Lb. plantarum* LB 1020 reduced expression levels of cancer-mediated genes such as Bcl-2, Bcl-xL and RTK by suppressing PI3K/Akt/mTOR signaling pathway in AGS cells. *Lb. plantarum* LB 1020 showed anti-bacterial activity against *H. pylori*. *H. pylori* infection activated inflammation and carcinogenesis related factors expression in AGS cells. However, treatment of *Lb. plantarum* LB 1020 inhibited the level of inflammatory factors containing TNF-α, IL-1b and NF-κB. Moreover, expression of integrin α5 and Integrin β1 involved in carcinogenesis was decreased, but expression of MUC5 involved in mucosal protection was increased by treatment of *Lb. plantarum* LB 1020.

T257 - Defining a new pathway for D-amino acid catabolism in *Pseudomonas putida*

Presenting Author - *Ronnie Fulton, University Of Georgia, United States*

Author/s – *Ronnie Fulton, Diana Downs*

Abstract Content

Background: In *P. putida*, PP_2246 is annotated as a homolog of the D-arginine oxidase, DauA, of *P. aeruginosa*. The genomic contexts of these presumed homologs differ, suggesting divergent roles. The locus in *P. putida* (named dbu herein) does not encode the additional oxidase, DauB, that is required for D-arginine utilization by *P. aeruginosa*. The dbu locus encodes a putative oxidase, Rid superfamily protein, transporter, and a regulator that together could form an uncharacterized D-amino acid catabolic pathway.

Objectives: This work was initiated to characterize the genes in the dbu locus and test the hypothesis that they comprise an uncharacterized D-amino acid catabolic pathway.

Methods: A biochemical-genetic approach was used to identify the function of each gene product encoded in the dbu locus of *P. putida*. Standard genetic tools were used to assess phenotypic consequences of mutations in each gene. The analyses include nutritional studies, biochemical assays and experiments to determine competitive fitness. Competitive fitness will be measured using fluorescent labelling and flow cytometry.

Results: Gene products in the dbu locus of *P. putida* are involved in the catabolism of D-branched chain amino acids. Data suggest the pathway is comprised of a transcriptional regulator (PP_2245/DbuR), D-amino acid oxidase (PP_2246/DbuA), Rid protein (PP_2247/DbuB), and a transporter (PP_2248/DbuC). Strains lacking DbuA are able to catabolize D-arginine, but not D-leucine or D-valine. Additionally, a plasmid expressing DbuA complimented phenotypes of *P. aeruginosa* dauA, suggesting overlap in activity. Continuing experiments aim to confirm roles for these proteins in the D-BCAA catabolic pathway of *P. putida*.

T258 - Purine and thiamine metabolism impacts the effect of a yggS mutation in *Salmonella enterica*

Presenting Author - *Kailey Ezekiel, University Of Georgia, United States*

Author/s – *Diana Downs*

Abstract Content

Background: The active form of vitamin B6, pyridoxal 5'-phosphate (PLP), is a cofactor of numerous metabolic enzymes in all domains of life. YggS (COG0325) is a highly conserved PLP-binding protein that has been implicated in B6 vitamer homeostasis. The loss of yggS homologs results in pleiotropic effects in a variety of organisms, and variants of this protein in humans (PROSC) are associated with B6-dependent epilepsy. In *Salmonella enterica*, yggS mutants accumulate PLP in their growth medium. We use S. enterica as a model to understand the biochemical function of this conserved protein family.

Objectives: This work was initiated to generate insights on the biochemical function of YggS and its role *in vivo*.

Methods: A pool of insertion mutations was screened for those that reduced or eliminated the accumulation of PLP in spent medium of a yggS mutant. Nutritional studies, sequencing, and genetic analyses are used to dissect the mechanism by which these mutations suppress PLP accumulation.

Results: Two suppressor mutations that reduced PLP accumulation in the medium of a yggS mutant were chosen for characterization. The first compromises purine biosynthesis and subsequent experiments determined that purine limitation was responsible for the suppressing effect. The second mutation generates a requirement for the thiazole moiety of thiamine. Current studies focus on the role of purines, thiamine, and amino acids in the accumulation of PLP in spent medium of yggS mutants. These studies will determine the molecular mechanism of suppression and provide insights into the metabolic function of YggS.

T259 - Physiology of trans-translation deficiency in *Bacillus subtilis*

Presenting Author - *Melissa Vázquez Hernández, Ruhr University Bochum, Germany*

Author/s – *Julia Bandow, Kenneth Keiler, Stephanie Leedon*

Abstract Content

Trans-Translation is a crucial process in bacterial physiology, responsible for the recycling of stalled ribosomes during protein synthesis. Due to its importance, it has become a target for new antimicrobials. However, not all bacteria rely solely on trans-translation for ribosome rescue. The model organism *Bacillus subtilis*, for example, has evolved alternative mechanisms that utilize the proteins BrfA or RqcH. In this study, we aimed to gain a deeper understanding of the role of trans-translation in the physiology of *B. subtilis* by examining the effects of its deficiency on growth, protein synthesis, and cellular behaviour. Using a gel-free label-free quantitative proteomics approach, we compared the proteomes wild type *B. subtilis* 168 and its trans-translation deficient strain (ΔssrA) during mid-log phase. We found that the growth rate of the ssrA deletion mutant was 20% lower in a chemically defined medium than that of the wild type. Additionally, protein synthesis rates were lower in the ΔssrA strain. Interestingly, in the mutant proteome, we observed an overrepresentation of ribosomal proteins and down-regulation of precursor supply in the mutant proteome, indicating a slowed ribosome recycling. Furthermore, the ΔssrA strain showed an altered motility and chemotaxis phenotype. Our findings provide new insights into the role of trans-translation in bacteria and its importance for growth and protein synthesis.

T260 - Intestinal microbiome variability during the production lifespan of two high-yielding laying hen breeds

Presenting Author - *Christoph Roth, University of Hohenheim, Germany*

Author/s – *Christoph Roth, Tanja Sims, Jana Seifert, Amélia Camarinha Silva*

Abstract Content

Intestinal microbiota is involved in nutrient digestion, pathogen inhibition, endocrine activity, and interaction with the intestine-associated immune system. The intestinal microorganisms constantly adapt to the intestinal morphological changes and birds growth. In hens, little is known regarding the microbiota shifts during the bird's lifespan and across the gastrointestinal tract (GIT). This study aimed to characterise the active intestinal microbiota of two breeds: Lohmann Brown-Classic and Lohmann LSL-Classic, during their productive life span. All birds were kept under the same diet and housing conditions. Crop, gizzard, duodenum, ileum, and caeca digesta were collected after 10, 16, 24, 30, and 60 weeks of life to represent the whole production period. 500 samples were analysed by target amplicon sequencing and a subset with shotgun metagenomics. Phylogenetic analysis of the bacterial sequences was assessed using Mothur, followed by multivariate statistical analysis. Shotgun metagenomic data was processed SqueezeMeta pipeline. A statistical significance was observed for the breed, GIT section, age and the combination of all factors ($p<0.05$). A genera abundance fluctuation depended on the breed, GIT section or production periods. A significant shift in the active microbiota was observed in early life and with the onset of laying between weeks 16 and 24. Functional profiling showed differences between the breeds with up- and down-regulated functions at the start of the laying phase. Breed and the onset of egg production were the drivers of intestinal microbiota dynamics.

T261 - *Escherichia coli* differ in virulence-associated genes between patients with colorectal neoplasia and healthy controls

Presenting Author - *Juraj Bosak, Masaryk University, Czech Republic*

Author/s – *Darina Kohoutová, Matěj Hrala, Paula Morávková, Stanislav Rejchrt, Jan Bureš, David Šmajs*

Abstract Content

Background: Pathogenic strains of *Escherichia coli* have been clearly identified as the causative agents of extraintestinal and diarrheal infections; however, the etiopathogenic role of *E. coli* in other conditions, including colorectal cancer, remains unclear. In our previous study, we found an increased prevalence of bacteriocin-producing *E. coli* strains in biopsies from patients with current or previous colorectal neoplasia.

Objectives: Since several bacteriocins were described as potential virulence factors, the aim of this follow-up study was to characterize mucosal *E. coli* isolates for the presence of genetic determinants encoding known virulence factors.

Methods: We analyzed mucosal *E. coli* isolates (n=246) for the presence of 35 known virulence-associated genes using multiplex-PCR.

Results: Virulence determinants encoding S-fimbriae (sfa), siderophore receptor (iroN), invasin (ibeA), and genotoxin (usp) were more prevalent among *E. coli* isolated from patients with neoplasia compared to the control group ($p < 0.05$). In addition, the prevalence of virulence determinants usp, sfa, iroN, and ibeA were increased in more advanced neoplasia stages ($q < 0.0125$). Half of the patients with current and previous neoplasia had *E. coli* strains with at least one of the abovementioned virulence factors, and combinations of these virulence factors were also common. Moreover, the prevalence of bacteriocins appears to correlate with strains harboring identified combinations of virulence factors. These findings suggest that *E. coli* strains isolated from patients with colorectal neoplasia possess several virulence factors, which could contribute to the development of neoplastic processes in the large intestine.

T262 - Application of direct bacterial 16S ribosomal RNA polymerase chain reaction on culture-negative clinical samples

Presenting Author - *Lily Shui Kuen Cheng, United Christian Hospital, Hong Kong*

Author/s – *Irene Lai Yan Lam, Barry Kin Chung Wong, Sandy Ka Yee Chau*

Abstract Content

Background: Early identification of bacterial pathogens is crucial in patient management by allowing timely administration of appropriate antibiotics. However, not all specimens collected from sites of infection are culture-positive, even with the advent of well-established culture system.

Objectives: To evaluate the performance of bacterial 16S ribosomal RNA (rRNA) polymerase chain reaction (PCR) directly from diverse clinical specimens in culture-negative patients who were assessed to have possible bacterial infections by microbiologists in clinical consultations.

Method: Requests for 16S rRNA PCR by microbiologists between April 2018 and March 2023 were retrieved from the laboratory records. Only the 16S rRNA PCR directly on culture-negative clinical samples from normally sterile sites, except serum, were included. A normally sterile site is defined as cerebrospinal fluid, peritoneum, pleura, bone, joint, or other internal body site. The analysis of 16S rRNA gene sequence, reports issued after clinical interpretation, and impacts on patient management were reviewed.

Results: A total of 288 samples from 202 patients were included. Fifty-eight (20.1%) samples from 48 (23.8%) patients were PCR positive, among which 49 (84.5%) gene sequencing met the 97% identity threshold and helped identify pathogens of clinical relevance. The most prevalent sequence-positive sample types were heart valve tissue (100%), liver and brain abscesses (50%), infective collection (30%) and pleural fluid (22.6%). Most bacteria identified (67.3%) were gram-positive cocci. The organism detection rate in samples with positive gram stains yet negative culture was 63.3%. The antibiotic regimes were changed in 61.2% of patients according to positive 16S rRNA results.

T263 - *Escherichia coli* piPolB promotes persister survival upon exposure to DNA crosslinking genotoxic agents.

Presenting Author - *Juan Jose Arredondo, Universidad Autonoma De Mdarid, Spain*

Author/s – *Diego Duarte-Zara, Esmeralda Solar-Venero, Irene Díaz-García, Modesto Redrejo-Rodríguez*

Abstract Content

Primer-independent PolBs (piPolBs) are a group of DNA polymerases belonging to the PolB family. These proofreading replicases are also endowed with translesion synthesis and DNA primase activities. Encoded in Mobile Genetic Elements called pipolins, which are present in Gram-positive and negative bacteria as well as in some fungi mitochondria, piPolBs are the only conserved gene among these elements. The lack of antimicrobial resistance genes in pipolins suggests that piPolB could provide some survival advantage.

Accordingly, insults with DNA crosslinking agents can stimulate its expression in *Escherichia coli*. To test a putative piPolB role in resistance or persistence against these agents, we constructed a piPolB deficient mutant. ΔpiPolB and wt strains are equally sensitive to treatment with genotoxic compounds. Nevertheless, the wt strain produced a higher number of surviving persisters than the ΔpiPolB mutant upon exposures to mytomicin C or mustine, two DNA crosslinking agents. Furthermore, no difference was observed when other genotoxic agents were used. Recombinant piPolB expression in a mutant background rescued persister survival while the surviving progeny showed similar sensitivity to genotoxic insult as the parental strains, demonstrating them to be true persisters. Our results show that pipolins contribution to bacterial survival to genotoxic insults is piPolB dependent. We hypothesize primer independent polymerases might collaborate with endogenous repair and/or damage tolerance systems, favouring persistent cells survival to DNA crosslinking agents.

T264 - Insights into piPolBs encoding elements mobilization dynamics.

Presenting Author - *Esmeralda Solar-Venero,* U*niversidad Autónoma De Madrid, Spain*

Author/s – *Modesto Redrejo-Rodríguez*

Abstract Content

Mobile genetic elements (MGEs) are important features of prokaryotic genomes, impacting bacterial metabolism, behavior, and pathogenicity. Pipolins are a recently discovered group of MGEs that encode a novel type of replicative DNA polymerase from family B (primer-independent PolBs or piPolBs) with de novo DNA synthesis capacity. Pipolins are often integrated into bacterial genomes or exist as episomal plasmids in some bacteria groups and mitochondria. Integrative pipolins encode for one or more integrases of the tyrosine recombinase family (Y-Recs). This study aims to investigate the mobilization dynamics of pipolins in diverse pathogenic strains of *Escherichia coli*.

To evaluate Pipolin excision, a PCR-based method was employed to detect circular pipolins and the corresponding excision scars in the genome. The episomal form was detected in all pipolin-harboring strains analyzed.

The role of Y-Recs in excision was furtherly assessed by constructing mutant strains of *E. coli* 3-373-03_S1_C2 pipolin's recombinase genes. The integrated and circular forms of pipolin were mapped by PCR in these strains, and the excision was quantified via qPCR. Strikingly, circular pipolins were detected in the wild-type strain and in the Y-Recs deletion mutants, downplaying the role of the recombinases in the pipolin mobilization under the assayed conditions.

Molecular mechanisms underlying pipolin integration were further evaluated and the expression of Y-recombinases was tested using RT-qPCR in response to different stimuli, including SOS-response inducing compounds. In all, our results shed light on the mechanisms driving pipolin mobilization and the role of their encoded recombinases, while also raising new questions regarding their biological function.

T265 - Hot and cold – the conformational dynamics of the periplasmic chaperone SurA and their link to SurA activity

Presenting Author - *Fabian Renschler, University Hospital and Faculty of Medicine Eberhard Karls University Tübingen, Germany*

Author/s – *Thales Kronenberger, Janes Krusche, Niklas Bohn, Sarah Akinci, Erwin Bohn, Monika Schütz*

Abstract Content

SurA, the main periplasmic chaperone of Gram-negative bacteria, shuttles unfolded outer membrane proteins (OMP) from the inner membrane SEC translocon to the outer membrane (OM). Due to SurA's central role in OMP biogenesis, it is crucial for virulence and OM integrity (1-3). SurA consists of three domains: NC-core and peptidyl-prolyl-isomerase domains PPI1 and PPI2, of which NC-core and PPI1 bind client OMPs (4,5). A distinct role of PPI2 is elusive but might be linked to an interaction with the beta-barrel assembly machinery inserting OMPs into the OM (6-8). Investigations of the SurA conformational landscape revealed a compact but flexible domain arrangement (4,5,8-10).

Hence, we aimed to investigate the link between PPI2 conformations observed *in silico* and SurA activity *in vitro*.

We created Alanine substitutions of residues involved in the PPI2:NC-core interface weakening this interaction. Activity of these SurA variants was evaluated using a novel SurA activity assay. This assay uses Luciferase (Luc) as a substrate to probe SurA holdase activity. Luc is partially unfolded in the presence of SurA by gentle heating. Active SurA prevents the refolding of Luc upon lowering the temperature. This results in a reduced luminescence signal once ATP and Luciferin are added. If SurA is inactive, Luc can refold and a high signal is observed.

By destabilizing the PPI2:NC-core interaction we were able to increase SurA activity *in vitro* . This is the first hint of the allosteric influence of PPI2 on SurA activity. We now seek to further verify these findings using living bacteria.

T266 - Mechanistic studies of the molecular response to O2 by the fumarate-nitrate reduction regulator (FNR) in Gram-negative bacteria

Presenting Author - *Eve de Rosny, Institut De Biologie Structurale, France*

Author/s – *Anne Volbeda, Juan C. Fontecilla-Camps, Roman Rohac, Fabien Chenavier*

Abstract Content

Fumarate nitrate reduction regulator (FNR) is a global regulator of facultative anaerobes. In addition to its role in modulating the switch between aerobic and anaerobic metabolism, it regulates virulence gene expression during host infection by pathogenic bacteria. FNR belongs to the dimeric CRP family, and contains one [4Fe-4S] cofactor per subunit. In Gram(-) bacteria, it modulates DNA binding through a monomer-dimer equilibrium, which depends on the integrity of the [Fe-S] clusters. Anaerobically, [4Fe-4S]-FNR binds to target DNA sequences and controls gene expression. Under O2, the cluster progressive disassembles into [2Fe-2S] species, leading to protein monomerization and its dissociation from DNA. Our laboratory is equipped with a series of anaerobic glove boxes, custom-made for bacterial growth, protein purification, crystallization and rapid kinetic studies. In 2015, we published the crystal structure of [4Fe-4S]-FNR, and postulated an O2-dependent signal propagation mechanism. More recently we have compared X-ray data set of [4Fe-4S]-FNR after different O2 exposure times, and have detected intermediates of the cluster degradation toward the [2Fe-2S] form. This work was combined with the analysis of FNR mutants, selected from both our FNR structure and previous data. Variants with altered O2 sensitivity were identified by kinetic measurements of cluster degradation, and phenotypically characterized, especially *in vivo* with reporter gene assays. Our recent results provide new insights on a possible molecular mechanism of signal transmission from the FNR [4Fe-4S]-binding site to an essential salt bridge found at the dimer interface, 15 Å away.

T267 - First application of reverse vaccinology on *Burkholderia* spp.: identification and characterization of antigen candidates

Presenting Author - *Samuele Irudal, University of Pavia, Italy*

Author/s – *Samuele Irudal, Viola Camilla Scoffone, Gabriele Trespidi, Giulia Barbieri, Silvia Buroni,*

Abstract Content

Background: *Burkholderia cepacia* complex (Bcc) bacteria can colonize immunocompromised, hospitalized and people suffering from Cystic Fibrosis. Since they are naturally resistant to antibiotics, empirical treatments are often unsuccessful. Considering the limitations in developing new antibiotics, vaccination could be used to prevent diffusion of resistant strains and protect fragile patients.

Objectives: As no vaccine for Bcc bacteria is available yet, we decided to apply the reverse vaccinology approach to search for antigen candidates.

Methods: The genomes of 16 Bcc strains were compared and, by applying specific criteria related to sequence conservation, extracellular or outer-membrane localization, immunogenicity, and function, a short-list of 24 protein coding genes was obtained. Three protein candidates -BCAL1524, BCAM0949, and BCAS0335- were selected for further characterization; their localization in the Outer Membrane Vesicles was assessed and different virulence-related phenotypes of the corresponding deletion mutants were investigated.

Results: While none of the proteins studied affected bacterial growth, BCAL1524, a collagen-like protein, promoted bacterial auto-aggregation and virulence in *Galleria mellonella*. BCAM0949, an extracellular lipase, mediated piperacillin resistance, biofilm formation in LB and Artificial Sputum Medium, rhamnolipid production, and swimming motility; its lipolytic activity was also assessed. BCAS0335, a trimeric autotransporter adhesin, promoted minocycline resistance, biofilm organization in LB, and virulence in *G. mellonella*. Each phenotype could be reverted by complementation, thus validating the role of each protein in virulence. Based on these promising results, additional experiments will be fundamental to evaluate the immunogenicity of these antigen candidates in a mouse model.

T268 - *Helicobacter pylori* employs a general protein glycosylation system for the modification of outer membrane adhesins

Presenting Author - *Mou-Chieh Kao, Taiwan*

Author/s – *Kai-Wen Teng, Kai-Siang Hsieh, Hong-Lin Chan, Wen-Ching Wang, Deng-Chyang Wu, Shau -Ku Huang, Chun-Hung Lin, Ji-Shiuan Hung, Chun-Jen Wang*

Abstract Content

Helicobacter pylori infection is associated with the development of several gastric diseases including gastric cancer. To reach a long-term colonization in the host stomach, *H. pylori* employs multiple outer membrane adhesins for binding to the gastric mucosa. However, due to the redundancy of adhesins that complement the adhesive function of bacteria, targeting each individual adhesin alone usually achieves nonideal outcomes for preventing bacterial adhesion. Here, we report that key adhesins AlpA/B and BabA/B in *H. pylori* are modified by glycans and display a two-step molecular weight upshift pattern from the cytoplasm to the inner membrane and from the inner membrane to the outer membrane. Nevertheless, this upshift pattern is missing when the expression of some enzymes related to lipopolysaccharide (LPS) biosynthesis, including the LPS O-antigen assembly and ligation enzymes WecA, Wzk, and WaaL, is disrupted, indicating that the underlying mechanisms and the involved enzymes for the adhesin glycosylation are partially shared with the LPS biosynthesis. Loss of the adhesin glycosylation not only reduces the protease resistance and the stability of the tested adhesins but also changes the adhesin-binding ability. In addition, mutations in the LPS biosynthesis cause a significant reduction in bacterial adhesion in the *in vitro* cell-line model. The current findings reveal that *H. pylori* employs a general protein glycosylation system related to LPS biosynthesis for adhesin modification and its biological significance. The enzymes required for adhesin glycosylation rather than the adhesins themselves are potentially better drug targets for preventing or treating *H. pylori* infection.

T269 - Targeting urocanate reductase to block imidazole propionate production: a potential therapeutic approach for type 2 diabetes mellitus

Presenting Author - *Hyunji Park, Pohang University of Science and Technology, Republic of Korea*

Author/s – *Na-Young Park, Ga Eun Ryu, In-Young Chung, Ara Koh*

Abstract Content

The gut microbiota can affect host health and disease by producing a variety of bioactive metabolites that can enter the systemic circulation. Recent studies have indicated that imidazole propionate (ImP), a histidine-derived metabolite, impairs glucose tolerance and insulin signaling. In addition, patients with type 2 diabetes mellitus exhibit increased levels of circulating ImP, implying that ImP may play a causal role in the onset of the disease. Despite the potential adverse impact of ImP on health, little attention has been given to exploring pharmacological interventions for regulating its production. In this study, we developed a urocanate reductase (UrdA)-lux reporter system to identify compounds that can decrease the expression of UrdA, a bacterial enzyme that mediates ImP production. We identified four compounds that reduce UrdA promoter activity, consequently inhibiting the production of ImP. Based on our findings, we propose that regulating microbial UrdA expression to inhibit ImP production could be a potential therapeutic approach for the prevention and treatment of type 2 diabetes mellitus.

T270 - High prevalence of heterogeneous vancomycin-intermediate *Staphylococcus aureus* in methicillin-resistant *S. aureus* and its impact

Presenting Author - *Mengting Chen, Institute of Antibiotics, Huashan Hospital, Fudan University, China*

Author/s – *Yaxin Fan, Jing Zhang*

Abstract Content

Background: Vancomycin remains the first-line agent for the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infections, but inappropriate use may lead to the emergence of vancomycin-intermediate *S. aureus* (VISA) or heterogeneous VISA (hVISA), whose prevalence has been increasing in recent years. We aimed to assess the prevalence and epidemiological characteristics of hVISA strains in MRSA pathogens, and then to evaluate the impact of hVISA on vancomycin treatment.

Methods: A total of 200 MRSA strains were collected in prospective, multicenter clinical studies conducted from February 2012 to June 2020, and these strains were subjected to vancomycin minimal inhibitory concentration (MIC) determination and population analysis profiling (PAP-AUC) for hVISA screening. Whole-genome sequencing of the hVISA isolates was performed. The collected strains were divided into hVISA and vancomycin-susceptible *S. aureus* (VSSA) groups to compare the clinical and microbiological efficacy of hVISA on vancomycin treatment.

Results: Out of the 200 MRSA strains, 113 (56.5%) were identified as hVISA strains and 4 (2.0%) were identified as VISA strains, including 99 (99/176, 54.0%) strains from adults and 18 (18/24, 75.0%) strains from pediatric patients. The predominant clone of both hVISA and VISA strains in adult and pediatric was identified as ST5-SCCmecII-agrII. Additionally, there was no significant difference observed in the clinical and microbiological efficacy of vancomycin between the hVISA and VSSA groups.

Conclusions: In this study, hVISA had no significant impact on vancomycin treatment in adult and pediatric patients, but its high detection rate highlights the need for global surveillance and more stringent infection control measures.

T271 - Probing the promoter activity for a periplasmic chorismate mutase in *Pseudomonas aeruginosa*

Presenting Author - *Luca Bressan, ETH Zurich, Switzerland*

Author/s – *Luca Bressan, Peter Kast*

Abstract Content

Aromatic amino acids are biosynthesized via the shikimate pathway that exists in the cytoplasm of bacteria, fungi, and plants. Interestingly, of the key enzymes involved, chorismate mutase (CM), has a periplasmic paralog in *Pseudomonas aeruginosa* (*PaeCM). *P. aeruginosa* is a highly persistent opportunistic pathogen, in part due to its biofilm-forming capabilities, and the main infectious agent in cystic fibrosis. The absence in the periplasm of the *PaeCM substrate chorismate and the non-obvious fate of the product prephenate suggests another role for *PaeCM than housekeeping aromatic amino acid biosynthesis. The goal of this project is to elucidate the role of secreted CMs, which may contribute to bacterial virulence (1). To study the function of *PaeCM and its gene expression profile under different lifestyles we generated in-frame genomic knock-out strains and several native promoter-reporter fusions. We established a promoter-activity assay with β glucuronidase (GUS) constructs on a pSEVA-type plasmid vector (2) for probing promoters in different mutant strain backgrounds. We also integrated the reporter construct genomically into wild-type *P. aeruginosa* PAO1 to preserve the native transcriptional configuration of the promoter region. Our plate-based medium-throughput assay design allows for rapid screening of inductive conditions in either complex or defined media. We use this reporter system to unravel *PaeCM gene expression and thereby its still mysterious purpose in the *P. aeruginosa* periplasm. A better understanding of *PaeCM in virulence could help combat *P. aeruginosa* as a major cause of nosocomial acquired infections.

T272 - Siderophore metabolizing genes profiling of *E. coli* causing urosepsis and assessment of their expression in urine and blood

Presenting Author - *Politechnika Gdanska, Gdansk University Of Technology, Poland*

Author/s – *Magdalena Burzyńska, Beata Krawczyk, Agnieszka Laskowska, Anna Stanisławska-Sachadyn, Paweł Wityk, Michał Markuszewski, Marek Bronk, Mariusz Siemiński*

Abstract Content

Background: Urinary tract infections (UTIs) are commonly caused by uropathogenic *Escherichia coli* strains (UPEC) presenting various virulence factors and fitness genes that facilitate its transmission from the lower to the upper urinary tract leading to urosepsis.

Objectives: The iron uptake system of UPEC in urosepsis was researched. We studied the profile of genes encoding siderophores and examined the expression levels of these genes depending on their habitat - urine and blood.

Methods: Patients with clinically confirmed urosepsis with positive *E. coli* blood monocultures and a control group with UTI, no sepsis, were studied. PCR-MP method was used for strains genotyping. Siderophore-associated genes were detected by PCR. Total siderophore activity was determined by CAS assay. The expression of siderophore synthesis genes was assessed by RT-PCR for strains cultured in either M9 medium, M9 medium with blood and artificial urine medium.

Results: In the one-dimensional analysis the aerobactin gene (iutA) was significantly more common in patients with UTI (no urosepsis) than in controls. Average siderophore activity in CAS assay were 86-95% (the highest in artificial urine). Gene expression of irp2 (yersiniabactin) and iucA (aerobactin) were significantly changed in strains from UTI when the medium was changed. In artificial urine, entC expression levels were significantly higher in urosepsis strains as compared to the control group, while the irp2, iucA and iroB expression levels were significantly lower. Chances of *E. coli* survival in urine at low iron levels are increased by higher siderophores synthesis or by increasing of the enterobactin expression.

T274 - The Korean Nucleotide Archive (KoNA) as a new data repositories for nucleotide sequence data

Presenting Author - *Youngmi Sim, Republic of Korea*

Author/s – *Seon-Young Kim, Byungwook Lee, Pan-Gyu Kim, Gunhwan Ko, Jae Ho Lee,*

Abstract Content

Over the previous decade, tremendous growth in high-throughput sequencing data, including amplicon sequencing and metagenome data, has posed challenges in enormous data transfer, storage, and sharing. Furthermore, to encourage data reuse, the Korean government recently announced that all biological data generated by government-funded R&D projects should be stored in the Korea BioData Station (K-BDS), which consists of different databases for the various data types. Here, we present the Korean Nucleotide Archive (KoNA, https://www.kobic.re.kr/kona/) and the Korean Read Archive (KRA) as a repository for nucleotide sequence data and that for next-generation sequencing (NGS) data, respectively. KRA, a subdatabase of the KoNA, had collected more than 591 TB of raw NGS data generated from 31,202 samples, as of 2023. In KRA, 11.3 TB out of 591 TB (1.9%) are microbiome data, corresponding to 4,450 samples (14.3% of 31,202 samples), collected from the Korea Post-Genome Project. Of note, microbiome data from 1,617 out of 4,450 samples can be used to reveal the microbial distribution of healthy Korean participants. The database complies with the International Nucleotide Sequence Database Collaboration's (INSDC) standard operating procedure (SOP) to assure data quality and compatibility. Our standard operating procedure includes using an automated pipeline to perform quality control on submitted data and metadata, followed by manual inspection. Moreover, users can employ GBox, a high-speed transmission system, enabling speedy and stable data transport. KoNA not only satisfies the unmet needs for a national sequence repository in Korea, but also provides datasets to researchers globally and contributes to advances in genomics.

T276 - Evaluation of the effect of Fe (III) and divalent cations on the M.ApeKI activity from hyperthermophilic archaeon

Presenting Author - *Mao Hayashi, , Japan*

Author/s – *Yoshinari Wada, Akira Yamamura, Yasuhiro Iida*

Abstract Content

In bacteria/archaea, DNA methylation has been mainly known the function as an immune system, Restriction-Modification system (R-M system). R-M system is constructed by restriction enzymes (REases) and DNA methyltransferases (MTases). In our previous works, M.ApeKI from *Aeropyrum pernix* K1 was indicated as strongly thermostability DNA MTase. It was estimated composition of R-M system in *A. pernix* K1 (1). For the prosthetic group, REases have been reported that Mg (II) is required as a cofactor. On the other hand, some DNA MTases activity is enhanced by Mg (II), but some DNA MTases activity is inhibited by Mg (II). In this study, we evaluated the influence of metal ions on the methylation activity of M.ApeKI. First of all, M.ApeKI was overexpression in *Saccharomyces cerevisiae* BY24036 and was purified by using His-tag affinity. And then, metal ions were removed from the enzyme by EDTA, and the metal removal was confirmed by Inductively coupled plasma atomic emission spectroscopy. In this study, Ca (II), Cu (II), Fe (II), Fe (III), Mg (II), Mn (II), and Zn (II) were investigated at 250 µM to 10 mM. The methylation activity level was measured by MTase-GloTM methyltransferase assay kit. As a result, the methylation activity of M.ApeKI was inhibited when over 5 mM of Cu (II), Mg (II), Mn (II), and Zn (II) were added. On the other hand, the methylation activity level was not decreased when Ca (II) Fe (II), and Fe (III) were added. From these results, several metal ions inhibit the methylation activity of M.ApeKI.

T277 - Gut microbiome and small RNA integrative-omic perspective of meconium and milk-FED infant stool samples

Presenting Author - *Helena Torrell, Fundació Eurecat, Spain*

Author/s – *Polina Kazakova, Helena Torrell, Nerea Abasolo, Nuria Canela*

Abstract Content

The human gut microbiome plays an important role in health, and its initial development is conditioned by many factors, such as feeding. It has also been claimed that this colonization is guided by bacterial populations, the dynamic virome, and transkingdom interactions between host and mi-crobial cells, partially mediated by epigenetic signaling. In this article, we characterized the bacte-riome, virome, and smallRNome and their interaction in the meconium and stool samples from infants. Bacterial and viral DNA and RNA were extracted from the meconium and stool samples of 2- to 4-month-old milk-fed infants. The bacteriome, DNA and RNA virome, and smallRNome were assessed using 16S rRNA V4 sequencing, viral enrichment sequencing, and small RNA sequencing protocols, respectively. Data pathway analysis and integration were performed using the R package mixOmics. Our findings showed that the bacteriome differed among the three groups, while the virome and smallRNome presented significant differences, mainly between the meconium and stool of milk-fed infants. Notably, the gut environment is rapidly acquired after birth, and it is highly adaptable due to the interaction of environmental factors. Additionally, transkingdom interactions between vi-ruses and bacteria can influence host and smallRNome profiles. However, virome characterization has several protocol limitations that must be considered.

T278 - Patrx2 : a paradoxal oxidoreductase implicated in alginate biofilm formation in *P. aeruginosa*

Presenting Author - *Marie Grandjean, Aix-Marseille University, France*

Abstract Content

Bacteria have a reducing cytoplasm in which abnormally oxidised cysteines are kept reduced by thiol-disulfide oxidoreductase (TDOR) systems. In the Gram-negative opportunistic pathogen *Pseudomonas aeruginosa*, we identified a cytoplasmic TDOR (Patrx2) with an unusual active site motif (CGHC) unique to eukaryotic protein disulfide isomerases (PDI). Based on our *in vitro* results, Patrx2 appears to be a cytoplasmic disulfide isomerase: A high redox potential for the cysteines in the active site was measured by NMR (-172mV), and it has weak disulfide reductase activity and good oxidase activity. To further investigate how disulfide isomerase activity might be possible in the reducing environment of *P. aeruginosa* cytoplasm, we examined the expression conditions of patrx2 using a transposon mutagenesis library. This approach showed that patrx2 is expressed in response to an envelope stress via the extracytoplasmic sigma factor AlgU and is overexpressed in clinical variants that form alginate biofilms and are termed « mucoid variants ». We then quantified alginate secretion in these mucoid *P. aeruginosa* variants and found that a catalytic mutant of Patrx2 produced three times less alginate. We are currently investigating potential Patrx2 substrates and have some preliminary data suggesting that Patrx2 may catalyse the formation of a disulfide bond at the first active enzyme of the alginate biosynthetic pathway. Overall, our results challenge the dogma that the bacterial cytoplasm only allows disulfide reductase activity and give us a new insight into the importance of redox conditions in mucoid biofilms of *P. aeruginosa*.

T279 - Characterization of the Type IX secretion pathway

Presenting Author - *Maelle Paillat, Laboratoire d'Ingénierie des Systèmes Macromoléculaires, France*

Author/s – *Caterina Comas Hervada, Stéphane Audebert, Mark McBride, Eric Cascales, Thierry Doan*

Abstract Content

T9SS substrates are secreted in two-step, first using the Sec machinery to cross the inner membrane. Effectors are then recruited by the T9SS thanks to a conserved C-terminal domain (CTD) to be secreted through the outer membrane. Recently, an in situ cryo-electron tomography approach revealed the architecture of *P. gingivalis* T9SS. However, only little is known regarding the secretion mechanism and the interactions driving substrates along the components of the T9SS during secretion.

Using proximity labeling, co-immunoprecipitation and bio-layer interferometry methods, I am trying to decipher the precise pathway followed by the effectors and to understand the role of each interaction in space and time. The idea is first to identify the interfaces of interaction between the T9SS components and the CTDs. Then, using a genetic approach with diverse mutants inhibiting specific interactions, the aim of my project is to identify the interactions that are essential for the CTDs to travel through the T9SS and to understand how they condition the following steps in the pathway.

I will present some of our first results, highlighting the importance of the interactions with GldM and GldN.

T281 - Short-hairpin RNA expressed from U6 snRNA promoters mediates RNA interference in *Acanthamoeba castellanii*

Presenting Author - *Yeonchul Hong, Republic of Korea*

Author/s – *So-Young Joo, Ja Moon Aung, Youn-Kyoung Goo, Dong-Il Chung*

Abstract Content

Background: The development of effective therapeutic agents for *Acanthamoeba* infection is based on the screening of factors that are essential for the proliferation and encystation of *Acanthamoeba*. However, this process has been hampered by the lack of available tools to regulate gene expression.

Objectives: The aim of this study is to develop a method for practical knockdown gene expression in *Acanthamoeba* using RNA interference.

Methods: Fluorescent reporter genes were integrated into the *Acanthamoeba* genome using the Cre/loxP recombinant system to monitor whether the target gene can be knocked down with shRNA. A novel U6 small nuclear RNA (snRNA) promoter was identified from the *Acanthamoeba* sequence database. Then, an episomal vector-based system was constructed for the target encoding genes using the *Acanthamoeba* U6 promoter to drive the expression of short hairpin RNAs (shRNAs) expression.

Results: The fluorescent reporter genes were successfully integrated into the genome by homologous recombination and expressed in *Acanthamoeba*. The putative *Acanthamoeba* U6 promoter is homologous to mammalian U6 snRNA promoter sharing the features of functional sequence elements for RNA polymerase III transcriptions. The shRNA against each fluorescent protein gene significantly reduced the expression level of the target protein. In conclusion, we report the first integration of an exogenous gene by homologous recombination into the *Acanthamoeba* genome and the identification of the *Acanthamoeba* U6 snRNA and its promoter. The use of the *Acanthamoeba* U6 promoter to drive the expression of the short hairpin RNAs is effective at knocking down protein expression in *A. castellanii*.

T282 - The iron-storing ferritin from *Acanthamoeba castellanii* protect DNA structure during its encystation

Presenting Author - *SeungHyeok Bang, School Of Medicine, Kyungpook National University, Republic of Korea*

Author/s – *Minsang Shin, Soyoung Joo, Yeonchul Hong*

Abstract Content

Acanthamoeba castellanii is a genus of amoebae that are commonly found in soil, freshwater, and other habitats. It caused keratitis and granulomatous amoebic encephalitis (GAE) diseases. When *Acanthamoeba* is exposed to harsh conditions, it changes to the cyst stage, which is a dormant and resistant stage. These cysts are highly resistant to antibiotics and drugs. Antibiotic effects are significantly decreased in the cyst stage. We found that the concentration of ferritin increased during the encystation. In general, ferritin stores iron for *Acanthamoeba* survival. To identify other function of ferritin we examined the characterization of ferritin using biochemical methods. We presented evidence of DNA protection by ferritin, a nonspecific DNA-binding protein from *Acanthamoeba castellanii*, against harsh conditions. We tested an oligomeric formation of ferritin with various divalent metal ions and DNA-ferritin complex formation using EMSA analysis. In particular, it showed high binding affinity to DNA at zinc ions. We confirmed that ferritin binds well to DNA due to oligomer formation in a high pH environment and through AFM analysis. We demonstrated that the role of ferritin is not only as an iron storage protein but also as a protector of DNA by binding to it. These results indicated that ferritin can protect DNA structure during the encystation. Ferritin may be a novel therapeutic target in *Acanthamoeba* infections.

T283 - Structural changes of phycocyanin from Atacama cyanobacteria associated to temperature adaptation

Presenting Author - *Alexandra Galetović, Chile*

Author/s – *Gabriel Peña, Milton Urrutia, Álvaro Olivera-Nappa, Andrés Marcoleta, Macarena Varas, Miguel L. Allende, Jan Ortíz-Bacigalupo*

Abstract Content

Background: Cyanobacteria can be classified as mesophiles or thermophiles, synthesize and accumulate a water soluble protein, phycocyanin (PC), an antenna pigment in the photosynthetic apparatus. PC is a blue protein composed by two polypeptide chains (αβ) covalently linked by cysteine residues to tetrapyrrole bilins chromophores.

Objectives: To compare the PC deduced aminoacid sequences between two thermophilic cyanobacteria strains *Nostoc* sp. GPT-12 and *Nodularia* sp. TAL-12 (isolated from two geothermal regions Tatio Geysers and Talabre) and mesophilic cyanobacteria to assess the stability at temperature.

Methods: Using bioinformatics tools and complementary purified PC from TAL-12 was exposed to 25-75°C. PC genes were sequenced by Nanopore and Illumina technologies, aligned and modeling by SWISS-MODEL.

Results: Phylogenetic analysis and modeling showed that TAL-12 and GPT-12 have highly conserved sequences close to PC from thermophilic microorganisms such as *Fischerella thermalis* and *Thermoleptolyngbya*. Additionally, an *in silico* experiment where a non-conserved region of mesophilic PC was replaced by a highly conserved sequence of thermophilic PC, suggested that this region determines the identity of thermophilic PC. Also, thirty-one aminoacid substitutions were involved in protein-phycobilin or intra and intermolecular interactions in PC polypeptidic chain. In the PC alpha subunit were found F28D and K33Q substitutions. Aspartate forms a salt bridge with lysine and glutamine forms hydrogen bonds to stabilize phycobilin. Also, preliminary data showed PC (TAL-12) was stable at high temperature (75°C, 24h). Therefore, the results suggest that PC from Atacama cyanobacteria present temperature adaptation mechanisms with potential biotechnological applications in pharmaceutical and food industries.

T284 - RNA-sequencing highlights major adaptation of key bacterial vaginosis-associated bacteria when grown in polymicrobial biofilms

Presenting Author - *Lúcia Sousa, University of Minho, Portugal*

Author/s – *Juliano Novak, Ângela França, Christina A. Muzny, Nuno Cerca,*

Abstract Content

Background: Bacterial vaginosis (BV) is the most common vaginal infection. BV etiology remains controversial, but a key feature is the presence of a polymicrobial biofilm, wherein *Gardnerella* spp. are the major constituent. In a previous RNA sequencing study, we found that when *Gardnerella* spp. shift from planktonic to biofilm growth, most detectable genes were repressed. However, little is known regarding additional interactions between key BV-associated bacteria (BVAB) in the polymicrobial biofilm.

Objectives: To determine if key BVAB influence each other's transcriptome when grown together in a polymicrobial biofilm.

Methods: Single- and triple-species biofilms composed of *Gardnerella vaginalis*, *Fannyhessea vaginae*, and *Prevotella bivia* were grown in New York City III media for 48h at 37℃ in anaerobic conditions. Thereafter, RNA was extracted, libraries constructed, and sequenced.

Results: Preliminary analysis revealed significant differences in gene expression levels for 207, 120, and 46 genes in *G. vaginalis*, *F. vaginae*, and *P. bivia*, respectively. When comparing the polymicrobial biofilm to each single-species biofilm, significant gene ontology enrichment was observed. Among down-regulated genes, in *G. vaginalis*, the most significant enrichment was found in amide biosynthetic processes, translation and ribosome components, in *F. vaginae*, ABC transporters and, in *P. bivia*, translation and ribosome components. Among up-regulated genes, significant enrichment was only found in *G. vaginalis* and *F. vaginae*, respectively, in proteins associated with the membrane, and in mannose transport and purine metabolism.

Conclusion: This preliminary analysis suggests that significant adaptations occur in the bacterial transcriptomes when these key BVAB grow together in a polymicrobial biofilm.

T286 - Physicochemical and molecular characterization of the adhesion mechanisms of some cutaneous bacteria

Presenting Author - *Ahmad Khodr, L'Oréal, France*

Author/s – *Xavier Janvier, Severine Jansen, Charleyne Prenom, Nabiha Khodabux, Sylvie Cupferman, Ahmad Khodr*

Abstract Content

Background: Skin microbiome is composed of diverse resident microorganisms like bacteria, fungi and viruses. Their adhesion to skin is an essential step in their colonisation and resilience. The first step of this reversible adhesion is driven by physicochemical interactions such as electrodynamic , hydrophobic or Lewis acid-base interactions.

Objectives: This work aimed at understanding at the physicochemical/molecular level, the mode of adhesion and the effect of different Raw Materials (RM's) on the first step of adhesion between some cutaneous bacteria and human skin. Those findings would help to elaborate novel ways to prevent skin colonization by undesired bacteria.

Methods: The Anti-adhesion effect was measured through a protocol developed on a 3D human skin model and three bacteria representative of the human skin microbiome. The physicochemical interactions involved are investigated by Goniometry and the MATS method (Microbial Adhesion To Solvents). Molecular pathways related to adhesion were also explored using RNA-Seq.

Results: Significant differences in the adhesion profile of these bacteria to the 3D skin model were demonstrated with and without RM's. A combination of physicochemical properties and adhesion molecular pathways expression explains the adhesion behaviour of the used bacterial model and the anti-adhesion characteristics of tested RM's.

T287 - Relevance of holdase chaperones and proteases in the response to heat shock in the acidophilic bacterium *A. ferrooxidans*

Presenting Author - *Gloria Levican, Universidad de Santiago de Chile y Fundacion Biociencia, Chile*

Author/s – *Katherin Izquierdo Fiallo, Claudia Muñoz Villagrán*

Abstract Content

Acidithiobacillus ferrooxidans is a chemolithoautothrophic acidophilic bacterium that belongs to microbial communities involved in the bioleaching of sulfide ores; in this environment, it must tolerate extreme conditions that can induce damage to the protein. The maintenance of protein homeostasis is carried out by the activity of ATP-(in)dependent molecular chaperones that prevent misfolding and aggregation, and proteases that degrade proteins that have lost their functionality. The relevance of these systems in acidophilic bacteria is still unknown. Here, using a bioinformatic approach, we identified the elements of the proteostasis in *A. ferrooxidans* ATCC23270. Using RT-qPCR we evaluated the expression levels of the encoding genes in cells exposed to heat shock. The intracellular ATP levels were also determined. The bioinformatic analysis showed a high redundancy of genes encoding holdases chaperones and proteases. Transcriptional analysis showed that all detected genes encoding ATP-independent holdase were up-regulated (hsp20.1, hsp20.2, hsp20.3, lon.1, lon.3, ridA.1, ridA.2, cnoX, slyD, hsp31, and hsp33) in cells exposed to 37°C for 2 h regarding the control culture (30oC). In the same way, two out three copies of the lon gene that encode protease Lon were up-regulated upon heat shock induction. Interestingly, the intracellular ATP levels showed a higher concentration in cells exposed to heat shock (147%) versus the control cultures (100%). These results suggest that in *A. ferrooxidans* holdases could contribute to the proteostasis under heat shock stress guarantying that ATP is available for other cellular processes. This work paves the way to understanding the proteostasis systems in extreme acidophilic bacteria.

T288 - Understanding translational fidelity in oral Streptococci

Presenting Author - *Indranil Biswas, University of Kansas Medical Center, United States*

Author/s – *Satya Deo Pandey, Saswati Biswas, Indranil Biswas*

Abstract Content

Translational fidelity, with an error rate of 10-4 to 10-3 per codon, is carefully controlled via correct codon-anticodon recognition on the ribosome and accurate pairing of each amino acids with its cognate tRNA by the aminoacyl-tRNA synthetase (aaRS). In addition, both aaRS and ribosome use proofreading mechanisms to correct mistakes and ensure fidelity. Various stress conditions such as nutritional and oxidative stresses often lead to mistranslation that leads to loss of fitness and cause growth defects in bacteria. Translation fidelity has been studied in only a few organisms such as *Escherichia coli*. Nothing is known about the role of translation fidelity in physiology of other bacteria including streptococci. We have been working on *Streptococcus mutans*, an oral pathogen, to study various aspects of protein sbiosynthesis and degradation. We recently identified a small hypothetical protein, called SprV, which is 90 amino acids with the DUF1021 domain. It is a highly conserved protein in streptococci and other Firmicutes. We found that SprV plays a pleotropic role in global transcription and translation. Genome-wide screening for pathogenesis has identified SprV as a potential virulence factor required for fitness and survival in *S. pyogenes* and other pathogenic streptococci. Recent. In this study, using various genetic and biochemical approaches, we demonstrate that how SprV is needed for ribosomal biogenesis and/or mature 70S production. Using a cell-free *in vitro* translation assay, we show that SprV is required for translation. Furthermore, we demonstrate how SprV and other ribosomal associated proteins are involved in the translational fidelity of streptococci.

T290 - Herbal antibiotics from *Ferula gummosa* essential oil in a natural nanocomposite

Presenting Author - *Ahmadfarhad Talebi, Semnan University, Islamic Republic of Iran*

Author/s – *Negin Valinezhad, Sanaz Alamdari*

Abstract Content

Herbal essential oils, due to the broad-spectrum and low tendency to generate resistance in microbial strains, are proper alternatives to existing antibiotics. In present investigation *Ferula gummosa* essential oil–chitosan (CS-FEO) nanocomposite was synthesized and physiochemical, optical, and antibacterial activity of nanocomposite has been studied. XRD pattern for pure CS confirm with its structure. In contrast, by presence of FEO, CS-FEO nanoparticles exhibited a reduction in peak. According to SEM/TEM images, spherical shape with particle size distribution of around 50 and 250 nm for composite nanoparticles was obtained. FTIR and EDX results confirmed the presence of expected elements and chemical groups in the structure of nanocomposite. PL measurement exhibited that addition of FEO caused a strong red emission in polysaccharide CS at room temperature. GC-MS analysis showed that the predominant component was alpha and beta pinene. Antimicrobial properties were also studied against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella* Typhimurium bacteria. Highest susceptibility belonged to *B. cereus* with minimum inhibitory and minimum bactericidal concentration 0.048 and 0.097 mg/ml, respectively. Based on the agar well diffusion method, this nanocomposite showed significant inhibitory potential against all strains; *S. aureus* with inhibitory zone of 33/43±0/41 mm, was the most susceptible strain. The largest zone diameter in the disk diffusion method was related to *P. aeruginosa* with a diameter of 31/36±1/72 mm. In colony count method, the highest susceptibility was related to *S. aureus* with inhibitory percentage of 85/3±4/6 %.

T291 - Mechanisms of predatory bacterium *Bdellovibrio bacteriovorus* to escape the surrounding bacterial prey cell remnants

Presenting Author - *Simona Huwiler, University of Zurich, Switzerland*

Author/s – *Ting F. Lai, Marc O. P. Amsler*

Abstract Content

Bdellovibrio bacteriovorus is a predatory bacterium that kills and invades Gram-negative (prey) bacteria like *Escherichia coli*. Once in the prey periplasm, the predator consumes the prey, divides and needs to exit the prey cell remnants. It has been shown that a predator lysozyme specific to lyse the deacetylated peptidoglycan of the bacterial prey cell wall enables exit of the predator from prey cell remnants. Further, second messenger cyclic guanosine monophosphate adenosine monophosphate (cGAMP) has been shown to be critical in controlling gliding motility at exit, as in its absence *B. bacteriovorus* remains stranded inside of the empty prey cell.

To understand this exit process in more detail we generated a shotgun proteomics dataset to reveal predatory proteins more abundant specifically during the prey cell exit. Among the most abundant proteins we found proteins involved in gliding as well as multiple proteases. Further, we confirm up regulation of the one gliding operon (out of five) identified by proteomics with transcriptional data. These new insights increase our understanding of predatory mechanisms specific to the bipartite interaction of predatory and prey bacterium. This is important in the context of using predatory bacteria as 'living antibiotics' or probiotics.

T293 - Transcriptome profiling of drug-susceptible clinical isolates of *Mycobacterium tuberculosis* by treatment duration

Presenting Author - *Ji-A Jeong, Korea Disease Control And Prevention Agency, Republic of Korea*

Author/s – *Eon-Min Ko, Seonghan Kim, Sungkyoung Lee, Hyungjun Kim*

Abstract Content

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (Mtb) is considered a major public health threat. The World Health Organization recommends treatment for 6 months for patients with drug-susceptible TB. However, there are patients with drug-susceptible TB who are not cured even after 6 months of treatment. RNA sequencing was performed to evaluate the difference between drug-susceptible Mtb isolated before treatment from TB patients who were not cured after 6 months of standard treatment (standard treatment delay group) and those who completed treatment within 6 months (standard treatment completion group). Transcripts of Mtb isolated from the standard treatment delay group were compared with transcripts from Mtb isolated from the standard treatment completion group. A total of 177 differentially expressed genes (DEGs, p-value < 0.05, |Fold change| ≥ 2) were identified in Mtb isolated from standard treatment delay group relative to the Mtb isolated from standard treatment completion group, of which 52 were induced and 125 were repressed. Gene ontology analysis showed that genes involved in 'Methyl-branched fatty acid biosynthetic process' and 'Fatty acid biosynthetic process' were upregulated. 'Defense response to virus' and 'Cell cycle', 'Cell division', and 'Peptidoglycan biosynthetic process' were downregulated. KEGG pathway enrichment analysis showed that 'Peptidoglycan biosynthesis' was enriched in the downregulated DEGs. In particular, the expression of the murECDF operon encoding ATP-dependent Mur ligase, which plays an essential role in peptidoglycan biosynthesis, was significantly reduced. These results suggest that downregulation of genes involved in peptidoglycan biosynthesis might affect the duration of treatment in TB patients.

T294 - Analysis of outer membrane vesicles (OMVs) produced by *Vibrio cholerae* under limitation and abundance of inorganic phosphate

Presenting Author - *Matheus Luchetta da Fonseca, Federal University Of Rio De Janeiro, Brazil*

Author/s – *Livia Carvalho Barbosa, Beatriz Ferreira de Carvalho Patrício, Carolina Neumann Keim, Wanda Maria Almeida von Kruger, Paulo M Mascarello Bisch*

Abstract Content

Vibrio cholerae, like other bacteria, produces vesicles of outer membrane (OMVs) that contain phospholipids, lipopolysaccharides, cytoplasm, periplasm and outer membrane and others. *V. cholerae* inhabits phosphorus-poor aquatic environments and colonizes the intestinal tract of the host, environments where it expresses various genes in response to limitation of inorganic phosphate (Pi). This information led us to verify whether *V. cholerae* OMVs produced *in vitro* under Pi limitation, could carry essential factors for the pathogenicity of the bacteria. GOALS:Molecular characterization of OMVs released by *V. cholerae* and its mutant phoB under limitation and abundance of inorganic phosphate and its relationship in pathogenesis of the bacteria. *V. cholerae* was cultured under Pi abundance and limitation and the OMVs were purified, observed by microscopy and quantified, OMVs was characterized through mass spectrometry and its potential pathogenic with *Galleria mellonella*.

Results: The results of this work provided information interesting facts about the protein and lipid constitution of OMVs released by strains of *V. cholerae* N16961Sr and its phoB mutant, WK10, under limitation and abundance of Pi.showed that several of the OMV proteins are important for the release of nutrients in the extracellular environment, for interaction with other cells, to protect the original bacteria against various stresses, among other functions, playing a role in survival and, in some cases, pathogenesis of *V. cholerae*. Furthermore, the OMVs generated by the strain wild in MGLP were lethal to the larvae *G. mellonella*.

T295 - Antibacterial activity of commercial hyaluronic acid-based gels against *Streptococcus oralis*

Presenting Author - *Neusa Silva, University of Lisbon, Portugal*

Author/s – *Ana Marques, Mariana Brito da Cruz, Joana Marques, António Mata*

Abstract Content

Background: Hyaluronic acid has been incorporated into oral gels for the treatment of oral aphthae due to its excellent biological properties.

Objective: The purpose of this *in vitro* study was to assess the antibacterial activity of various commercially available hyaluronic acid-based aphthae treatment gels against *Streptococcus oralis*.

Methods: *Streptococcus oralis* CECT 907T strain was cultured on an enriched blood agar plate at 37°C for 72 hours under anaerobic conditions (10% CO2, 10% H2, and balance N2). A single colony was grown in 10 mL of Brain-Heart Infusion Modified Medium (BHI-2) at 37°C under anaerobic conditions. After reaching the exponential phase, suspension growth was confirmed by measuring the optical density (OD) at 550 nm. The suspension was then diluted

1:10 and 100 µL was spread onto the blood agar plates. Four equidistant wells were made in each plate using a sterile 4.1 mm diameter circular scalpel and 50 µL of hyaluronic acid-based aphthae treatments gels (Bexident Aftas®-BA, Gengigel®-GG, Afta Clear®-AC, and Aloclair-AL) was inoculated in each punch, in the following concentrations 100%, 75%, 50%, and 25%. Chlorhexidine 2% (Bexident Gengivas®-BG) was used as a positive control. Plates with *S. oralis* suspension and no topical gels were used as negative controls. After incubation time, the diameter of the inhibition zone was measured (in mm) with a metal ruler using a stereoscopic microscope. Group comparisons were made through ANOVA with the Tukey post-hoc test and the significance was set at ($p<0.05$).

Results: Of the tested gels, AC (0,63 ± 0,08 mm)and AL (0,94 ± 0,16 mm) showed the highest antibacterial activity at 100% concentration ($p<0.05$), comparable to BG (0,94 ± 0,12 mm) which was used as a positive control. No statistical differences between AL and BG antibacterial effects were observed for all concentrations ($p>0.05$). BA and GG showed no antibacterial activity, as observed by the absence of an inhibition halo. It was also observed that the antibacterial effect of BG, AC, and AL was dose-dependent, and AC was not present for concentrations below or equal to 50%.

Conclusions: Among all tested hyaluronic acid-based aphthae treatment gels, AC and AL were found to be more effective against *Streptococcus oralis*.

T296 - Using optical fibers functionalized with specifically designed oligonucleotide probe to detect LAMP amplicons of *Escherichia coli* malB gene

Presenting Author - *Ljiljana Janjušević, Biosense Institute, Serbia*

Author/s – *Ljiljana Šašić Zorić, Maria João Camacho, Débora Cristina Albuquerque, Verónica C. Martins, Mila Djisalov, Robert S. Marks, Ivana Gadjanski*

Abstract Content

Background: Current pathogen detection approaches show an acute need for novel, rapid, on-site biosensing systems for early detection of pathogens infecting animals, plants and fungi as well as for the freshwater quality monitoring with high sensitivity and specificity. To achieve this goal, the development of highly specific genosensor is of great importance.

Objectives: The aim of this study was to design a specific oligonucleotide probe for detection of the loop-mediated isothermal amplification (LAMP) products of malB gene for *Escherichia coli* and the functionalization of the optical fiber with the same probe. Such functionalized chemiluminiescent optical fiber would serve as a highly specific genosensor for detection of *E. coli* in the water.

Methods: Amplification of malB gene of *E. coli* was performed using LAMP protocol. The design of a specific probe was done based on criteria defined in Viveiros, S.; Rodrigues, M.; Albuquerque, D.; Martins, S.A.M.; Cardoso, S.; Martins, V.C. Multiple Bacteria Identification in the Point-of-Care: An Old Method Serving a New Approach. Sensors 2020, 20, 3351. The functionalization of the optical fiber was done according to the procedure described in the work of Ye et al., 2017. The light signal of chemiluminiescent optical fiber was interpreted as relative light intesity measured by a photomultiplier tube detector in a confined black box.

Results: We functionalized the optical fiber with the designed probe and measures relative light intensity. The obtained results, a high chemiluminescent signal indicated successful detection of LAMP products of *E. coli* malB gene. This kind of genosensor, with specifically designed oligonucleotide probe, has potential to be adapted for detection of other microbial patogens.

T297 - FcrX, a new global regulator of cell cycle in free-living conditions and during symbiosis in *Sinorhizobium meliloti*

Presenting Author - *Sara Dendene, Centre National de la Recherche Scientifique (CNRS), France*

Author/s – *Shuanghong Xue, Quentin Nicoud, Odile Valette, Angela Frascella, Anna Bonnardel, Romain Le Bars, Mickaël Bourge, Peter Mergaert, Matteo Brilli, Benoît Alunni, Emanuele G. Biondi*

Abstract Content

Sinorhizobium meliloti is model organism for the study of bacterial differentiation and a soil bacterium that establishes a symbiosis with Medicago sativa, colonizing nodules where it fixes the atmospheric nitrogen into ammonia and obtains carbon sources in return from the plant. In this symbiosis, *S. meliloti* undergoes a drastic cellular change leading to an intracellular terminal differentiation (bacteroid) characterized by genome endoreduplication, cell enlargement and high membrane permeability, suggesting that bacterial cell cycle regulation is implicated in this process. Indeed, in free-living cells, the bacterial regulator CtrA, among other functions, activates cell division (controlled by constriction ring forming FtsZ), and inhibits DNA replication, while during symbiosis CtrA and FtsZ downregulation is essential for bacteroid differentiation. So far, little is known about regulators controlling CtrA and FtsZ in *S. meliloti* and about their role during bacteroid development.

This study focuses on a new factor, FcrX, that controls both CtrA and FtsZ. Depletion of the essential gene fcrX leads to minicell formation in which levels of FtsZ and CtrA are abnormally high. Using several techniques, we showed that FcrX (a alpha-helix-rich protein) is able to interact with FtsZ and CtrA via a still unknown mechanism. Further, we showed that, despite a weak homology with FliJ-like proteins, only closely-related species FcrXs are able to complement *S. meliloti* fcrX deletion. Finally, mutants of FcrX showed abnormal symbiotic behaviors in plants suggesting a putative role of this factor during bacteroid differentiation.

T298 - Prioritisation of molecular targets for antifungal molecule 1,6-diphenoxyhexa-2,4-diyne derivative of eugenol against *A. fumigatus*

Presenting Author - *Lovely Gupta, Amity University, India*

Author/s – *Asish Bhattacharya, Pooja Vijayaraghavan*

Abstract Content

Background: *Aspergillus fumigatus* is one of the major pathogenic fungal species, causing life-threatening infections. Due to a limited spectrum of available antifungals, exploration of potential antifungal molecules and new drug targets has become pertinent.

Objective: We have analysed the antifungal drug targets for synthesised 1,6-diphenoxyhexa-2,4-diyne derivative of eugenol against *A. fumigatus*.

Method: Screening of antifungal activity of the diyne derivatives against *A. fumigatus* ATCC 46645 strain via *in vitro* susceptibility. On the basis of minimum inhibitory concentration (IC50), one diyne derivative (compound 3a) was identified and analysed for cell cytotoxicity. Biochemical and electron microscopic studies were carried out to understand changes in *A. fumigatus* cell surface morphology and associated structural components. Relative expression of virulence genes responsible for adherence and cell wall integrity was studied via qRT-PCR. Total proteome analysis was carried out to study differentially expressed proteins. Further, gene-protein interaction was studied for drug target prioritisation.

Result: Compound 3a was found to be exhibiting promising antifungal activity with IC50 value of 7.75 µM, non-cytotoxic on lung epithelial cell line; thereby suggesting that these types of scaffolds could pave the way for developing as new antifungal agents. It was observed that compound 3a interacted with the conidial surface proteins and altered its surface characteristics. Its treatment led to significant down-regulation of virulence genes and cell wall integrity protein which were differentially expressed. Combine transcriptional and proteomics data of the regulatory genes/proteins can be used to predict potential molecular target and to map the pathways to combat *A. fumigatus* resistance.

T300 - Towards soil microaggregates stabilisation by *Bacillus amyloliquefaciens* pellicles

Presenting Author - *Emmanuelle Baudu, Université Toulouse III - Paul Sabatier, France*

Author/s – *Yassine Nait-Chabane*

Abstract Content

Bacillus amyloliquefaciens is a soil bacterium known for its role in promoting plant growth. Under lab experimental conditions, it can produce a resistant biofilm at the air-liquid interface that exhibits highly hydrophobic properties at the air interface, and hydrophilic character at the liquid interface. In soil, the presence of bacteria producing extracellular polymeric substances (EPS) can have an impact on the stability of microaggregates. The role of polysaccharides has been extensively studied, but that of proteins remains largely unexplored. Although hydrophobicity has been linked to soil stability, the role of hydrophobic proteins needs to be examined.

Pellicles of *B. amyloliquefaciens* strain L17 were studied, focusing on EPS and hydrophobic properties. Pellicles were produced in glucose minimal medium (GMM) at 30°C and were harvested at different time. After drying, each side of the pellicle was analysed by attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR). The EPS composition of pellicles was also explored by a sequential extraction protocol with quantitative analysis of extracellular proteins, polysaccharides, and DNA.

B. amyloliquefaciens L17 formed a thick pellicle after a few hours that sustained for several days. EPS analyses showed a variation in the protein/polysaccharide ratio over time and highlighted the difference in composition between the biofilm-air and the biofilm-liquid interface. Recent work in the laboratory has clearly shown the highly hydrophobic character of the pellicle. For this reason, we have undertaken preliminary experiments to establish a link between this hydrophobic character and the stability of soil micro-aggregates. The results obtained are very encouraging.

T301 - PNPase, a conserved exoribonuclease fundamental for biofilm formation of *Escherichia coli*

Presenting Author - *Manuel Condinho Carvalho, ITQB NOVA, Portugal*

Author/s – *Manuel Condinho, Cecília Maria Arraiano, Vânia Pobre*

Abstract Content

Bacterial communities constantly deal with stress situations, having to quickly regulate their gene expression to ensure survival. Biofilm formation is one of these response mechanisms, as it gives bacterial aggregates protection against harsh environments. Switch from planktonic to a sessile lifestyle is a highly controlled process in bacteria and is dependent on RNA regulators. Ribonucleases (RNases) are the enzymes that regulate all RNA levels in the cell, being responsible for their degradation, maturation and processing. RNases regulate various processes in bacteria, including biofilm formation, aggregation, adhesion, among others. In our work we described the role of PNPase in the biofilm formation of *Escherichia coli*. Initially, we observed that the PNPase deletion mutant (Δpnp) forms less biofilm than the wild type. The Δpnp has slower growth and has an elongated shape, compared to the wild type, which may explain the difficulties in forming biofilm. We found that the PNPase mutant has its aggregation compromised, which is a fundamental process at an initial stage of biofilm formation. To comprehend the molecular role of PNPase in this process we quantified by mass spectrometry the intracellular levels of c-di-GMP, which is a signaling molecule that regulates biofilm growth in several bacteria. We observed that the Δpnp has decreased levels of c-di-GMP compared to wild type, which agrees with the observed biofilm phenotype. In summary, with our work we established a link between PNPase and biofilm formation in *E. coli*, revealing the decisive role of PNPase in this process.

T302 - Early emergence of biofilm phenotypic heterogeneity in ribosomal gene regulation

Presenting Author - *Rohit Dutta, Indian Institute of Science, India*

Author/s – *Rohit Dutta, Rahul Roy*

Abstract Content

Bacterial biofilms often contain multiple phenotypically heterogeneous subpopulations. They are well characterized for inducible biofilm markers that are involved in locomotion, surface adherence, appendage expression, matrix expression, and antibiotic resistance. Level and induction kinetics of heterogeneous basal gene expression, however, is poorly understood. Translational regulation via ribosomal proteins is a key mechanism for the emergence of heterogeneity in global protein expressions. Perturbation of ribosomal subunit assembly or translocation along the mRNA, both mediated by the ribosomal proteins 3,4, have been shown to affect polysome formation, growth rates and protein production.

To investigate the emergence of ribosomal protein expression, we employ S13 promoter, PrpsM driven fast maturing GFP. To capture early dynamics in the biofilm ribosomal gene expression at single cell level, *E. coli* biofilms grown in 96 well plates are characterized by imaging and flow cytometry. While GFP expression remains relatively constant in planktonic cultures, GFP expression decreases over 24-48 hours in biofilms. This effect is cell density-dependent suggesting cell-cell communication in biofilms involved in regulating this transient bifurcation. We further characterize this phenotypic heterogeneity between the states using global protein analysis and metabolic reporter dyes. Such emergence of early-phase biofilm heterogeneity can reveal new avenues for controlling biofilm communal populations.

T303 - Single-step gene deletion: a new approach for creating gene knockout in *Mycobacteria*

Presenting Author - *Yahav Bracha, Faculty of Dental Medicine, Hebrew University of Jerusalem, Israel*

Author/s – *Daniel Barkan*

Abstract Content

Introduction: Gene deletions in *Mycobacteria* are important to study their physiology and genetics, but are challenging due to the complexity of available methods: many are inefficient or require multiple, technically complicated steps, limiting their use to highly specialized laboratories. Therfore, we developed a simple, efficient, single-step approach based on the Che9c recombineering enzymes. In a previously described method, these enzymes were expressed from a plasmid and regulated by an inducible acetamidase promoter, necessitating pre-insertion of that plasmid, induction, and subsequent plasmid loss.

Methods: We expressed the Che9 genes from a constitutive promoter, and placed it on the same linear fragment used for the gene deletion itself. We've constructed "stock" plasmids allowing simple cloning of long flanking regions and subsequent linearization and electroporation into target mycobacteria.

Results: We've successfully applied our method to *Mycobacterium smegmatis* and *Mycobacterium abscessus*. We were able to delete genes by a single-step procedure, including an ABC transporter in *M. smegmatis* (33/45 correct colonies), and a small RNA from *M. abscessus* (4/4 correct colonies). The system is currently being tested in *M. tuberculosis*.

Importance: Targeted gene deletions in mycobacteria are invaluable in genetic research, but the complexity of current methods limits these techniques to highly specialized laboratories – and there too the procedure is lengthy and labor intensive. We developed a simple and effective tweak to an existing method, making the procedure technically and conceptually simple, effective, and time-saving across multiple mycobacteria, thus making it accessible to any laboratory with basic molecular biology capabilities.

T304 - The role of TnaA in the formation of *E. coli* biofilms

Presenting Author - *Alicia Liu, University of Cambridge, United Kingdom*

Author/s – *Alicia Liu, Ashraf Zarkan*

Abstract Content

A key aspect of the antibiotic resistance crisis is the ability of bacteria to form biofilms, which are structural barriers against antibiotics and the host immune system. Biofilms are implicated in recurrent infections, such as urinary tract infections (UTIs) which are predominantly caused by *Escherichia coli* (*E. coli*). Tryptophanase (TnaA) is a promiscuous amino acid degrading enzyme in *E. coli*, and it's role in the formation of biofilms has been previously demonstrated. However, the exact amino acids that can be degraded by TnaA remains unclear, and the contribution of each degradation pathway in the formation of *E. coli* biofilms is unknown. Here we describe a set of enzymatic assays to assess the potential interaction of TnaA with all 20 proteinogenic amino acids that can be synthesised by *E. coli*. To investigate the contribution of each degradation pathway in *E. coli* biofilms, we describe a set of biofilm assays in minimal media supplemented with one amino acid at a time. This work investigates the mechanisms underlying the role of TnaA in the formation of *E. coli* biofilms, which may provide a potential route to combat biofilm formation in recurrent UTIs.

T306 - The overwhelming influence of propionic acid on the proton ATPase activity: the significance of potassium ions

Presenting Author - *Tamara Abaghyan, Department of Biochemistry, Microbiology, and Biotechnology, Yerevan State University, Armenia*

Author/s – *Heghine Gevorgyan, Margarita Mirumyan, Konstantin Yenkoyan, Karen Trchounian*

Abstract Content

Propionic acid (PPA) is an inducer for autism spectrum disorders and have a negative impact on cell viability triggering K+ leakage. ATPase activity provides energy to the cell and maintains its viability. The impact of K+ ions on proton ATPase activity depending on different concentrations of PPA (11.7 and 33.4mM) was investigated in gram-negative *Escherichia coli* K12 and gram-positive *Enterococcus hirae* ATCC9790. ATPase activity was determined by the amount of inorganic phosphate (Pi) produced in the reaction of membrane vesicles with ATP. To determine proton ATPase activity N,N'-dicyclohexylcarbodiimide (DCCD) was used.

The proton ATPase activity was ~365 nmol Pi (min μg protein)-1 in *E. coli*. Its' variation was negligible in the assays with the addition of PPA. Moreover, proton ATPase activity was reduced by ~17% and ~20% in the presence of K+ ions when PPA with 11.7mM and 33.4mM concentrations was added. Proton ATPase activity was ~195 nmol Pi (min μg protein)-1 in E. hirae. It was decreased with addition of 11.7mM PPA, and was unchanged with addition of 33.4mM PPA. Proton ATPase activity was increased by ~28% in the presence of K+, while it was decreased by ~38% and ~35% with addition of 11.7mM and 33.4mM PPA, respectively.

Taken together, the influence of PPA on the interruption of bacteria homeostasis managed by K+ ions, mediated by the interaction between K+ transport systems and proton ATPase in the membrane. This suggested mechanism functioned in both gram-negative and gram-positive bacteria.

T307 - A multi-omics approach to identify molecular pathways associated with remission in pediatric Crohn's disease

Presenting Author - *Nienke Koopman, University of Amsterdam, Netherlands*

Author/s – *Nienke Koopman, Yorrick Jaspers, Pim van Leeuwen, Lucas Bresser, Kay Diederen, Anje A. te Velde, Angelika Kindermann*

Abstract Content

Although the etiology of Crohn's disease (CD) is still under investigation, it is thought that an interplay between genetic predisposition, environmental factors and microbiome triggers inflammation. Insight in the mechanisms underlying the transition to remission is of major importance in the development of novel therapies. For this study, 59 pediatric patients (9-21 years) with active CD or in remission (characterized by fecal calprotectin levels), were included. The fecal microbiome was determined by both ITS1 (fungi) and 16S (bacteria) analysis. In addition, metabolomics analysis with HILIC-QTOF-MS was performed on fecal, urine and plasma samples and proteomics with LC-MS/MS on fecal samples. Distinct signatures for the two patient groups were observed for the metabolomes (both fecal and plasma), and proteome. The bacteriomes of patients in remission scored a higher Shannon-diversity index and were more similar to each other, as shown by Bray-Curtis dissimilarity, while fungal data did not reveal large differences between all individuals. Integrating the different datasets in a Manifold Mixing for Stacked Regularization model, specifically designed for multi-omics datasets resulted in a model that separates the interactome of the two patient groups. The bacteriome, fecal and plasma metabolome showed to have the strongest effect on this separation. In active disease an inflammatory profile of proteins was observed which is positively correlated with amplicon sequence variants belonging to the *Hungatella* and *Lachnoclostridium* genera and *Streptococcus oralis*/*sanguinis*/*parasanguinis*. Further investigation of the underlying pathways may provide more insight in the mechanisms involved in remission of Crohn's disease.

T308 - Revealing the changes in *Escherichia coli* membrane treated with Ib-M peptides

Presenting Author - *Ana Elvira Farfán-García, , Colombia*

Author/s – *Ana Elvira Farfán-García, Indira Paola Hernández-Peñaranda, Oscar G. Gómez-Duarte, Edgar Javier Rincón-Barón, Andrés Gerardo Torres-Rodriguez, Johanna Marcela Flórez-Castillo,*

Abstract Content

For decades, antimicrobial peptides have been considered the most promising alternative for the treatment of multiresistant bacteria, and there has been a growing interest in this research area. Recently, the bactericidal activity of Ib-M peptides against *E. coli* was established, but how they exert their action on membranes is still unknown. We evaluated the peptides Ib-M antibacterial and cytotoxic activity, alterations in permeability and membrane potentials. In this study, the morphological changes were reviewed by scanning and transmission electron microscopy (SEM and TEM); and Ib-M secondary structure by circular dichroism also were reviewed. The minimal inhibitory concentration of Ib-M peptides was 12,5 µM and minimal bactericide concentration did not exceed by more than two times MIC. In addition, Ib-M treated with monovalent cations retained their anti-bacterial activity under physiological conditions. Ib-M were not cytotoxic to NCM-460. The peptide secondary structure prediction showed two α-helix linked by a random structure consistent with circular dichroism spectrum assay. The permeability of the outer and inner membranes of bacteria was observed with N-Phenyl-1-naphthylamine and O-nitrophenyl-β-D-galactopyranoside respectively. Also, the peptides caused depolarization of the *E. coli* membrane at the concentrations tested. In SEM, the cells treated with Ib-M presented shrinkage, roughness, deep invaginations, pore formation, blebbings, and loss of bacillary structure. TEM studies revealed cell wall detachment, blebbing, electron-dense inclusions, focal lysis of bacterial cytoplasm. Our results contribute to knowledge of the mechanisms of action of Ib-M peptides in *E. coli*, and suggest that Ib-M permeabilizes and cause ultrastructural alterations on the *E. coli* membrane.

T309 - Deciphering the transcriptional stress response to DNA damage in *Bacteroides* gut commensals

Presenting Author - *Lucia Margara, European Molecular Biology Laboratory, Germany*

Author/s – *Anastasiia Okhtienko, Carlos Geert Pieter Voogdt, Maria Zimmermann-Kogadeeva, Michael Zimmermann*

Abstract Content

DNA damage is a common threat to living cells. To cope with such damage, bacteria have developed a transcriptional program known as SOS response, regulated by the transcriptional repressor LexA. Here, we used *Bacteroides thetaiotaomicron* (Bt) as a model with the aim at characterising the stress response to DNA damage in the prevalent and abundant human gut commensal bacterial *Bacteroides* genus. We identified an uncommon presence of two putative LexA proteins in Bt (Bt_lexA1 and Bt_lexA2). Protein structure predictions revealed main features of the LexA repressor: the helix-turn-helix domain for DNA binding and the peptidase domain, necessary for the self-cleavage during SOS induction. RT-qPCR measurements demonstrated that both factors are induced by DNA damage indicating their involvement in the SOS response. Further, genetic deletion of these genes (lexA1, lexA2 and lexA1lexA2) and gene expression measurements identified Bt_lexA1 as a master regulator of the SOS response repressing both the expression of itself and Bt_lexA2. Employing transcriptomics analysis of genetically modified wild-type bacteria under DNA damaging and control conditions allowed us to systematically map the SOS response in Bt. Eventually, we bioinformatically analysed the genomes of 50 *Bacteroides* genomes of the human gut to demonstrate that the existence of multiple lexA(s) copies is a common phenomenon in these gut commensals. Hence, our work sheds light on a common mechanism of the DNA damage response in *Bacteroides*, employing a hierarchy of transcriptional repressors, each regulating a distinct subset of the SOS stress response.

T310 - Mutational analysis of clarithromycin- and levofloxacin-resistant genes from clinical *Helicobacter pylori* isolates in Croatia

Presenting Author - *Ivica Šamanić, University Of Split, Croatia*

Author/s – *Blanka Dadić, Jonatan Vuković, Ana Maravić, Marija Tonkić, Pavle Vrebalov Cindro, Mia Dželalija,*

Abstract Content

Background: *Helicobacter pylori* is a pathogenic bacterium that causes gastrointestinal diseases such as gastritis, peptic ulcers, and stomach cancer in humans. In the last two decades, *H. pylori* resistance to various antibiotics, including clarithromycin (CAM) and levofloxacin (LVX), has increased significantly. The causes of resistance to clarithromycin and levofloxacin are point mutations in the genetic sequence that disrupt the cellular activity of antibiotics by altering drug targets.

Objectives: We performed genetic molecular analysis of three genes in *H. pylori* whose mutations are associated with resistance to CAM and LVH as first-line antibiotics for therapy of *H. pylori* infections.

Methods: *H. pylori* strains were isolated from gastric biopsy specimens of patients with gastric diseases at Split University Hospital. To explore the mutational characteristics of *H. pylori* genes associated with resistance to the antibiotics clarithromycin and levofloxacin, 23 s rRNA, gyrA, and gyrB genes were amplified by PCR and sequenced.

Results: In the CAM-resistant isolates, the mutation sites in the 23s rRNA gene were A2514C, A2514G, and A2515G. In addition, mutations D91G and D91N were identified in DNA gyrase subunit A, which are associated with resistance to levofloxacin. No mutations critical for levofloxacin resistance were identified in DNA gyrase subunit B. Multidrug resistance was also observed, suggesting successful development of resistance to multiple antibiotics and thus an increased risk to human health.

T311 - Identification of anode mediators enabling bio-electrochemical cultivation of *Dehalococcoides mccartyi* strain CBDB1

Presenting Author - *Marie Eberwein, Helmholtz Center For Environmental Research Leipzig - UFZ, Germany*

Author/s – *Darja Deobald, Ronny Frank, Lorenz Adrian*

Abstract Content

Halogenated compounds, including chlorinated and brominated benzenes, dioxins, and phenols, pose a significant environmental threat as they are persistent and difficult to remove once released into the environment. However, the strictly anaerobic bacterium *Dehalococcoides mccartyi* strain CBDB1 has the ability to biodegrade these compounds through a process called organohalide respiration (OHR). OHR is a complex metabolic process that takes place at the bacterium's membrane-bound, modular OHR-complex, consisting of three modules. The hydrogenase module oxidizes hydrogen and takes up electrons, while the reductive dehalogenase (RdhA) module is responsible for the reductive dehalogenation step. During the OHR, electrons originating from the hydrogen oxidation are transferred to the corrinoid cofactor in the active side of the RdhA and then onto halogenated compounds. We aim to identify and characterize anode mediators shuttling electrons between the RdhA and an anode, which is crucial for establishing a method for the bio-electrochemical cultivation of strain CBDB1. Since the OHR complex is not suitable for direct electron exchange with the anode, the identification of suitable anode mediators plays a critical role in realizing this goal, as it allows for the transfer of electrons between the RdhA and the anode obviating the utilization of often toxic halogenated compounds. Our results show that metal complexes containing cobalt are capable of reversibly shuttling electrons between the anode and the respiratory complex of strain CBDB1.

T312 - Gene expression and disruption of *Halomonas* sp. A020 using the endogenous plasmids

Presenting Author - *Mei Hirata, Kindai University, Japan*

Author/s – *Ayaka Tsuji, Yoshinao Azuma*

Abstract Content – *Halomonas* species are aerobic, alkaliphilic, and moderately halophilic bacteria. Many species were isolated from high saline environments such as the seas and salterns aiming for industrial biochemical productions, such as polyhydroxybutyrate (PHB) and ectoine by fermentation due to their magnificent abilities including high-density culturing capacity and low-risk contamination. However, the genetic tools to modify the metabolism of *Halomonas* are still limited. In this study, we used *Halomonas* sp. A020 for the establishment of genetic manipulation. A020 was isolated from the wastewater of a pickled plum factory and showed the highest production of PHB and the most stress-tolerant in our laboratory isolates. After the determination of the whole genome sequence of A020, we found that the genome contains two plasmids, and two shuttle vectors were prepared using the endogenous plasmids. Using the vectors, the cas9 gene of *Streptococcus pyogenes* and guide RNAs for A020 pyrEF genes, homologs of yeast URA3 and 5, were introduced into A020 using the electroporation method. As result, pyrEF were successfully disrupted and the mutants exhibited 5-fluoroorotic acid resistance and uracil auxotroph. This genetic tool was promising for understanding metabolic regulation and engineering applications in *Halomonas*.

T313 - Influence of osmotic stress on H2 production in *Escherichia coli* during fermentation of mixed carbon sources at acidic pH

Presenting Author - *Anush Babayan, Department of Biochemistry, Microbiology, and Biotechnology, Yerevan State University, Armenia*

Author/s – *Karen Trchounian*

Abstract Content

Molecular hydrogen (H2) is one such alternative energy sources, and the use of H2-producing microorganisms for this purpose is of great interest. *E. coli* evolves H2 via multiple [Ni–Fe]-hydrogenases (Hyds). This activity under hyper-osmotic stress has been investigated by the use of mutant strains lacking different Hyd subunit during glycerol and glucose fermentation. Data obtained has been compared with stress-free assays.

The redox potential (Eh) in bacterial suspension was determined with a potentiometric method using a pair of redox, titanium–silicate (Ti–Si) and platinum (Pt) electrodes. The difference in the values of Ti-Si և Pt electrodes allows determining the generation of H2 (VH2) in bacterial suspension.

During glycerol fermentation at pH 5.5 wild type and mutant strains showed the same VH2 low rate under stress-free and hyper-osmotic stress, while during glucose fermentation stress-free samples of wild type showed high rate of VH2. H2 production was absent in hycE in all samples besides stressed cells during formate fermentation at pH 5.5, suggesting that Hyd-3 were responsible for H2 generation. Under hyper-stress in formate assays VH2 was inhibited in hycE ~ 3.2 fold, compared to wild type, but DCCD inhibition was ~ 1.5 fold. These results suggest that Hyd-3 plays a role in the response to osmotic stress, which could result in interaction with other proteins including F0F1 to stabilize the cell turgor.

These findings may help to understand the mechanisms of cell osmolality for the development of H2 production biotechnology.

T314 - Identification of enzymes responsible for anaerobic transformation of sulfamethoxazole in sulfate reducing bacteria

Presenting Author - *Yu Liu, Helmholtz Center For Environmental Research Leipzig – UFZ, Germany*

Author/s – *Chang Ding, Lorenz Adrian*

Abstract Content

Sulfamethoxazole has been frequently detected in the environment due to its extensive usage in animal farming and human healthcare as well as its recalcitrance to degradation. Anaerobic transformation of sulfamethoxazole into two transformation products (reduced or isomerized) has been observed in the sulfate reducer *Desulfovibrio vulgaris* Hildenborough. In addition to *D. vulgaris*, two other sulfate reducers *Desulfosporosinus meridiei* and *Desulfovibrio desulfuricans* were also proved to be capable to transform sulfamethoxazole into the same two transformation products as yielded by *D. vulgaris*. However, the underlying enzyme transformation processes are still unknown. Here, we investigate transformation of sulfamethoxazole in the above-mentioned sulfate reducing bacteria, using a combination of enzymatic, proteomics, and genomics approaches. We developed *in vitro* activity assay, allowing for sensitive and reliable detection of sulfamethoxazole transformation activity in crude extracts from all three strains. Ultrafilters and fast protein liquid chromatography (FPLC) were applied to separate crude extract into fractions which were used in activity assay. Transformation of sulfamethoxazole was observed in some of the fractions. The proteome of the FPLC fractions was analyzed by shotgun proteomics via LC-MS/MS and label-free quantification. The comparison of protein abundances in the FPLC fractions with the corresponding sulfamethoxazole transformation rates revealed several protein candidates that may catalyze the transformation of sulfamethoxazole. Comparative genomics of the three strains also indicated the existence of functional genes that could be responsible for sulfamethoxazole transformation. Findings obtained in this study are significant for comprehensive understanding on transformation of sulfamethoxazole and effective elimination of sulfamethoxazole in the environment.

T315 - Formate-nitrite transporters of *Naegleria fowleri*: potential drug targets?

Presenting Author - *Moira Möller, University of Kiel, Germany*

Author/s – *Moira Möller, Anjan Debnath, Eric Beitz*

Abstract Content

Naegleria fowleri, a globally distributed rhizopod of the Vahlkampfiidae family, is the causative agent of primary amoebic meningoencephalitis. 97% of the known cases are fatal due to the lack of suitable treatment. *N. fowleri* is mainly found in the tropics and subtropics but also in warmed freshwaters of temperate zones. Infection occurs by the trophozoite form through the nasal mucosa. The parasite then travels along the olfactory nerve through the cribriform plate into the brain and causes severe tissue damage and necrosis. Here, we describe the identification of three *N. fowleri* genes encoding formate-nitrite transporters, FNT1-3. Recently, we discovered the vital lactate-releasing FNT in the malaria parasite [1], and a novel class of small, drug-like FNT inhibitors with nanomolar antimalarial efficiency [2]. Treatment of *N. fowleri* trophozoites in culture with inhibitors of our set killed the parasites in the single-digit micromolar range. NfFNT1-3 share high sequence similarity with the FNT from malaria parasites. To test whether the compounds target the *N. fowleri* FNTs, we obtained codon-optimized open reading frames, and expressed NfFNT1-3 in *Saccharomyces cerevisiae* yeast. Confocal microscopy of NfFNT1-3 with C-terminal GFP fusions indicated plasma membrane localization. However, in initial assays, NfFNT1-3 failed to transport the 14C-labeled monocarboxylates formate, acetate, and lactate. Other than malaria parasites, Naegleria species do not release lactate as a metabolic end product. Besides glucose, fatty acids are preferred as an energy source as well as acetate that are eventually shuttled into the Krebs cycle. Future work will address the identification of NfFNT1-3 transport substrates.

T316 - Effects of essential oil and cetylpyridinium chloride mouthwash on oral microbiota and salivary biomarkers

Presenting Author - *HyunWoo Son, Kyungpook National University, Republic of Korea*

Author/s – *HyunWoo Son, Vineet Singh, Dokyung Lee, Sihyun Park, Yu-Jin Hyun, Jae-Ho Shin,*

Abstract Content

The oral environment is an important aspect of overall health and well-being, as it is the gateway to the rest of the body and can serve as a reflection of overall health. In order to maintain good oral health, oral microbiome, and salivary factors work in combination. However, studies based on the effects of mouthwashes used for oral health and their impact on oral microbiota and salivary factors are lacking. Therefore, to study the effect of mouthwash on oral microbiota and salivary factor, a 7-day study was conducted using mouthwash containing essential oil and cetylpyridinium chloride, which are representative components of mouthwash. In the present study, subjects were randomly divided into three groups (Control, essential oil, and cetylpyridinium chloride) with equal representation (n = 10). In the week-long study, 4-day mouthwash treatment showed significant ($p<0.05$) oral microbiome changes. Analysis showed that microbial diversity is significantly different in people using mouthwash ($p<0.05$), including lower Shannon's diversity in the essential oil group. We also found an abundance of Streptococcus in the essential oil mouthwash group. In addition, the concentrations of various salivary factors that are related to oral health and host health were different. Results of the salivary factor, buffer capacity, stimulus salivation amount, potassium, and calcium were lowered in the mouthwash group, while glucose and lactate concentrations were increased. Our findings showed that the use of mouthwash induces significant variation in the oral microbiome along with salivary factors, and many of these changes have the potential to negatively affect oral health.

T317 - Cross-talk between the c-di-GMP modulating Wsp pathway and Cfc chemosensory system in *Pseudomonas putida*

Presenting Author - *María Isabel Ramos-González, Estación Experimental del Zaidín-CSIC, Spain*

Author/s – *María Luisa Travieso, María Isabel Ramos-González*

Abstract Content

Pseudomonas putida KT2440 is a plant beneficial bacterium that colonizes efficiently solid surfaces (both biotic and abiotic). In this bacterium, three CheR/CheB pairs of chemosensory proteins, with respectively metyltransferase and methylesterase activities, are present, indicating the existence of three chemosensing pathways in this strain. One of them governs its chemotactic behavior and the second is homologous to the Wsp system, which has been characterized as regulating the intracellular second messenger c-di-GMP and therefore biofilm in diverse bacteria of the *Pseudomonas* genus. However, no function has been found so far for the third pathway, which is conserved among plant-colonizing pseudomonads. The CheR/CheB pair of this pathway is encoded by cfcB and cfcC in the same gene cluster as cfcA. CfcA is a multisensory hybrid histidine kinase that phosphorylates the response regulator with diguanilate cyclase activity CfcR (1, 2). This gene organization suggests a possible functional connection of CfcB/CfcC with c-di-GMP signaling via the CfcA/CfcR pathway. Inactivation of the genes encoding CfcB/CfcC was performed and no major effect upon the phenotypes related with multicellular lifestyle (i.e. c-di-GMP levels, biofilm formation and efficiency of rhizosphere colonization) was observed. However, results compatible with cross-talk between Wsp and Cfc pathways were observed under certain growth conditions and environmental stimuli when single elements of both pathways were simultaneously inactivated. The molecular mechanisms sustaining this cross-talk are being investigated.

T318 - Parageobacillus sp. H-70 as a promising thermophilic exopolysaccharide producing strain

Presenting Author - *Diana Ghevondyan, Department of Biochemistry, Microbiology, and Biotechnology, Yerevan State University, Armenia*

Author/s – *Armine Margaryan, Nils-Kare Birkeland, Annarita Poli, Hovik Panosyan,*

Abstract Content

Background: Exopolysaccharides (EPSs) produced by thermophilic microbes have many potential applications in various industries. Geothermal habitats are considered valuable sources of thermophilic microbes that produce EPSs1.

Objectives: This study focused on the selection of the EPS producers among the thermophilic strains isolated from various geothermal springs in Armenia and on revealing it's EPS production, the chemical composition of produced polymers, as well as genes encoding EPS production.

Methods and Results: The screening of EPS producers among the strains belonging to *Anoxybacillus*, *Geobacillus*, *Parageobacillus*, *Ureibacillus*, *Brevibacillus* and *Bacillus* genera was done by plating on the sugar containing media. The mucous consistency of the colonies on the medium was used as an indicator of potential EPSs production. As a result, a Gram-positive, motile, spore-forming bacterium with Topt=60°C and pHopt=7 and identified based on 16S rRNA, *Parageobacillus* sp. H-70 (MK418383), has been selected as the best EPS producer2. EPS production was investigated under different time, temperature and culture media's composition. The highest EPSs production was observed after 24 h when glucose, arabinose, xylose or maltose were used separately as sole carbon sources at 60°C and pH 7.0. The chemical composition of partially purified biopolymer determined by TLC indicated that EPS is a heteropolymer composed of xylose as a major monomer unit. Whole genome analysis (3.26 Mb) was revealed genes (epsA, epsB, epsE, and epsF) responsible for EPS biosynthesis.

T319 - Antibiotic-coated gold nanoparticles and their antibacterial mode of action

Presenting Author - *Andre Schultz, Ruhr University Bochum, Germany*

Author/s – *Tianyi Zhou, Milena John, Pascal Dietze, Nils Metzler-Nolte, Jürgen Scherkenbeck, Julia Bandow,*

Abstract Content

For the last 100 years modern medicine could rely on antibiotics for the treatment of bacterial infections. But this effective method is progressively endangered as few new antibiotics are being developed while multi-resistant pathogens are spreading (Hutchings et al. 2019). In this study, we investigated the potential of small gold nanoparticles to function as a new drug delivery system for antibiotics. To this end, we investigated the mode of action of antibiotic-coated gold nanoparticles using gel-based proteomics against the model organisms *Bacillus subtilis* and *Escherichia coli*. The gold nanoparticles were coated with a derivative of the peptide deformylase (PDF) inhibitor actinonin (Bandow et al. 2003). We showed that the actinonin derivative has broad activity against different gram-positive and gram-negative bacteria. The gel-based proteomic results showed that the derivative inhibits PDF, resulting in the expected shift of newly produced proteins to a lower pI. Furthermore, we found that the mode of action of the actinonin derivative is preserved even when applied as gold nanoparticle conjugate. Interestingly, 35S-methionine incorporation assays showed that gold nanoparticles themselves (without antibiotic load) also caused a decrease in protein synthesis rates.

T320 - Envelope stress response in *Brucella abortus*

Presenting Author - *Adélie Lannoy, University of Namur - URBM, Belgium*

Author/s – *Xavier De Bolle, Jonathan De Stercke*

Abstract Content

Bacterial envelope integrity is crucial for survival and growth, making envelope stress response system essential. In *Brucella abortus*, these systems are under-investigated. Tn-seq analysis, with and without the soft anionic detergent deoxycholate (DOC), identified a two-component system (TCS) CenK-CenR and a homologue of the Mla pathway are required to grow on DOC. According to a previous Tn-seq analysis, these mutants are also attenuated in macrophages suggesting that mutants failing to adapt to envelope stress are indeed impaired for their ability to survive and/or grow inside host cells.

CenK-CenR TCS was first identified in Caulobacter crescentus where it plays an essential role in maintaining cell envelope integrity. In Brucella this TCS is poorly characterized. We generated markerless ΔcenR and ΔcenRΔcenK mutants which present a growth defect phenotype on DOC. Indicating that they are involved in envelope stress sensing. Interestingly constitutively active CenR mutant presents the same growth phenotype than the WT strain. CenK deletion strain and constitutively inactive CenR mutants could not be obtained, suggesting that the unphosphorylated CenR is probably bactericidal or bacteriostatic for *B. abortus*.

In *Escherichia coli* the Mla pathway maintains lipid asymmetry and membrane integrity by phospholipids transport between both membranes. In *B. abortus* the deletion strains for Mla components have a growth defect on DOC indicating that this system is involved in envelope integrity. Moreover, Mla mutants seem unable to infect macrophages, which could be due impaired intracellular survival, or a defective entry. These results confirm that Mla pathway plays a role in *B. abortus* virulence.

T321 - Beneficial effects of enriched polyphenols culture of *Lactobacillus helveticus* on the intestinal *Candida albicans* growth

Presenting Author - *Silvia Rizzo, Università Cattolica Del Sacro Cuore, Italy*

Author/s – *Maura Di Vito, Paola Mattarelli, Laura Micheli, Claudia Mazzuca, Roberto Rosato, Maurizio Sanguinetti*

Abstract Content

Background: *Candida albicans* (CA) is an opportunistic pathogen, commensal member of the intestinal microbiota. However, specific pathophysiological conditions can predispose to invasive, mostly nosocomial, CA infections. Probiotics, such as *Lactobacillus helveticus* (LH), are known for their beneficial effects on the human gastrointestinal system. Polyphenols (PPs), in addition to having antioxidant and anti-inflammatory properties, interact with microbial metabolism, exerting health effects through their related metabolic derivatives. PPs are molecules of secondary plant metabolism also identified in the residual water (RW) present in the main tank of the distiller at the end of essential oils production (different from hydrolates).

Objectives: This study aimed to investigate the interfering effect between LH and PP in modulating CA growth.

Methods: The main PP content in the lyophilized RW obtained from the hydro-distillation of Origanum vulgare was evaluated by HPLC. The 24h growth curves of LH and CA in the presence or absence of PPs (4 mg/ml and 0,5 mg/ml) were obtained. CA was co-cultured with LH and PP dilutions or LH supernatant, and relative CFU/mL were evaluated. The LH secretome content in the presence of PPs and their PPs metabolic derivate were studied through biochemical analyzes.

Results: Rosmarinic acid is the main PP identified. Growth curves show that only LH is stimulated by the presence of PPs. In presence of LH and PPs the growth of CA is significantly inhibited. Supernatant and biochemical studies indicate that the inhibiting activity is exerted by both the secretome and the contact inhibition given by LH.

T322 - Characterization of a regulatory network governing a flotillin-containing operon in *Pseudomonas aeruginosa*

Presenting Author - *Martín Sastre Gallardo, National Centre for Biotechnology (CNB-CSIC), Spain*

Author/s – *Ana Isabel Rico Errazquin, Daniel López Serrano*

Abstract Content

Flotillins are ubiquitous membrane proteins that organize the Functional Membrane Microdomains (FMM) in bacteria. Thus, their production is necessary for the correct function of a myriad of bacterial processes. In Gram-negative bacteria, flotillin genes typically form a two-genes operon with the flotillin-associated gene nfeD. Here we show that the flotillin operon of the human pathogen *Pseudomonas aeruginosa* is exceptionally constituted by a four-gene cluster. The regulation of this operon is controlled by the global post-transcriptional regulator RsmA, which responds to specific membrane stresses. In addition, we show that the two extra genes of the operon, PA14_16140 and PA14_16150, generate a tight regulatory network of flotillin in response to other cellular cues. We have implemented a CRISPR-Cas9 recombineering technique in the laboratory to generate different operon mutants and study their physiological implications within an infection context. Our work reveals an intricate regulatory network for the flotillin operon in *P. aeruginosa* with potential significance in the establishment of infections.

T323 - Molecular characterization of phototropin signalosome regulating photophysiological processes in green algae

Presenting Author - *Sunita Sharma, Jawaharlal Nehru University, India*

Author/s – *Kumari Sushmita, Sibaji K. Sanyal, Irina Sizova, Peter Hegemann, Suneel Kateriya,*

Abstract Content

Phototropin is a blue light-sensing photoreceptor, composed of two LOV domains that non-covalently bind FMN as chromophore and a serine-threonine kinase domain at its C-terminus. Phototropin is well-established in higher plants and aquatic algae. However, the photoreceptor biology of the evolutionary important green alga has not yet been established. We have characterized the photoactivated LOV domain of phototropin from an evolutionary important green alga and compared unique characteristics with the aquatic green algae, *Chlamydomonas reinhardtii* phototropin (CrPhot) LOV domain.

The intraflagellar transport (IFT) machinery responsible for bidirectional movement of proteins in cilia or flagella is involved in trafficking of CrPhot. We investigated the molecular component and mechanistic basis of phototropin trafficking using immunoblotting and immunolocalization experiments with different IFT mutants.

The CrPhot knock-out strains showed reduced photomotility, and decreased levels of other interacting proteins like ChRs and, 14-3-3 in comparison to wild-type strain. Various approaches like protein and RNA profiling (qRT-PCR), and proteomics were applied to delineate its role in light-mediated physiology in C. reinhardtii.

We have also studied the photochemistry, oligomerization, and photodynamic behavior of LOV1 domain of a novel terrestrial algal phototropin using UV-visible, CD, AFM, and fluorescence spectroscopy methods. Our findings emphasize the unique characteristics of LOV domain in terms of unusual long dark recovery time. Different variants of LOV1 protein were produced and photochemistry was compared with respect to the wild-type protein. We proposed the optogenetic potential of the engineered LOV domain in modulation of diverse cellular signaling pathways like ciliogenesis and apoptosis.

T324 - Dynamic changes in protein structure and transcriptional levels of a toxin-antitoxin system from *Pseudomonas putida*

Presenting Author - *Frederik Oskar Henriksen, Department of Biological and Chemical Engineering, Denmark*

Author/s – *Ragnhild Bager Skjerning, Ditlev Egeskov Brodersen*

Abstract Content

Bacteria live in complex and competitive environments, constantly faced with threats they must overcome to survive. This has led to the evolution of numerous survival mechanisms. Toxin-Antitoxin (TA) systems are a class of common two-gene operons encoding a metabolically deactivating toxin and its cognate antitoxin. In the most studied TA group, type II, the antitoxin inhibits the toxin by direct protein-protein interactions and plays a role in autoregulation of the TA operon. *Pseudomonas putida* encodes a type II TA system, xre-res, where the TA complex displays an unusual 2:4 stoichiometry with each Xre antitoxin dimer forming a domain structurally similar to the λ phage Cro repressor.

We aim to understand transcriptional regulation of the xre-res operon through investigation of promoter requirements for Xre-RES binding and transcriptional activity.

In silico promoter prediction has suggested recognition elements of both σ70 and σ54, which overlay well with one of four repeated sequence elements in the xre-res promoter. *In vivo* expression analysis using a GFP reporter assay showed that the predicted promoter region was essential for transcription, and Xre-RES expression represses transcription only when a different repeat was present. *In vitro* analytical size exclusion chromatography (SEC) and SEC-multiple angle light scattering (MALS) showed specific binding of Xre-RES with this repeat. Finally, we use mass photometry to suggests that the Xre-RES complex can exist in two states; a 2:4 DNA-binding complex as seen in the crystal structure, and a putative 2:2 non-binding complex. This suggests regulation by a translational-responsive model, which we are currently investigating.

T325 - Effect of antimicrobial toothpaste on healthy salivary microbiota

Presenting Author - *Veronika Chuchmová, Masaryk University, Czech Republic*

Author/s – *Kristýna Brodíková, Martin Krsek*

Abstract Content

The salivary microbiota is a natural part of the oral cavity. It reflects the state of health and the influences that affect it. When the salivary microbiota is in balance, it plays a protective role against pathogen overgrowth and benefits the entire human body. Investigating the impact of various factors to which the salivary microbiota is exposed is essential for its healthy maintenance.

In this research the effect of antimicrobial toothpaste on healthy salivary microbita was investigated.

Eleven generally healthy respondents, non-smokers, without antibiotic treatment, with excellent oral hygiene and without periodontal disease or dental caries, were selected.

Antimicrobial toothpaste with Chlorhexidine 0.06% and Cetylpyridine Chloride 0.05% was used for 12 weeks; during that time respondents did not use any other dental products.

Saliva samples were collected before and after the use of antimicrobial toothpaste. Salivary microbiota analysis was performed by sequencing targeting the V4 region of the 16S rRNA gene.

Although there were changes in the salivary microbiome of individuals at different taxonomic levels before and after antimicrobial toothpaste use, statistical analysis showed statistically significant changes only for some specific groups of bacteria at individual taxonomic levels.

T326 - In Salmonella, PagR regulates its own synthesis and the expression of genes encoding transketolase C

Presenting Author - *Regan McCormick, University Of Georgia, United States*

Author/s – *Regan McCormick, Anastacia Parks, Jorge Escalante-Semerena*

Abstract Content

Background: The enteropathogen *Salmonella enterica* subsp. Enterica sv. Typhimurium str. LT2 (S. Typhimurium) utilizes genes encoded within the pathogenicity island 2 (SPI-2) to proliferate inside macrophages. Expression of SPI-2 is controlled by a complex network of transcriptional regulators and environmental cues, including the PagR protein. In conditions mimicking those within a macrophage, PagR ensures SPI-2 induction by upregulating the transcription of slyA, a known activator of SPI-2 (Jiang et al., 2020). Adjacent to pagR is a predicted five-gene operon encoding three genes (stm2342-4) whose function remains hypothetical, plus the subunits of transketolase TktC (i.e., tktD, tktE) (Shaw et al., 2018).

Results: We hypothesized that PagR regulated the expression of this potential operon. We determined that tktDE were part of the hypothetical operon, by performing reverse-transcription PCR (RT-PCR) across the genetic boundaries of all genes in the predicted operon. We also used quantitative RT-PCR on RNA from S. Typhimurium strains with and without PagR to show that tktDE were repressed by PagR. Electrophoretic mobility shift assays (EMSAs) and DNase I footprinting demonstrated that PagR bound to the intergenic region between stm2344 and pagR. Based on our results, we established binding sites for PagR. Finally, we used a pagR-lacZ reporter to show that PagR also regulated its own expression.

T327 - Structure of the cyclic beta-1,2-glucan synthase

Presenting Author - *Jaroslaw Sedzicki, Biozentrum of the University of Basel, Switzerland*

Author/s – *Dongchun Ni, Frank Lehmann, Henning Stahlberg, Christoph Dehio*

Abstract Content

Cyclic beta-glucans (CBGs) are circular polysaccharides found in the periplasm of many bacteria. In Rhizobiales, which include pathogens of mammals (*Brucella*) and plants (*Agrobacterium*), CBG plays a key role in the establishment of host-microbe interactions. CBGs are synthesized by the cyclic glucan synthase (Cgs), a massive multi-domain membrane protein of 320 kDa. So far, the structure of Cgs as well as the mechanisms underlining CBG synthesis have not been clarified. We used cryo-electron microscopy and functional approaches to study Cgs from *Agrobacterium tumefaciens*. We determined the full-length structure of this complex protein machinery and clarified key aspects related to CBG synthesis. Our research opens new possibilities for combating pathogens that rely on polysaccharide virulence factors. In addition, mechanistic understanding of Cgs may lead to new synthetic biology approaches for producing complex cyclic sugars.

T328 - Effect of copper supplementation of feed on microbiota and metabolites production in the piglet gut

Presenting Author - *Rafal Kolenda, Quadram Institute Bioscience, United Kingdom*

Author/s – *Rebecca Ansorge, Falk Hildebrand, Marwa Mohsen Hussain Ali Hassan, Roberto La Ragione, Rob Kingsley*

Abstract Content

Background: Copper sulphate is increasingly used as feed additive in the pig industry since the EU-wide ban on the use of antibiotics as growth promoters. It was considered as beneficial alternative to antibiotics, as it decreased the incidence of post-weaning diarrhoea and increased the rate of fattening. Widespread use of copper led to development of copper-resistant pathogens and environmental pollution. New alternatives are required to provide sustainable and environmental-friendly pig production.

Objectives: To understand the effect of copper sulphate as growth promoter we investigated the effect of high copper supplemented pig feed on the development of microbiome and metabolite production of 4–6-week-old piglets.

Methods: Four-week-old piglets(60) were separated into two groups and fed on diet with high(therapeutic) or low(nutritional) levels of copper sulphate. Faecal samples were collected immediately prior to and on day 7 and 14 following placing piglets on low or high copper diet. Metagenome sequence of total fecal DNA was determined using short read shotgun sequencing and metabolite levels were determined by NMR.

Results: Higher alpha diversity in the feces of piglets fed with normal copper diet was observed. Differences between high and low copper diet were observed for *Bifidobacterium*, *Clostridium*, *Escherichia*, *Holdemanella*, *Lactobacillus* and *Succinatimonas*. NMR analysis revealed effect of high copper diet on levels of formate, succinate, BCAA and xanthine. Functional analysis showed altered abundance of genes responsible for copper homeostasis, formate and BCAA metabolism. Our data indicate strong influence of high copper supplementation as habitat filtering factor on piglet intestinal microbiota and their function.

T329 - Transcriptome analysis of persistent cell formation in *Staphylococcus aureus*

Presenting Author - *Eon-Min Ko, Korea Disease Control And Prevention Agency, Republic of Korea*

Author/s – *Seonghan Kim*

Abstract Content

In *Staphylococcus aureus*, understanding of persistent cell formation is important for elucidation the mechanism of antibiotic response. In this study, persistent cell formation was confirmed when treated with ciprofloxacin (CIP). Transcripts of 2 h, 4 h, 6 h, 8 h and 24 h CIP-treated samples were compared with those of 0 h CIP-treated samples to evaluate genes important for the formation of persistent cells. A total of 745, 1,017, 1,132, 1,183, and 1,166 differentially expressed genes (DEGs, p-value < 0.05, |Fold change| ≥ 2) were identified in 2 h, 4 h, 6 h, 8 h, and 24 h samples relative to the 0h sample, respectively. 453 common DEGs were identified across all DEG sets. Of the 453 genes, 145 were induced and 308 were repressed. Gene ontology analysis showed that genes involved in 'Stress response to cadmium ion', 'Stress response to copper ion', 'Nucleotide-excision repair', and 'Inorganic phosphate transmembrane transporter activity' were upregulated, and 'Negative regulation of DNA-templated transcription, termination', 'Maltodextrin transport', 'Phosphoribosylformylglycinamidine synthase activity', and 'Nitrate reductase activity' were downregulated. Clusters of orthologous groups analysis using eggNOG database revealed that the categories 'Inorganic ion transport and metabolism' and 'Transcription' were significantly enriched in the upregulated common DEGs and downregulated common DEGs, respectively, except for the 'Function unknown' category. These results imply that upregulation of genes involved in inorganic ion transport and downregulation of genes involved in transcription are important for persistent cell formation.

T330 - Unexplored pathways of arsenate response in yeast

Presenting Author - *Sofia Silva, Universidade Nova de Lisboa - Instituto de Tecnologia Quimica e Biologica Antonio Xavier, Portugal*

Author/s – *Sofia M. da Silva, Teresa Pissarro, Américo G. Duarte, Catarina Amaral, Catarina Pimentel*

Abstract Content

The development of sustainable and environmentally friendly processes able to mitigate arsenic contamination, a major public health concern, relies on the comprehensive knowledge of arsenic detoxification pathways. In *Saccharomyces cerevisiae*, a powerful eukaryotic model organism, the arsenate (AsV) reductase Acr2, the arsenite (AsIII) permease Acr3 and the aquaglyceroporin Fps1 are key players in arsenic detoxification, and their mode of action is well documented. However, our recent data indicate that arsenic detoxification is far more complex than generally assumed. We found that the deletion of both ACR2 and ACR3 genes increased *S. cerevisiae* sensitivity to AsV and led to a massive cellular accumulation of arsenic, which does not occur in Δacr2 single mutant. These results challenge the accepted model for AsV detoxification in yeasts, and suggest the direct involvement of the Acr3 transporter in AsV detoxification. On the other hand, although the deletion of FPS1 from Δacr2 background also renders the cells more sensitive to AsV, it does not lead to arsenic accumulation. Instead, it triggers the accumulation of large amounts of glycerol in the presence of AsV, suggesting that osmolyte export is required to avoid AsV toxicity.

In order to determine if AsV is indeed a substrate of the Acr3 permease we expressed a recombinant form of the protein on yeast Δacr2Δacr3 cells and prepared plasma membrane vesicles by two-phase partitioning. We then evaluated the ability of the vesicles, expressing the recombinant Acr3, to transport AsV as well as other metals, and determined the parameters of the translocations.

T331 - The expression gradient of integron cassette arrays is shaped by cassette identity

Presenting Author - *André Carvalho, Complutense University of Madrid, Spain*

Author/s – *Alberto Hipólito, Lucia Garcia-Pastor, Ester Vergara, Aránzazu Buendía-Andrés, Teresa García-Seco, José Antonio Escudero,*

Abstract Content

Background: Integrons are one of the major contributors to the dissemination of antimicrobial resistance in gram-negative bacteria. Specifically, class 1 integrons are composed of a stable platform and a variable cassette array which can contain up to several integron cassettes encoding mostly antibiotic resistance genes. Interestingly, most of these cassettes are promoterless, with their expression being granted by the Pc promoter located upstream of the array. This results in an expression gradient where the cassettes more proximal to the Pc promoter have higher expression levels.

Objectives: We sought to study how this expression gradient may vary according to the identity and order of the cassettes in the array and how it can impact antibiotic resistance levels.

Methods and Results: We transformed *Escherichia coli* MG1655 with a vector containing a class 1 integron-like structure with a variable cassette array composed of an antibiotic resistance cassette in first position followed by a gfp gene. We were able to study the impact of 135 different antibiotic resistance cassettes on GFP expression through flow cytometry and RT-qPCR. We show that fluorescence varies between a factor of 0.01 and 3, depending on the cassette located immediately upstream. Such variations correlate well with gfp mRNA levels suggesting a transcriptional basis for this phenomenon. Importantly, when we exchanged the gfp gene for a specific antibiotic resistance cassette we observed that the MIC for that antibiotic also changed in function of the identity of the preceding cassette. Thus, integron array identity may shape the resistance profiles of bacteria.

T332 - An integrated transcriptomics–functional genomics approach reveals fitness-relevant ncRNAs in *Bacteroides thetaiotaomicron*

Presenting Author - *Daniel Ryan, Helmholtz Institute for RNA-based Infection Research, Germany*

Author/s – *Elise Bornet, Gianluca Prezza, Shuba Varshini Alampalli, Taís Franco de Carvalho, Hannah Felchle, Titus Ebecke, Regan Hayward, Adam M. Deutschbauer*

Abstract Content

Gene expression plasticity allows bacteria to adapt to diverse environments, tie their metabolism to available nutrients, and cope with stress. This is particularly relevant in a niche as dynamic and hostile as the lower human intestine, yet transcriptional networks remain largely unknown in gut *Bacteroides* spp.

Here, we map transcriptional units and profile their expression levels in the predominant human gut commensal *Bacteroides thetaiotaomicron* over a suite of 15 defined experimental conditions that are relevant *in vivo*, such as variation of temperature, pH, and oxygen tension, antibiotic stress, and growth on simple carbohydrates or on mammalian mucin-derived glycans. Thereby, we infer stress- and carbon source-specific transcriptional regulons, including conditional expression of capsular polysaccharides and polysaccharide utilization loci, and expand the annotation of small regulatory RNAs (sRNAs) in this organism. Integrating this comprehensive expression atlas with transposon mutant fitness data, we identify conditionally important sRNAs. One example is MasB, whose inactivation led to increased bacterial tolerance of tetracyclines. Using MS2 affinity purification coupled with RNA sequencing, we predict targets of this sRNA and discuss their potential role in the context of the MasB-associated phenotype.

Together, this transcriptomic compendium in combination with functional sRNA genomics—publicly available through a new iteration of the 'Theta-Base' web browser—constitutes a valuable resource for the microbiome and sRNA research communities alike.

T333 - Plasmid-derived beta-lactamase expression induces collateral sensitivity to azithromycin and colistin

Presenting Author - *Laura Álvaro Llorente, Instituto Ramón Y Cajal De Investigación Sanitaria (irycis), Spain*

Author/s – *Cristina Herencias, Ada Muñoz, Laura Jaraba, Álvaro San Millán, Jerónimo Rodríguez-Beltrán*

Abstract Content

The dissemination of antimicrobial resistance genes and the shortage of new antibiotics highlights the need for new therapeutic approaches to combat antibiotic-resistant bacteria. Collateral sensitivity (CS) —defined as the increased susceptibility to one antibiotic after the acquisition of resistance to a second antibiotic— is a promising strategy that capitalises on the event of resistance acquisition. Recently, it has been described that the acquisition of clinical resistance plasmids induces CS, increasing sensitivity to non-directly related antimicrobials by a mechanism yet to be characterised. Most of the plasmids in which CS was observed carried beta-lactam resistance genes suggesting that beta-lactamase expression could be responsible for the CS phenotype. In this work, we hypothesised that plasmid-derived beta-lactamase expression might trigger CS. To test this hypothesis, we built a library of plasmids driving the expression of clinically relevant beta-lactamases under the control of an arabinose-inducible promoter and expressed them in *E. coli* BW25113. Our results suggest that the expression of clinically relevant beta-lactamases of plasmid origin induces CS to the antibiotics azithromycin and colistin. Moreover, by measuring CS in a range of representative strains, we show that beta-lactamase-induced CS is conserved across the *E. coli* phylogeny. These results pave the way for designing new combination therapies to combat plasmid-mediated antimicrobial resistance while immediately suggesting mechanistic insights that identify beta-lactam resistance as a potential new therapeutic target.

T334 - Tse1 mobilized by T6SS of *Pseudomonas* induces sporulation of *Bacillus* via σW

Presenting Author - *Alicia Isabel Pérez-Lorente, IHSM-UMA-CSIC, Spain*

Author/s – *Carlos Molina-Santiago, Antonio de Vicente, Diego Romero*

Abstract Content

The extracellular matrix and sporulation are defensive mechanisms used by *Bacillus* cells when they interact with *Pseudomonas* strains expressing a type VI secretion system (T6SS) (Molina-Santiago et al., 2019). Here, we define Tse1 as the main toxin mobilized by the *Pseudomonas* T6SS that triggers sporulation in *Bacillus*. We characterize Tse1 as a peptidoglycan hydrolase and electron microscopy analysis and the use of diverse chemical probes, let us visualize malfunction of the *Bacillus* cell membrane. By performing RNA-seq and immunocytochemistry analyses, we also delineate the response of *Bacillus* cells to Tse1, which through the coordinated actions of the extracellular sigma factor σW and the cytoplasmic histidine kinases KinA and KinB, culminates in activation of the sporulation cascade. We propose that this cellular developmental response is conserved in Bacilli to defend against the toxicity of T6SS-mobilized Tse1 effector.

T335 - Genetic analysis identifies candidate genes that suppress SCV phenotype of *Pseudomonas aeruginosa* Δorn

Presenting Author - *Sookyoung Kim, University of Maryland, College Park, United States*

Author/s – *Husan Turdiev, Mona Orr, Vincent Lee*

Abstract Content – RNA degradation is a fundamental process to recycle nucleotides in the cell. This is a sequential process in which RNA is cleaved by endonucleolytic enzymes to generate RNA fragments. Eventually, these RNA fragments result in a dinucleotide product that must be cleaved into mononucleotides. Orn is the only enzyme that catalyze this terminal step in RNA degradation that cleaves dinucleotides to mononucleitudes. In γ-proteobacteria, c-di-GMP is linearized into pGpG by phosphodiesterase. The pGpG intermediate is further hydrolyze into two GMP by Orn. For *P. aeruginosa*, Δorn mutant displays small colony variant (SCV) phenotype which led us to hypothesize that the accumulation of dinucleotides in Δorn directly act on protein targets to cause toxicity to the cell. To investigate SCV in Δorn, we carry out transposon mutagenesis to suppress the Δorn SCV phenotype Whole genome sequencing of these suppressors verified orn gene deletion and the transposon locations. In-frame deletion of the genes with transposon insertion in combination with Δorn failed to recapitulate SCV suppression indicating that these genes are not responsible for the suppression. When complementing the Δorn strain with a plasmid containing the genomic library of the transposon suppressors, a number of suppressors were identified containing an overlapping 4 gene fragment. Cell lysates of these suppressors can hydrolyze pGpG, unlike the lysate of Δorn. Current studies seek to understand the mechanism of suppression.

T336 - Novel cationic backbone antimicrobial peptides to address AMR

Presenting Author - *Anupam Mishra, University Of Delhi, India*

Author/s – *Kantaraja Chindera, satish Kumar Awasthi*

Abstract Content

The widespread prevalence of antimicrobial resistance (AMR) among bacterial pathogens complicates treatment and adds to economic loss. AMR is considered a “global health and development threat” affecting both human and veterinary medicine. There is an urgent need for the development of new classes of antimicrobials as alternative to antibiotics.

Among several alternatives antimicrobial peptides (AMPs), synthetic analogues, inorganic antimicrobials and polymers are important candidates. The primary mechanism of antibacterial action of these compounds involves initial binding to negatively charged phospholipids or lipopolysaccharides on the bacterial membrane to create pores resulting in leakage of cellular components and cell death, they are less prone to bacterial resistance mechanisms.

Current AMPs have positive charge arising from cationic amino acids or analogues, wherein the cationic moiety is on side chain hanging from the peptide backbone. We hypothesised that, it’s possible to create new generation AMPs by introduce the cationic group on the peptide backbone instead on side chain, this strategy would enhance antimicrobial activity. We have developed a method for synthesis of novel unnatural amino acids, yielding peptides with a novel moiety on the peptide backbone, yielding net positive charge on the peptide backbone. We have produced two methods of producing the cationic backbone peptides. Most of the peptides synthesised exhibit potent antimicrobial activity against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. Moreover, our method can be used to generate a new library of AMPs to provide novel therapeutics for drug-resistant bacterial infections.

T337 - Differential chain length in xylan-derived fibers drive the divergence in gut-derived microbial consortia

Presenting Author - *Ming-Hsu Chen, , Taiwan*

Author/s – *Sainan Zhao, Raymond Lau*

Abstract Content

Xylan, an abundant polysaccharide in the plant cell wall, is commonly consumed as a source of dietary fiber. Although researchers have known that the composition and structural property of xylan affects its digestion fate in the human colon, the mechanism of xylan degradation and the involved microbial taxa are not well understood. In this study, we employed a sequential batch fermentation approach to elucidate the impact of xylan chain length on the assembly of the gut-derived microbial consortia. Five xylan-derived substrates (F0, F1, F2, F3, F4) with varying degrees of polymerization (DP > 40; DP20–40; DP10–20; DP5–10; DP2–4) were fermented with human gut microbiota along with xylan (polymer control). Sequential passages were conducted to generate substrate-specific microbial consortia; cultures were transferred into fresh media every 24 h at a dilution rate of 50 for 3 times. Dynamic changes of microbial communities in each lineage were monitored using amplicon sequencing. Results indicated that the fecal inoculum treated with substrates with larger DP (xylan, F0, F1) displayed resilience to the dilution pressure, whereas the fecal inoculum treated with substrates with smaller DP samples (F2, F3, F4) was reduced in the measures of species evenness over time. Dominant microbial taxa in each consortium were revealed; cultures treated by xylan had a high abundance of Firmicutes; substrates F0 and F1 were particularly favored by Bacteroidetes, whereas Actinobacteria were dominant in F3 and F4 cultures. To conclude, chain length is a crucial determinant for selecting gut microorganisms to utilize xylan-derived fibers.

T339 - New moderately halophilic bacteria from hypersaline soils: *Terrihalobacillus insolitus* gen. nov., sp. nov, and *Aquibacillus salsiterrae*

Presenting Author - *Antonio Ventosa, Universidad de Sevilla, Spain*

Author/s – *Cristina Galisteo, Rafael R. de la Haba, Cristina Sánchez-Porro*

Abstract Content

Metagenomic studies on hypersaline soils of Odiel Saltmarshes Natural Area, South-west Spain have permitted to determine the prokaryotic diversity and their activities. Recently, we described new Fodinibius species, which based on the metagenomic data, were determined to be one of the major taxa on these habitats. However, a large proportion of the existing prokaryotes on these saline soils have not been isolated in pure culture.

In this study we describe the isolation and characterization of 32 new strains belonging to the phylum Bacillota, which represents a minor fraction of the prokaryotic population of these soils. These strains were isolated by the dilution-plating technique on R2A medium supplemented with 7.5% salts, after 3 months of incubation at 28ºC. These isolates were most closely related to species of the genus *Aquibacillus*, but constituted two clusters, clearly separated of species of this genus. Representative strains were analysed based on their genome sequences as well as phenotypic and chemotaxonomic features. These analyses confirmed that these two groups constituted a new genus and a new species, respectively, for which we propose the names *Terrihalobacillus insolitus* gen. nov., sp. nov, and *Aquibacillus salsiterrae* sp. nov.

Genome recruitment analysis, when compared to a large number of metagenomic databases from different hypersaline environments, indicate that species of the genera *Terrihalobacillus* and *Aquibacillus* belong to the rare biosphere on these habitats. However, they could play an important role, we determined that they present a molybdenum cofactor biosynthesis pathway as well as a salt-out mechanism of osmoregulation.

T340 - Genomics and taxonomy of the “tumorigenes” clade of the family Rhizobiaceae

Presenting Author - *Nemanja Kuzmanovic, Julius Kühn Institute (JKI), Institute for Plant Protection in Horticulture and Urban Green, Germany*

Author/s – *George C. diCenzo, Boyke Bunk, Cathrin Spröer, Anja Frühling, Meina Neumann-Schaal, Jörg Overmann, Kornelia Smalla*

Abstract Content

Background: Tumorigenic bacteria of the family Rhizobiaceae are responsible for crown gall disease of numerous agricultural crops worldwide. These pathogens are primarily identified within the genus Agrobacterium. However, in our previous work, we identified a distinct Rhizobium clade named as “tumorigenes”, which includes the tumorigenic species Rhizobium tumorigenes, as well as strains causing crown gall disease on rhododendron.

Objectives: The objective of this study was to gain insights into genomic diversity of the “tumorigenes” clade of the family Rhizobiaceae, and to taxonomically characterize the rhododendron strains.

Methods: To obtain high-quality closed genomes, representative strains were subjected to whole-genome sequencing using a combination of long- (PacBio) and short-read (Illumina) sequencing technologies. Using the resulting genome sequences, comparative genomic and phylogenomic analyses were performed. Additionally, the phenotypic traits of selected strains were analyzed.

Results: Polyphasic taxonomic characterization revealed that tumorigenic strains isolated from rhododendron represent a novel species of the genus Rhizobium for which the name *Rhizobium rhododendri* sp. nov. is proposed. *R. rhododendri* can be clearly distinguished from closely related species *R. tumorigenes* based on overall genome relatedness indices. Both *R. tumorigenes* and *R. rhododendri* contain multipartite genomes, including a chromosome, putative chromids, and megaplasmids. However, these two species showed distinct genome architecture. Comparative genomic analyses indicated that a large putative chromid of *R. rhododendri* resulted from the cointegration of an ancestral megaplasmid and two putative chromids, following its divergence from *R. tumorigenes*. Additionally, evidence of inter-replicon DNA exchange between putative chromids of one *R. tumorigenes* lineage was detected.

T341 - Insights into the phylogenetic inconsistencies of the genus *Amazonocrinis* and description of *Amazonocrinis malviyae* sp. nov.

Presenting Author - *Aniket Saraf, R J College of Arts, Science and Commerce, India*

Author/s – *Naresh Kumar, Sagarika Pal, Deeksha Mishra, Prashant Singh*

Abstract Content

Background: The genus *Nostoc* represents a highly polyphyletic lineage and is therefore considered a challenging group to study taxonomically. The advent of the polyphasic approach has led to the description of numerous *Nostoc*-like genera that have morphological similarities with *Nostoc* but are phylogenetically distinct from the *Nostoc* sensu stricto. Interestingly, the taxonomic studies on *Nostoc* and *Nostoc*-like taxa from India using a polyphasic approach are limited. Therefore, conducting taxonomic studies of cyanobacteria from the underexplored region of Jammu and Kashmir using a polyphasic approach is significant.

Objective: To characterize epilithic cyanobacterium strain 19C-PS isolated from the J&K using a polyphasic approach.

Methodology: Strain 19C-PS was first subjected to morphological characterization followed by phylogenetic analysis using the 16S rRNA gene and 16S-23S ITS region, and 16S-23S ITS secondary structure analysis.

Results: In the phylogenetic trees inferred using the 16S rRNA gene, the genus *Amazonocrinis* was split into two clades. The splitting of *Amazonocrinis* is probably because of the addition of phylogenetically related sequences that were absent in earlier studies. However, in the 16S-23S ITS phylogenetic analysis, members of both clades were intermixed. Further, phylogenetic positioning of 19C-PS within *Amazonocrinis* sensu stricto, unique folded secondary structures of D1-D1', BoxB, V2, and V3 regions of 16S-23S ITS and the presence of heterocytes in series indicated 19C-PS to be a novel species of *Amazonocrinis*; and is described as *A. malviyae*. This study highlights the importance of taxon sampling and advocates the need for further studies to resolve the taxonomic discrepancies within *Amazonocrinis*.

T342 - *Helicobacter kumamotonensis* sp. nov. isolated from human specimens could be misidentified as *Helicobacter equorum*, horse origin

Presenting Author - *Yoshiaki Kawamura, Aichi Gakuin University, Japan*

Author/s – *Ryo Kutsuna, Junko Tomida, Kei-ichi Yamamoto, Tohru Miyoshi-Akiyama, Hiroshi Tsutsuki, Tomohiro Sawa, Miki Okuno, Yoshitoshi Ogura, Masao Matsuoka, Tatusya Kawaguchi*

Abstract Content

Background: We isolated gram-stain-negative, spiral bacterium from the blood of a patient with diffuse large B-cell lymphoma. This isolate resembled *Helicobacter equorum*, which had been thought to be related to only horses.

Objectives: We want to clarify the taxonomical position of our human isolate.

Methods and Results: 16S rRNA gene sequence analysis showed our isolate was very closely related to *Helicobacter equorum* LMG 23362T (99.1% similarity). We found another human isolate PAGU 1750 (=CCUG 41437) very also close to PAGU 1991.

23S rRNA gene and GyrA amino sequences supported the close relationship between the two human isolates and the horse strain. However, the whole genome analysis (ANI, dDDH) demonstrated that the two human isolates formed a single species, distinct from *H. equorum*. Biochemically and morphologically, human isolates could be distinguished from closely related species. We concluded that the two human isolates were a novel species. We proposed *Helicobacter kumamotonensis* sp. nov., with the type strain PAGU 1991T (=GTC 16810T = CCUG 75774T).

In this study, we proposed a new species based on two well-distinct isolates; however, Minimal standards for new species belonging to Campylobacteraceae and *Helicobacter*aceae: *Campylobacter*, *Arcobacter*, *Helicobacter*, and *Wolinella* spp state that descriptions should ideally be based on not fewer than five isolates. Indeed, our proposal using two isolates had been complicated. Under these circumstances, some new species candidates have not been proposed due to a lack of the number of isolates. This prevents us from understanding biodiversity. We want to discuss this issue with the participants.

T343 - Animals as reservoirs for diverse novel *Helicobacter* species

Presenting Author - *Bojan Papić, University of Ljubljana, Slovenia*

Author/s – *Darja Kušar, Igor Gruntar*

Abstract Content

Background: The genus *Helicobacter* currently comprises 51 validly published species. Whereas *H. pylori* is a well-known human pathogen, many gastric or enterohepatic *Helicobacter* species have been described in various animal species, suggesting their widespread presence and possible zoonotic significance. We recently described four novel *Helicobacter* species, namely *H. labacensis*, *H. mehlei* and *H. vulpis* from gastric mucosa of red foxes in 2020, and *H. colisuis* from pig cecum in 2022.

Objectives: We systematically characterized seven spiral-shaped isolates characteristic of *Helicobacter* sp., which were obtained from cloacal swabs of migratory birds of the genera *Sylvia* and *Acrocephalus*.

Methods: A polyphasic approach including WGS, MALDI-TOF and biochemical tests was applied.

Results: The seven studied isolates comprised a novel *Helicobacter* species, for which we propose the name *H. passerinus* sp. nov. The pairwise average nucleotide identity (ANI) values between the studied isolates were 99.0–99.7 %. The core genome phylogeny revealed that *H. passerinus* is most closely related to *H. anseris* (ANI < 72.7 %). Of note, 16S rRNA gene proved to be a poor marker for phylogenetic reconstruction of the genus *Helicobacter*. The present findings confirm that migratory birds and wildlife in general harbor diverse Helicobacters, including novel species.

T344 - *Serratia grimonti* sp. nov. isolated from human and comparative genomics with *Serratia nevei*

Presenting Author - *Christiane Bouchier, Institut Pasteur - Paris, France*

Author/s – *Sandrine Favre-Rochex, Christiane Bouchier, Estelle Mühle, Dominique Clermont, Olivier Chesneau*

Abstract Content – Recently, *Serratia nevei* and Serratia bockelmannii have been described as novel species closely related to *Serratia marcescens*, *Serratia nematodiphila* and *Serratia ureilytica*. In order to update the nomenclature of *S. marcescens* strains hosted for a long time in the Collection of Institut Pasteur, Paris, France, a taxonomic study was undertaken. The studied strains were isolated from human clinical samples between 1967 and 2005, and roughly identified as *S. marcescens* according to physiological and biochemical tests done at the time of their deposit. Based on whole genome sequence (WGS) data and phylogenomic analysis, these eight strains have been shown to delineate a novel taxon well-separated from its nearest neighbor *S. nevei*, and the name *Serratia grimonti* sp. nov. is proposed. A comparative genomic analysis between *S. nevei* and *S. grimonti* was conducted and revealed that the latter is devoid of the urease operon genes and tet(41) resistance gene.

T345 - The architecture of the Asgard Archaea Rag GTPase heterodimers: tracing the origin of the eukaryotic Gtr/Rag GTPases

Presenting Author - *Fatima Alsheikh, University Of Gezira, Sudan*

Author/s – *Robert Robinson, Wipa Alsheikh,*

Abstract Content

Background: The origin of the small GTPases superfamily is poorly understood. Nonetheless, the discovery of Asgard archaea provided new insights into the process of eukaryogenesis and the origin of the eukaryotic signature proteins, due to the uniqueness of their genomes among prokaryotes in harboring a substantially large number of small GTPases. Thus, allowing the reinvestigation of small GTPases origin and early evolution.

Objectives: Our study aims to identify Gtr/Rag GTPases heterodimers among the Asgard archaea clade and determine their structural similarities and differences to the eukaryotic Gtr/Rag GTPases.

Method: The Asgard Rag GTPases were identified by homology searching. Then the heterodimeric pairs were predicted using AlphaFold2. The phylogenetic analysis was conducted with a selected representative set of small GTPases. The Asgard Rag heterodimer was co-expressed in *E. coli* BL21(DE3).

Results: Rag GTPases are widely distributed in lokiarchaeota and helarchaeota phyla. The phylogenetic analysis consistently grouped the eukaryotic Gtr/Rag GTPases as sister to the identified archaeal sequences, both in a bifurcating node from within the larger Arf-like superfamily. Among these, AlphaFold2 was able to predict the formation of 21 heterodimers, which were predicted to share a similar structure model to the eukaryotic Gtr/Rag dimers. Generally, the two GTPase subunits dimerize through their C-terminal domains while their N-terminal G-domains are not involved in the heterodimer formation. And unlike their eukaryotic counterparts, the G domains of the heterodimers lack key catalytic eukaryotic residues, suggesting a distinct mode for subunits communication. The formation of the heterodimer was confirmed via size exclusion chromatography.

T346 - Investigation of the antimicrobial potential of *Streptomyces* from the bark beetle microbiome

Presenting Author - *Anne Goll, University of Freiburg, Germany*

Author/s – *Andreas Bechthold*

Abstract Content

Background: Actinobacteria, particularly the genus *Streptomyces*, are an excellent source of potent natural products with antibiotic and antifungal properties. In the face of increasing antibiotic resistance, sources other than soil are being sought. It is hoped that challenging environments (such as marine sediments, plant roots or insects) will force *Streptomyces* to adapt well and thus offer great potential for the formation of bioactive secondary metabolites. It has already been shown that *Streptomyces* living with insects such as fungus-growing ants, bees and bark beetles, e.g. the North American pine beetle, are able to produce interesting new antimicrobial compounds [1].

Objectives: In this context, the microbiome of central European bark beetles will be isolated and incubated to search for novel species. Our aim is to screen these *Streptomyces* by a genome mining approach for the presence of putative biosynthetic gene clusters (BGCs) and to associate them with compounds found in the extracts.

Methods: We analysed 71 *Streptomyces* from the microbiome of European bark beetles, such as the European spruce bark beetle, by 16S rRNA sequencing and taxonomic analysis by phylogenetic tree construction. Three potentially novel *Streptomyces* were identified and whole genome sequencing was performed. Crude extracts were purified and screened for bioactive compounds.

Results: Three novel *Streptomyces* species were identified. Bioinformatic analyses suggest that they have a high potential for the production of antimicrobial agents.

T347 - SPINGO 2.0: Improved identification and taxonomic classification of 16S rRNA amplicon reads

Presenting Author - *Kardokh Kaka Bra, University College Cork, Ireland*

Author/s – *Marcus Claesson*

Abstract Content

Ribosomal ribonucleic acid (rRNA) genes have been extensively utilized as genetic markers for the taxonomic classification of microorganisms. Specifically, the small subunit 16s rRNA gene is commonly utilized for taxonomic assignment at the species or genus level. The SPINGO software tool was introduced as a solution for extracting partial rRNA sequences from large-scale sequencing datasets and mapping them to their bacterial or archaeal origin. This study presents an updated version of SPINGO (version 2.0), which offers extended functionality, including support for an integrated reference database, combining the latest iterations of the Ribosomal Database Project (RDP) database version 18 and SILVA database version 138, and a significantly improved classifier that allows for classification down to the genus or species level with enhanced precision and without incurring substantial computational costs. A comprehensive performance evaluation of SPINGO 2.0 was conducted, comparing its results to those of other widely utilized taxonomic classifiers, utilizing simulated, mock, and real-life samples. The results indicate that SPINGO 2.0 often performs better than current methods in terms of correct predictions, while continuing to maintain a low misclassification rate and speedy performance.

T348 - Discovery of novel *Psychrobacter* strains DM4 and DM8 from shrimps, *Lebbeus groenlandicus* and *Pandalopsis japonica*

Presenting Author - *Kyoung-Ho Kim, Pukyong National University, Republic of Korea*

Author/s – *Sang-Eon Kim, Hye-Jin Park*

Abstract Content

The shrimp species, *Lebbeus groenlandicus* and *Pandalopsis japonica*, are found in the East Sea region of Korea and are characterized by their ability to live in deep, low-temperature waters. Psychrophilic bacteria have been isolated, resulting in the discovery of two novel strains, DM4 and DM8. These strains were obtained from the stomachs of *Lebbeus groenlandicus* and *Pandalopsis japonica*, respectively, and were cultured on marine agar at 4°C for 5 days to isolate white colonies. Analysis of the 16S rRNA gene sequence showed that both DM4 and DM8 belonged to the genus *Psychrobacter* and had similarities of 98.75% and 98.02% to *Psychrobacter glacincola* and *Psychrobacter alimentarius*, respectively. Whole genome sequencing revealed that DM4 and DM8 were novel strains, as indicated by the analysis of average nucleotide identify (ANI), average amino acid identity (AAI), and digital DNA–DNA hybridisation (dDDH). DM4 is an aerobic, gram-negative, cocci bacterial strain, with diphosphatidylglycerol and sphingoglycolipid as its major polar lipids. On the other hand, DM8 is an aerobic, gram-positive, short-rod bacterial strain, with diphosphatidylglycerol as its major polar lipid. The characterization of psychrophilic bacteria can provide the knowledge to understand the role of microbial community in their hosts.

T349 - Isolation and characterization of a novel bacterium from the intestine of *Chionoecetes japonicus* in the East Sea of Korea

Presenting Author - *Sang-Eon Kim, Pukyong National University, Republic of Korea*

Author/s – *Hye-Jin Park, Kyoung-Ho Kim*

Abstract Content

Among the many crustaceans present in the Korean sea, *Chionoecetes japonicus* (red snow crab) belongs to the family Oregoniidae and lives in the deep and cold waters of the East Sea. It mainly inhabits rocky or muddy bottoms and has a close relationship with *Chionoecetes opilio* (snow crab), which shares the same living environment. A novel strain, Pro17, a gram-positive rod, was isolated from the anterior intestine of *Chionoecetes japonicus*. The yellow colony of Pro17 was obtained after incubation at 25℃ for 3 days on marine agar. Its genome was composed of two circular contigs, sized 4,189,217 bp and 48,614 bp. The 16S rRNA sequence similarity and genomic analysis revealed that it belongs to the genus *Roseovarius* and represents a potential new species. Strain Pro17 exhibited optimal growth at pH 7.5 and 3% NaCl (w/v), was catalase-positive, weakly oxidase-positive, and non-motile. The major polar lipids identified were phosphatidylglycerol and sphingoglycolipid. The purpose of this study was to identify the characteristics of microorganisms and their relationship with the host.

T350 - Purple sulfur bacteria with unique near-infrared light harvesting capabilities between 900-1000 nm

Presenting Author - *Steven Kuzyk, DSMZ German Collection of Microorganisms and Cell Cultures, Germany*

Author/s – *Anika Methner, Jörn Petersen, Sabine Bauer, Henner Brinkmann, Katja Sichau, Jörg Overmann, Steven B. Kuzyk, Anika Methner, Jörn Petersen, Sabine Bauer, Henner Brinkmann, Katja Sichau, Gerhard Wanner, Jacqueline Wolf, Meina Neumann-Schaal, Petra Henke, Marcus Tank, Cathrin Spröer, Boyke Bunk, Jörg Overmann*

Abstract Content

Photosynthesis provides most organic material to the base of foodwebs, but limited information exists for the range of light utilized. Specialized cultivation techniques were employed to isolate bacteria that could absorb energy in the 900-1000nm near-infrared spectrum, yielding the purple-sulfur bacterial strain 970, isolated from the shoreline of Baltrum island, Germany. While containing bacteriochlorophyll (BChl) a as its relatives, it absorbed light maximally at 963nm, which represents the furthest documented infrared-shift for BChl a-containing light-harvesting complexes known to date. Through a polyphasic approach that encompassed complete genome sequencing, as well as biochemical and physiological properties, strain 970 was closely related to *Thiorhodovibrio winogradskyi* DSM 6702T by 26.5, 81.9 and 98.0% similarity via dDDH, ANI, and 16S rRNA gene comparisons, respectively. The photosynthetic properties of strain 970 were unlike other *Thiorhodovibrio* spp., which contained typical LH absorbing characteristics of 800-870nm, as well as a newly discovered absorption peak at 908nm. Photosynthetic operons were strikingly different between strain 970 and the other species of the genus. Furthermore, strain 06511 was found to be divergent from *Trv. winogradskyi* DSM 6702T, with 25.3, 79.1, and 97.5% similarity via dDDH, ANI, and 16S rRNA gene homology, respectively. Strain 06511 was thereby described as *Thiorhodovibrio litoralis* sp. nov., and the unique strain 970 as *Thiorhodovibrio frisius* sp. nov.

The new species *Trv. litoralis* and *Trv. frisius* represent novel taxons of the same genus that show distinct and unusual absorption bands between 900-1000nm. These novel phototrophs aid our understanding of photoautotrophy and the evolution of photosynthesis.

T351 - Comparison of three methods/two modules for direct bacterial identification from positive blood culture bottles by MALDI-TOF MS

Presenting Author - *Dilan Karadag, Department of Medical Microbiology, Faculty of Medicine, Dokuz Eylül University, Turkey*

Author/s – *Mahmut Cem Ergon*

Abstract Content

Background: Rapid and accurate identification of microorganisms causing sepsis reduces mortality.

Objectives: We aimed to analyse the performance of three different preparation methods [MBT-Sepsityper IVD kit (Bruker Daltonics, Germany), Sodium dodecyl sulfate (SDS) lysis and differential centrifugation+protein extraction (PE)] and compare in standard and Sepsityper modules of the Bruker Biotyper MALDI-TOF MS for direct identification of bacteria from positive blood culture bottles.

Methods and Results: In present study, 240 positive blood culture bottles of BACTEC FX (Becton Dickinson, USA) were included.

By using standard module, correct identification at species level (score≥2) was determined in 46.7% of the samples with SDS, 44.2% with Centrifugation+PE and 25.4% with Sepsityper-kit. These ratios at genus level (1.7≤score≤1.99) were 34.6%, 31.3% and 32.5%, respectively (Table 1, 2, 3). With SDS (195), more bacteria were identified correctly than Centrifugation+PE (181) and Sepsityper-kit (139). A statistically significant difference was found between SDS and Sepsityper-kit, Centrifugation+PE and Sepsityper-kit ($p<0.001$ for both).

By using Sepsityper module, correct identification at species level (score≥1.8) was determined in 74.2% of the samples with SDS and Centrifugation+PE each, and 55% with Sepsityper-kit. These ratios at genus level (1.6≤score≤1.79) were 16.3%, 10% and 19.2%, respectively. With SDS (217), had significantly higher identification rates than Centrifugation+PE (202) and Sepsityper-kit (178) ($p=0.028$ and $p<0.001$). A statistically significant difference was also observed between Centrifugation+PE and Sepsityper-kit ($p<0.001$).

Best performance was obtained with SDS among the methods. Although better performance was achieved by using Sepsityper software module, risk of misidentification by using this module should not be ignored.

T355 - Polypeptide hydrogels for sustained release of oxazolidinone-based antibacterial compounds

Presenting Author - *Polina Ilina, University of Helsinki, Finland*

Author/s – *Anna Gasa Mir, Frederica Secco, Pauli Wrigstedt, Vladimir Iashin, Jesus Perea-Buceta, Silvia Marchesan, Päivi Tammela*

Abstract Content

Background: Peptide-based supramolecular hydrogels hold a great promise for drug delivery applications due to their biocompatibility, biodegradability and potential responsiveness to external stimuli. Their structure allows flexible design to fine-tune self-assembly process and drug release in response to environmental and biological signals.

Objectives: The aim of this work was to investigate the potential of tripeptide self-assembling hydrogels as drug delivery systems for novel oxazolidinone-based antibacterial compounds. We also aimed to correlate their release kinetics with the molecular structure of the compound.

Methods: We studied a set of five N-aryl-oxazolidinone analogs highly active against a panel of Gram-positive bacterial species (Cruz et al., 2021). Using an optimized procedure for the hydrogel formation, we confined the compounds in the D-Leu-L-Phe-L-Phe hydrogel and studied their release kinetics in a phosphate-buffered saline using high-performance liquid chromatography. We then investigated the behavior of this system in biologically relevant solutions at concentrations that were shown to have antibacterial effect. We utilized inhibition zone assay, colony-forming units (CFU) counting assay and broth microdilution assay to study release kinetics and antibacterial activity of the released compounds against *Staphylococcus aureus* ATCC 29213. In addition, we assessed the cytotoxicity of these compounds on human skin fibroblasts as a model of potential topical applications.

Results: Molecular structure of the compound clearly affected the release kinetics. At biologically relevant conditions, two boron-containing analogs demonstrated sustained release from the hydrogel for prolonged periods, and possessed low cytotoxicity to mammalian cells.

T360 - Insight into the synthesis and function of membrane vesicles in the extremophilic bacteria *Acidithiobacillus ferrooxidans*

Presenting Author - *Matias Castro-Gonzalez, Millennium Institute of Oceanography (IMO), Chile*

Author/s – *Marcela Montoya*

Abstract Content

The acidithiobacilli are chemolithoautotrophic bacteria that thrives in environments with extremely low pH and high heavy metals concentrations. Acidithiobacillia class members obtain energy from the oxidation of reduced inorganic sulfur compounds releasing sulfuric acid and generating polluted acidic metal-rich drainage waters. *Acidithiobacillus ferrooxidans* is one of the most late-diverging members of Acidithiobacillia class and possess additional capabilities such as ferrous iron and hydrogen oxidation, and cell-cell communication through a Quorum Sensing system.

Membrane Vesicles (MVs) are nano-sized proteoliposomes derived from the bacterial envelope that perform a broad diversity of functions. Despite the molecular mechanism of MV synthesis in bacteria is not well understood, the secretion and reception of MVs is recognized as a widespread intercellular communication system. However, our current knowledge about MVs in Acidithiobacillus is scarce.

Here, to evaluate the production of MVs of *A. ferrooxidans*, we isolate the MVs produced by *A. ferrooxidans* ATCC 53993 grown on elemental sulfur and determine their size distribution by NanoTracking Analysis and Transmission Electron Microscopy. Furthermore, to gain insight into MVs molecular function we determine their cargo by LC-MS/MS proteomic analysis.

TEM and NTA analysis showed nanometric (90 to 200 nm) round particles, which agree with morphology of MVs. MVs protein cargo suggest the presence of both outer-membrane vesicles and outer-inner membrane vesicles. Relevant functions include Transport/efflux (RND, ABC, Porins), Electron transport (Cytochromes), Signaling transduction (c-di-GMP), Surface attachment (type 4 pili), DNA mobilization (integrase, excinuclease, recombinase), membrane curvature promotion and vesicle fusion. Potential mechanisms for MVs synthesis and function are proposed.

T361 - Treatment outcome in *M. genitalium* infections from Bulgaria following implementation of macrolide resistance-guided therapy

Presenting Author - *Ivva Philipova, National Center Of Infectious And Parasitic Diseases, Bulgaria*

Author/s – *Maria Mademova, Viktoriya Levterova, Elena Birinjieva*

Abstract Content

Background: *Mycoplasma genitalium* (MG) raises a major public health concern because resistance and treatment failures to both first and second-line treatment (azithromycin and moxifloxacin, respectively) have been increasingly reported internationally and no optimal alternative therapies can be suggested at present. From Bulgaria, previous study described high azithromycin (47.6%) failure rate in 2020.

Objectives: Macrolide resistance-guided therapy (RGT) was implemented in 2022 and the aim of this study was the evaluation of RGT, by comparing before and after treatment failure rates and times to microbiological cure.

Methods: MG diagnostics was conducted in patients visiting Bulgarian health clinics in 2022, for whom testing was indicated. Clinical samples were tested for MG using the AmpliSens® *Mycoplasma genitalium*-FRT (Ecoli s.r.o., Slovak Republic). Positive samples were then tested for macrolide-resistance mutations (MRMs) by ResistancePlus® MG assay (SpeeDxPty.Ltd) and specific antimicrobial treatment was recommended accordingly (MRM-negative infections received azithromycin and MRM-positive received moxifloxacin). Treatment failure was defined as MG positive at test-of-cure and time to microbiological cure was defined as time to MG negative test from the first positive result.

Results: Among patients given RGT (n=17), the overall treatment failure rate was 1/17 (5.9%). This was significantly lower than 10/21 (47.6%) observed in patients treated pre-RGT (p=0.002). The time to microbiological cure was 29.4 days (CI 24.5 – 34.3) compared to 45.2 days (CI 36.5 – 53.7) pre-RGT (p=0.001). These results confirm that RGT has a beneficial effect on treatment outcome in MG infections from Bulgaria and could serve the antimicrobial stewardship.

W1 - Identification and metabolic characterization of perchlorate-degrading halophile microorganisms from Chilean hypersaline lakes.

Presenting Author - *Bernardo González, Adolfo Ibáñez University, Chile*

Author/s – *Luis Cid Cid, Gustavo Rodríguez-Valdecantos, Felipe Torres-Rojas, José Eduardo González-Pastor*

Abstract Content

Halophiles can tolerate some understudied pollutants, such as perchlorate. The presence of perchlorate in water and soil samples from the Atacama Desert and Arica´s plateau in Chile has been identified. We report that potentially new bacterial species from the phyla Pseudomonadota, Bacillota and Actinomycota have been isolated and identified by 16S rRNA gene sequencing and phylogenetic analysis. The potential of isolates to grow in perchlorate-enriched media has been determined in ranges between 1 and 400 mM of sodium perchlorate. Moreover, these isolates showed perchlorate resistance in different salinity ranges, from 5% to 25% SW medium. Results showed that all isolates could grow in 1 mM of ClO4- medium, but for higher concentrations of 200-400 mM, only bacteria of the genera *Exiguobacterium*, *Psychrobacter*, *Halomonas* and *Pantoea* showed significant growth rates. All the isolates have an aerobic metabolism, which is a novelty compared to previous studies in which perchlorate degradation has only been reported in strains from anaerobic environments. Consistently, PCR-based detection of the well-known perchlorate reductase ABC genes cluster from anaerobic species did not yield positive results, which implies a new way to remove perchlorate in aerobic environments.

In addition, the ability to degrade other pollutants, for instance, phenol, 4-hydroxybenzoate, and 2,4-dichloro phenoxy acetate), tested positive in only a couple of isolates: E. undae AT-3 and Halomonas sp. AT-7.

W2 - Long-term evaluation of phytoremediation and bacterial community dynamics in oil-contaminated soil

Presenting Author - *Kyung-Suk Cho, Ewha Womans University, Republic of Korea*

Author/s – *Yun-Yeong Lee, Soo Yeon Lee*

Abstract Content

Petroleum hydrocarbons (PHs) persist for a long time once they introduced into the soil environment because they are stable and can be trapped in soil pores as an immobile. *Festuca arundinace*a (tall fescue) is a suitable plant for the long-term restoration of PHs contaminated soil because it is not only a perennial plant but also able to remove organic pollutants including PHs. Long-term phytoremediation and bacterial community structure dynamics were explored during the phytoremediation of oil-contaminated soil planted with tall fescue. The soil was contaminated with diesel oil at a total petroleum hydrocarbon (TPH) concentration of 24,000 mg-TPH·kg dry soil-1. Tall fescue seedlings were cultivated in the contaminated soil for 571 days. The residual TPH concentration in the soil gradually decreased with time, reaching the maximum removability of 63.6–75.6%. The bacterial community structure was distinguishable over the period. On day 0, *Acinetobacter* was absolutely dominant (62.4%) in the community. Genera *Pseudomonas* (3.3–16.7%), Immundisolibacter (9.2–12.1%), and *Umboniibacter* (3.1–12.9%) showed a high relative abundance at the initial period of phytoremediation (days 33–117). On the other hand, *Pseudomonas* (12.8–58.3%) and *Sphingomonas* (4.6–17.0%) were dominant during days 210–411. Finally, *Sphingomonas* (6.5–12.2%) was still dominant in the community at the final period of the phytoremediation (days 443–571), followed by *Marinobacter* (2.7–13.4%) and *Aquabacterium* (1.6–8.4%). Most of these dominant bacterial genera are known to have PHs biodegradability by producing biosurfactants. These results are useful to develop practical long-term phytoremediation technology in PHs-contaminated soil.

W3 - Microbiota of the ancient Roman limestone monument Mitrej

Presenting Author - *Ivica Dimkić, University of Belgrade, Serbia*

Author/s – *Tamara Janakiev, Nikola Unković, Aleksandar Knežević, Milica Ljaljević Grbić, Janez Kosel, Črtomir Tavzes, Ivica Dimkić, University of Belgrade, Faculty of Biology, Department of Biochemistry and Molecular Biology, Studentski trg 16, Belgrade, Serbia*

Abstract Content

Background: Mitrej above Rožanec is a limestone monument (in the forest of Judovje, Slovenia), which represents the god Mithras performing the sacrificial act. Significant biodeterioration has occurred on the surface of the relief and remedial action should be taken.

Objectives: To identify the microbiome associated with the damaged surfaces of the monument to obtain a more comprehensive picture of microbial-induced deterioration.

Methods: Total bacteriobiota and mycobiota were analyzed by using the NovaSeq 6000 sequencing platform. In addition, a comprehensive bioinformatic and computational biological analysis was performed at the ASV, genus, family, and the phylum level.

Results: According to the alpha diversity indices, there were no statistically significant differences in the richness and diversity of the microbiome associated with the studied monument. Beta diversity analysis of fungal and bacterial communities showed significant variability among samples. The bacteriobiota on the monument (relief and around the relief) was characterized by high relative abundance (RA) of the phyla Cyanobacteria, Proteobacteria and Actinobacteria. The bacterial genera that were statistically significantly most abundant or only present around the relief were *Flavisolibacter* and *Blastocatella*, and on the relief the genera *Howardella* and Truepera. The mycobiome of the entire monument was characterized by a high RA of the phylum Ascomycota and, to a lesser extent, Basidiomycota. The most abundant genera around the relief were *Verrucaria* (19.56%), *Gyalecta* (8.44%), *Acremonium* (5.64%), *Cladosporium* (6.20%), and *Coprinellus* (3.71%). The most abundant genera on the relief were *Acremonium* (27.86%), *Coprinellus* (9.41%), *Cladosporium* (7.76%), *Bagliettoa* (6.78%), and *Verrucaria* (5.12%).

W4 - Microbial communities and nitrogen cycle response to short-term flooding in a riparian forest soil

Presenting Author - *Mikk Espenberg, Unversity of Tartu, Estonia*

Author/s – *Kristel Reiss, Thomas Schindler, Maarja Öpik, Kaido Soosaar, Ülo Mander,*

Abstract Content

Background: The impact of short-term floods on different ecosystems is of increasing interest in the context of climate change and the increase in extreme rainfall. The nitrogen cycle is one of the most crucial nutrient cycles, and nitrogen management is economically, ecologically and environmentally critical. The quantity and distribution of nitrogen are controlled through biogeochemical processes. However, the need for knowledge regarding microbial processes governing nitrogen cycling hinders climate-change impact estimations of forests.

Objectives: The aim of the study was to assess how short-term flooding impacted nitrogen cycle processes and microbial communities and nitrous oxide (N2O) emissions while also considering the correlation with physicochemical parameters in riparian alder forests.

Methods: The peat sampling from topsoil was carried out in 2017 and 2018 at riparian alder forests. Real-time polymerase chain reaction (qPCR) and sequencing were applied to evaluate the processes and communities. Physicochemical parameters and in situ, N2O emissions were measured.

Results: The short-term flooding significantly affected the abundance of bacteria and microbes with archaeal amoA, n-damo(nitrate/nitrite-dependent anaerobic methane oxidation)-specific 16S rRNA and nosZII genes. Several relationships were identified between marker genes and N2O emissions. The bacterial and fungal communities showed a shift in the community because of the short-term flooding. Sudden changes in soil moisture influenced the patterns of marker genes of nitrogen cycle processes and microbial communities.

W5 - Population dynamics of filamentous bacteria in a full-scale activated sludge reactor by metabarcoding

Presenting Author - *José Alonso, Universitat Politècnica de Valencia Spain, Spain*

Author/s – *Andrés Zornoza, Pablo Alonso, Paula Barbarroja*

Abstract Content

Background: Filamentous bacteria usually are present in the activated sludge in small amounts. Under specific conditions, they proliferate to such an extent that they markedly affect the treatment plant performance causing, sludge bulking or foaming (Eikelboom 2000). It is necessary to understand the dynamics of the filamentous bacteria community and their seasonal variations for predicting undesirable changes in the functional diversity of activated sludges, for a better control over the operational parameters of WWTPs.

Objectives: This study aims to explore the contribution of environmental parameters to the variability observed in the filamentous structure.

Methods: A total of 29 samples of activated sludge from a bioreactor belonging to a municipal wastewater treatment plant were collected every fifteen days for 16 months. PhotonMasterTM Luminometer from Luminultra® was used to measure the adenosine triphosphate of activated sludge samples. Filamentous bacteria were investigated using high-throughput short-read (Illumina) 16S rRNA gene amplicon sequencing. To assess the contribution of the temperature and operational parameters to the variability observed in the filamentous bacteria structure we carried out distance-based linear models (DISTLM) and distance-based redundancy analysis (dbRDA).

Results: A total of 39 filamentous bacteria genera were identified using Illumina sequencing. Analysis of the core filamentous bacteria community at the genera level showed that *Nocardioides*, *Haliscomenobacter*, *Ca. Villigracilis*, *Mycobacterium*, *Ca. Amarithrix*, *Ca. Catenibacter*, *Trichococcus*, *Streptococcus*, *Turicibacter*, *Defluvicoccus* and, *Sphaerotilus* were found in all 29 activated sludge samples. The dbRDA results revealed a significant influence of temperature in the population dynamics of filamentous bacteria.

W7 - PepA: an extracellular manganese oxidase induced by lignin in *Pseudomonas putida* KT2440

Presenting Author - *Helena Gómez-Álvarez, Spanish National Research Council, Spain*

Author/s – *Laura Castro, Pablo Iturbe, Juan Nogales, Manuel Carmona, Eduardo Díaz*

Abstract Content

Background: Manganese (Mn) oxides play a key role in the oxidation of recalcitrant organic carbon, including lignin (1), and have also emerged as a potential material in a wide range of technological applications, e. g, energy storage or catalysis. A transcriptomic study aimed to identify lignin-induced genes in the environmental model bacterium *Pseudomonas putida* KT2440 revealed pepA, encoding an animal-heme peroxidase-like enzyme homologous to certain Mn oxidases (2), as one of the main induced genes.

Objectives: To characterise the mechanisms of secretion and action of PepA in *P. putida* KT2440.

Methods: A collection of *P. putida* mutant strains was designed to unequivocally asses the function of pepA and that of the adjacent operon coding for a type-1 secretion system (T1SS). Liquid Chromatography coupled to tandem mass spectrometry (LC-MS/MS) was used to confirm localization and processing of the mature enzyme. Transmission (TEM) and scanning (SEM) electron microscopy techniques were applied to detect and characterise manganese oxides nanoparticles (MnOx NPs). Superoxide generation by PepA was tested by making use of a chemoluminescent probe.

Results: This work shows that PepA is an extracellular heme-dependent enzyme that oxidizes Mn(II) by generating superoxide. A specific T1SS encoded adjacent to pepA is involved in PepA transport and processing of the mature form. A PepA-overproducer *P. putida* strain allowed the characterisation of the extracellular MnOx NPs generated. These results expand the knowledge on bacterial manganese oxidases and their potential role in lignin depolymerization, and broaden the biotechnological utilities of the model *P. putida* KT2440 bacterial chassis.

W8 - Glycerol and *Limosilactobacillus reuteri* supplementation enhance butyrate production by broiler chicken cecal microbiota in vitr

Presenting Author - *Anna Greppi, ETH Zurich, Switzerland*

Author/s – *Paul Tetteh Asare, Annelies Geirnaert, Alessia Pennacchia, Angela Babst, Christophe Lacroix*

Abstract Content

The administration of probiotic strains of *Limosilactobacillus reuteri* in poultry has been shown to improve poultry performance and health, partly associated with the gut production of reuterin from glycerol, a broad-spectrum antimicrobial compound. In this study, the effect of high-reuterin-producing chicken-derived *L. reuteri* PTA5_F13 and glycerol on broiler chicken cecal microbiota composition and metabolism were evaluated in the continuous PolyFermS model recently developed to mimic chicken cecum fermentation. Three PolyFermS models were inoculated with different separately immobilized cecum microbiota and used to investigate the impact of glycerol addition (50 and 100 mM) in the medium, with and without daily supplementation of viable *L. reuteri* PTA5_F13 (107 CFU/mL final concentration). The addition of glycerol alone and combined with *L. reuteri* PTA5_F13 resulted in a reproducible 18 to 25% increase of butyrate production in the three models. Concurrently, a microbiota dependent response was observed, resulting in a reduction of Enterobacteriaceae by glycerol. The combined treatment resulted in significant 1,3-PDO accumulation in the effluent medium, therefore confirming the conversion of glycerol via the reuterin pathway. Collectively, our in vitro results suggest that the co-application of *L. reuteri* PTA5_F13 and glycerol could be used to promote chicken gut health by increasing butyrate production and potentially decreasing Enterobacteriaceae which have been previously shown to be highly sensitive to the antimicrobial effect of reuterin.

W9 - Ecology and interaction between bacteria and phytoplankton at the microscale (a modeling study)

Presenting Author - *Ferdi Hellweger, Free University of Berlin, Germany*

Author/s – *Falk Eigemann, Jutta Hoffmann, Charlotte Schampera, Shuting Liu, Craig Carlson, Stephen Giovannoni,*

Abstract Content

Microbes are important players of surface waters, yet a quantitative mechanistic understanding of these complex ecosystems remains a grand challenge. Concentrations of microbes can be observed and their functions may be estimated with next-generation methods, but specific interactions and the ecological role of various traits have to be inferred from models. Existing microbial ecosystem models are too coarse to resolve the microscale structure of these systems. Here we present a novel model that simulates a 1-mL 3D-cube with ~6.0e5 no./mL individual microbes (three phytoplankton size classes with healthy, senescent and dead lifecycle stages, copiotrophic and oligotrophic bacteria) and extracellular substrate at 50-µm resolution, using a hybrid Lagrangian – Eulerian approach, at ecologically-relevant timescales. This quantitative representation of the ecosystem shows: (1) copiotrophs grow mostly attached to larger phytoplankters and get almost all (99%) of their carbon from them, whereas oligotrophs get most (90%) of their carbon from exudates and lysates of small phytoplankters; (2) diel patterns with an earlier appearance of substrate in the phycosphere than ambient, and corresponding time-lag in growth between particle-associated copiotrophs and free-living oligotrophs, which is consistent with observations; (3) shear and phytoplankter sedimentation reduce chemotactic efficiency and fitness of the copiotroph, and increase fitness of the oligotroph; and (4) chemotaxis and attachment provide substantial benefit, and those traits are dependent, i.e. chemotaxis aids attachment. Our study provides insights into the microscale ecology of marine bacteria and the open-source code is a tool for further research in this area.

W10 - Comparison of bacterial communities and their functional profiling in the inherent serpentine-associated sites

Presenting Author - *Bing-Mu Hsu, National Chung Cheng University, Taiwan*

Author/s – *I-Sen Tsai, Suprokash Koner, Bashir Hussain*

Abstract Content

In the study, the collected samples included parent rock, weathering soil, plants, and rhizosphere in the serpentine area (Central Mountains Range and Coastal Mountains Range) of Taiwan. These samples were carried out the microbial metabolomics analysis followed by the process of heavy metal analysis, bacterial community, EcoPlate carbon metabolism analysis, and qPCR. This study highlighted that the heavy metal accumulation in the serpentine soil system could significantly restricted the resident microbial community's physiological profiling/carbon source utilization patterns. Further, the bacterial diversity differed in serpentine soil and endemic plant rhizosphere soil samples which were denoted by the variance in abundance of the Proteobacteria, Bacteroidota, and Acidobacteriota groups, respectively. It is noteworthy that the functional bacterial genera associated with the N, C, S, CH4, and Fe biogeochemical cycles and metabolic pathways were identified, and interestingly, they were found enriched in non-serpentine as well as the endemic plant rhizosphere soil compared to serpentine bedrock rock and soil. This study concludes that the heavy metal concentrations in a serpentine ecosystem could influence and select specific microbiome signatures with unique CLPP. The heavy metals and nutrient limitation-driven dual stresses in the serpentine settings of the endemic soil could significantly influence the functional bacterial diversity, and their potentiality for the elemental cycling and metabolic repertoires which could be detrimental for the soil fertility and supporting local vegetation.

W13 - Copper resistance of *Staphylococcus aureus* isolates in liquid culture

Presenting Author - *Clemens Kittinger, Medical University Of Graz, Austria*

Author/s – *Gernot Zarfel*

Abstract Content

Background: Heavy metals are used in manifold ways on surfaces, as an anti-infective tool for fighting bacteria. Although there are an increasing number of protocols and ISO standards for evaluating the antibacterial efficacy of surfaces, it is not known much on the change of growth behavior and tolerance of bacteria to heavy metal in liquid culture.

Objectives: The aim of the study was to test heavy metal tolerance of *Staphylococcus aureus* clinical wound isolates (MSSA as well as MRSA) in liquid culture over an extended time, to determine growth behavior under increasing copper concentrations.

Methods: 120 isolates of *Staphylococcus aureus* were analyzed in a Bioscreen™ over a 72-hour period. They were incubated with copper concentrations up to 20 mM copper chloride. TRIS-buffer was added to the growth medium to maintain stable pH-conditions. In addition, the presence of copA and copB was determined.

Results: The isolates of all groups showed recurrent changes in their growth behavior with rising copper concentrations. Depending on the copper concentration, the lag phase was lengthened dramatically (up to 70 hours), but the minimal doubling time after entering the log phase did not or only slightly decrease in comparison with the log phase in media without copper. Isolates with a prolonged lag phase that started to grow late, were able to grow immediately when transferred to media containing copper. Some of the isolates studied were able to tolerate high copper concentrations (up to 9mM).

W15 - Filling the gap: bacteria associated with Plakinidae sponges as paramount sources of agarases

Presenting Author - *Marinella S Laport, Federal University Of Rio De Janeiro, Brazil*

Author/s – *Isabelle Rodrigues Lopes, Tamires Avilla de Souza Clemente, Guilherme Muricy, Bruno Francesco Rodrigues de Oliveira,*

Abstract Content

The enzymatic repertoire of the poriferan microbiome constitutes a promising reservoir of industrial biocatalysts, notably the carbohydrate-active enzymes (CAZymes). Among them, agarases have been scarcely studied from sponge-associated microorganisms, despite the wide range of bioactivities of their hydrolysis products, the agaro-oligosaccharides. In this context, we investigated the production of agarases by bacteria isolated from cave-dwelling Plakinastrella and Plakortis sponges and a β-agarase from *Pseudoalteromonas lipolytica* PA2MD11, isolated from *Plakina cyanorosea* using a coupled fermentation-based and molecular cloning approach. Among 262 strains isolated from 16 Plakortis specimens and 285 strains from 9 Plakinastrella specimens, 160 (61.1%) and 139 (48%) had EI ≥ 2 in Hu agar, respectively, indicating potential producers of this CAZyme. The potential producer strains were identified by MALDI-TOF MS [n=252 (84.2%)], and the dominant genus was *Vibrio*, representing 55.5% of strains isolated from Plakortis sp. and 83.1% from Plakinastrella sp. The PA2MD11 strain exhibited an activity of 0.345 U/mL after 72 h of growth in liquid minimal medium at 28°C and pH 7.2. Regarding the agarolytic repertoire of the PA2MD11 strain, the GH16 β-agarase (50-kDa) is encoded by aga890 gene. The agarase was heterologously expressed and purified by affinity chromatography on a nickel-loaded agarose resin column. The molecular mass and the degree of purity were verified by SDS-PAGE and total protein concentration was determined (6.12 mg/mL). Thus, this study reinforces the exploration of the sponge microbiome for novel CAZymes with biotechnological applications.

W16 - Single-amplified genomes reveal most streamlined free-living marine bacteria.

Presenting Author - *Mario López-Pérez, UMH, Spain*

Author/s – *Jose M Haro-Moreno, Juan J Roda-Garcia*

Abstract Content

Background: Evolutionary adaptations of prokaryotes to the environment sometimes result in genome reduction. This evolutionary scenario, commonly found in intracellular parasites or endosymbionts, is facilitated by the loss of biosynthetic pathways that can be replaced by the utilization of nutrients derived from the host cell. However, the precise ecological drivers and the limits for genome streamlining among free-living bacteria remain enigmatic.

Objectives: We address the dynamics and limits of genome reduction by examining one of the most abundant bacteria in the ocean, the SAR86 clade. Despite its abundance, comparative genomics has been limited by the absence of pure cultures and the poor representation in metagenome-assembled genomes.

Methods and Results: We co-assembled multiple single-amplified genomes to obtain the first complete genomes from members of the four families defined by our phylogenomic analyses. All families showed a convergent evolutionary trajectory with characteristic features of streamlined genomes, most pronounced in the TMED112 family. This family has a genome size of ca.1Mb and negative median intergenic distance, exceeding levels found in other abundant microbes such as SAR11 and Prochlorococcus. This genomic simplification led to a reduction in the biosynthesis of essential molecules, DNA repair-related genes, and the ability to sense and respond to environmental factors which could suggest an evolutionary dependence on other co-occurring microbes for survival (Black Queen hypothesis). Therefore, these reconstructed genomes within the SAR86 clade are ideal candidates for unravelling the evolutionary mechanisms behind simplified genomes and provide new insight into the limits of genome reduction in free-living microbes.

W17 - Dynamics of wood decay on fungal interactions are visible in metabolome and transcriptome responses

Presenting Author - *Taina Lundell, University of Helsinki, Finland*

Author/s – *Eero Kiviniemi, Tuulia Mali, Janina Österman-Udd*

Abstract Content

Wood decay Basidiomycota fungi are unique in their ability to colonize and degrade wood and solid plant biomass. The fungal mycelia confront and combat for substrates and space in their habitats. Fungi may occasionally perform mutualistic interactions and even supportive effects to advance wood degradation and nutrient cycling in forest ecosystems.

We approached the diversity of fungal interactions experimentally studying a selection of species with different decay strategies (white, brown or intermediate rot, litter-decomposition) and substrate specifications. These simulations are compared to forest deadwood samples from a long-term field study. Combinatory cultivations on wood substrates of fungal species were followed by assaying enzyme activities, production of metabolites and sugars, and differences in gene expression. Selected co-cultures and decayed forest deadwood samples were subjected to RNA-Seq meta-transcriptomics.

The fungal and microbial consortia in decaying wood are under continuous transformation along with advancement of wood decay. Combinations of fungi presented white rot signifying oxidoreductase activities and mycelial dominance at the late stage of wood decay (one year of growth on wood) whereas earlier stages were dominated by brown rot species and biochemistry. Combinations of three species or more stimulated production of fungal secondary metabolites. Among the compounds, candidates with antioxidant and antimicrobial activities were identified.

Our findings pinpoint that deadwood is not only a changing substrate and nutrient for fungi but also a source for an array of valuable metabolites and fungal-converted wood components. Our next aim is to dissect the meta-genomes driving the metabolic and transcriptomic responses in naturally decaying deadwood.

W18 - White rot fungi for controlled biodegradation of lignin

Presenting Author - *Linda Mezule, Riga Technical University, Latvia*

Author/s – *Anna Civzele, Alise Anna Stipniece-Jekimova*

Abstract Content

Lignocellulose containing biomass can be used as a source for energy, fuel, and valuable chemical production. At the same time practical and industrial scale application is still limited due to either high conversion costs, low efficiency or environmental issues (unsustainable technologies for conversion like concentrated acid hydrolysis). From all technologies, biological approaches have been recognized as the most sustainable ones, however, the need for specific and expensive lignocellulose degrading enzymes and generally slow conversion rates (from one to several days) still set this technology aside. Research has shown that enzyme products obtained from white rot fungi, especially *Irpex lacteus*, and other wood decay fungi can demonstrate comparable cellulose conversion efficiency in less than 30 hours at mild environmental conditions. Simultaneously, degradation of lignin fractions has not been thoroughly assessed for their industrial use. The aim of the study was to determine the rate and impact of various lignin degrading enzymes produced by *I. lacteus* to engineer the biological pre-treatment process of lignocellulose. During preliminary screening studies, it was observed that *I. lacteus* has the highest laccase productivity, even under low carbohydrate conditions. Furthermore, during 4 weeks of growth *I. lacteus* was able to reduce straw and hay biomass by 12,52 ± 3,84% and 22,17 ± 5,31% respectively. Active production of lignin degrading enzymes was observed via correlation of laccase concentration with total protein content in the samples. At the same no significant decrease in total carbohydrates was observed, thus, indicating on degradation of lignin components in the biomass.

W19 - Nordic Seas metabarcoding and eDNA taphonomy

Presenting Author - *Ngoc-Loi Nguyen, Institute Of Oceanology Polish Academy Of Sciences, Poland*

Author/s – *Joanna Pawłowska, Jan Pawłowski*

Abstract Content

Recent development of DNA metabarcoding led to spectacular accumulation of metabarcoding data, especially for microbial and meiofaunal biomes. However, despite rapidly increasing number of DNA metabarcodes, their taxonomic assignment is still very limited. The large proportion of metabarcodes remains unassigned even at higher taxonomic level, impeding their ecological interpretation and sometimes making it difficult to distinguish between planktonic and benthic taxa. To overcome this issue, we propose to establish a reference database of barcodes obtained from morphospecies known to be present in the Nordic Seas and metabarcodes obtained in this and other eDNA studies from the same area. We will target selected taxonomic groups that are of particular interest to this study, e.g. foraminifera, diatoms, and copepods. Moreover, we will assign metabarcodes to plankton and benthic community, based on their occurrence and relative frequency in water column and sediment DNA datasets. We expect that this will help to better understand the taphonomic processes involved in a transfer of DNA from water column to the sediment, and to determine whether all planktonic taxa are equally recorded in sedimentary DNA.

W20 - Dynamics of active bacterial community in a multi-stage sludge treatment process

Presenting Author - *Heewook Ryu, Soongsil University, Republic of Korea*

Author/s – *Kyung Suk Cho, Jeonghee Yun, Hyung Pan Kim*

Abstract Content

A multi-stage digester system using longer sludge retention time and low food to microorganism ratio was developed for solid sludge treatment. The multi-stage system consisted of a pre-biodigester, three biodigesters, and a submerged membrane bioreactor. To better the understand sludge treatment mechanisms in this process, it is necessary to interpret changes in sludge characteristics and active microbes. In this study, sludge reduction performance and bacterial community dynamics in the process were characterized using mass balance analysis and an RNA-based pyrosequencing method. The process achieved no sludge discharge except sampling at a total loading sludge of 4700 kg-MLSS for 281 days. Excess sludge can be efficiently reduced through a strategy that maximizes maintenance energy by keeping biomass (sludge) in the bioreactors in a saturated state. The dominants in the pre-biodigester were *Chujaibacter soli* (11.8%) and *Owenweeksia hongkongensis* (8.2%). In the biodigesters, *C. soli* was the most abundant with a relative abundance range of 22.5%, followed by Romboutsia timonensis (3.5%), *Rhodanobacter glycinis* (3.3%), *Niastella hibisci* (3.0%), and *Gemmata obscuriglobus* (2.9%). In the submerged bioreactors, the dominant bacteria were A*quisphaera giovannonii, G. obscuriglobus, Trichocoleus desertorum, Rhodopila globiformis, Thermostilla marina*, and *Roseiarcus fermentans*. Functional bacteria contributing to reduced sludge were as follows: (i) aerobic and anaerobic heterotrophs for sludge decomposition, (ii) heterotrophs with hydrolytic activities for sludge lysis, and (iii) fermentative bacteria for solubilizing particulate inorganic matters (PIM). Hydrolysis, PIM solubilization, and maximizing maintenance energy are proposed as sludge reduction mechanisms in the multi-stage sludge treatment process.

W21 - Genome shuffling mutant of *Streptomyces diastatochromogenes* for substantial improvement of toyocamycin production

Presenting Author - *Xuping Shentu, China Jiliang University, China*

Author/s – *Yang Song, Zixuan Zhang, Xiangli Zhang, Jiayi Yao, Xiaoping Yu*

Abstract Content

Toyocamycin, a nucleoside antibiotic, is a fungicide with the potential to control plant pathogens. In this study, three rounds of genome shuffling screening were applied to enhance the toyocamycin production in *Streptomyces diastatochromogenes* 1628. After three rounds of genome-shuffling screening, the toyocamycin production increased by 10.8-fold that of wild-type, and 2.64-fold of its parental strain. By optimization of its nutrition condition in medium, the highest production of toyocamycin reached 1173.6 mg/L in TY-producing medium. In addition, the mechanism for the improvement of shuffled strains was investigated. Recombinants with increased toyocamycin production exhibited higher transcriptional level of the toy cluster and product resistance. Furthermore, the rise of ATP hydrolysis rate indicated that intracellular ATP exhibit a significant role in tuning the toy cluster by an ATP-binding pathway-specific regulator. In all, we obtained *S. diastatochromogenes* mutants with enhanced toyocamycin production, and provided a valuable clue for the activation of secondary metabolites.

W22 - Identification of indicator genes for monitoring antibiotic resistance and pathogens in wastewater

Presenting Author - *Rafael Tavares, University of Coimbra, Portugal*

Author/s – *Elsa T. Rodrigues, Marta Tacão, Isabel Henriques*

Abstract Content

Background: Few indicators are available for feasible and cost-efficient monitoring of antibiotic resistance (AR) in Wastewater Treatment Plants (WWTPs). Notwithstanding, it is known that WWTPs are important hotspots to determine the spread of antibiotic resistance (AR) and pathogens.

Objectives: This study aims to evaluate the reliability of integron-related genes as possible indicators of AR and putative pathogens in wastewater.

Methods: Influent (n=11) and effluent (n=25) samples were collected from a municipal WWTP over 2020. Whole-community DNA was extracted, and integrase genes, antibiotic resistance genes (ARGs) and putative pathogenic groups were quantified by standard qPCR (5 targets) and high-throughput qPCR (48 targets).

Results: Thirty-six of the 40 screened ARGs were detected, mainly blaOXA-10, strB and ermB. Influent and effluent resistomes were significantly different ($p<0.05$). For influent, lower ARG diversity was determined in winter. For effluent, the summer ARG profile was distinct from winter and spring ($p<0.05$). *E. coli* and Bacteroidetes were identified both in influent and effluent, but Bacteroidetes was enriched in the outflow. Relative abundances of intI1 and intI3 were strongly correlated with the sum of all ARGs detected in each sample ($\rho=0.92$ and $\rho=0.87$, respectively). Beta-lactam and aminoglycoside ARGs were better correlated with integron-encoded blaGES ($\rho=0.96$ and $\rho=0.91$, respectively) and blaVIM ($\rho=0.90$ and $\rho=0.89$, respectively), than with intI1 ($\rho=0.82$ and $\rho=0.86$, respectively) and intI3 ($\rho=0.82$ and $\rho=0.88$, respectively), and no relevant correlations were found between these genes and putative pathogens.

Conclusion: intI1, blaGES and blaVIM appear to be promising targets for monitoring AR in wastewater.

W23 - Fertilization regimes modulate bacterial diversity and the enrichment of antibiotic resistance genes at different trophic levels

Presenting Author - *Kunal Jani, Institute of Microbiology and Biotechnology, Germany*

Author/s – *Karoline Jetter, Rostand Chamedjeu, Patrick Schäfer, Christian Riedel, Lena Wilfert, Simone Sommer,*

Abstract Content

Background: The intensive administration of organic fertilizers represents one of the major drivers impacting the function of agricultural ecosystems. Considering microbiomes, in addition to the biodiversity and functional diversity of bacterial communities, antibiotic resistance, and its spread is a grave concern in agricultural landscapes.

Objectives: Here, we study the impact of different fertilization regimes on the function and biodiversity of microbiomes across different levels of the trophic chain in grassland ecosystems.

Methods: We monitor changes in bacterial communities via amplicon sequencing in order to deduce the extent of ecological perturbation and the proportional increase in antibiotic-resistance genes. The functional potential of bacterial communities was derived by using imputed metagenomics.

Results: Assessment of the bacterial communities under different land use regimes, i.e. control agricultural lands and those fertilized with biogas, cow manure, and pig slurry revealed significant differences in the bacterial diversity (Shannon, $p= 0.01$). The observed divergence was consistent between the studied organisms i.e. earthworms, bumblebees, and voles ($p\leq 0.01$), representing different trophic levels. Metagenomic imputation revealed that gene families involved in the biosynthesis of streptomycin and tetracycline were abundant and that their abundance was particularly high in voles, indicating a potential enrichment of antibiotic-resistance genes at higher trophic levels. This poses important challenges in ensuring animal and human health.

W24 - Exploration of the role of microbial associates in different species of ambrosia beetles

Presenting Author - *Juan Carlos Cambronero-Heinrichs, University of Padova, Italy*

Author/s – *Giacomo Cavaletto, Giacomo Santoiemma, Antonino Malacrino, Peter Biedermann, Andrea Battisti, Davide Rassati, Christopher M. Ranger*

Abstract Content

Ambrosia beetles (Curculionidae: Scolytinae) are fungus growing insects that mainly develop in wood tissue. These beetles are distributed worldwide, and some of them are recognized as successful invaders. Most ambrosia beetles preferentially attack dying or stressed and ethanol-emitting trees, but if substances beside ethanol can affect host selection and colonization is still unclear. In addition, galleries of ambrosia beetles are inhabited by multiple associates including filamentous fungi, yeasts, and bacteria. Hardly any of these microbes, besides nutritional symbionts, have been described or associated to functional capabilities. In a study performed at a forest site in Veneto, Italy, we investigated whether host selection and colonization success changed in eight tree species under two different treatments, a real stress (flooding) vs. a simulated stress (ethanol-injection). We studied the effect of abiotic stress on endophytic communities and microbial isolation was conducted. Isolated fungi were confronted and the interactions between the insect associated microbes, endophytes, and pathogens were described. We observed plant colonization by five species of ambrosia beetles: *Xylosandrus crassiusculus, Xylosandrus germanus, Xyleborinus saxesenii, Xyleborus monographus,* and *Anisandrus dispar*. Interaction between microbes suggest the presence of protective symbioses within the gallery associates that may benefit the insects, of mycopathogens cohabiting the insect galleries, and endophytes that successfully compete against the insect associated fungi. We also found closely related bacteria species in the galleries of the different insects. These bacteria might be functionally relevant for insect colonization success. Our findings help aid in understanding the multikingdom symbiotic web associated to ambrosia beetles.

W25 - Physiology and transcriptional analysis of ppGpp-related regulatory effects in the *Streptomyces diastatochromogenes* 1628

Presenting Author - *Yang Song, China Jiliang University, China*

Author/s – *Xuping Shentu, Xiaoping Yu*

Abstract Content

ppGpp is a ubiquitous small nucleotide messenger that mediates cellular self-protective responses under environmental stress. However, the mechanisms of ppGpp to control transcription and other metabolic process depend on species, and even ppGpp regulate the same process via different mechanisms. The level of ppGpp is regulated by RelA/SpoT homolog (RSH) enzymes that both synthetase and hydrolyze the alarmone. Here, we constructed a ppGpp0 strain and monitored the effects of ppGpp on the transcriptional level, physiology and secondary metabiotic production in the antibiotic producer *Streptomyces diastatochromogenes* 1628. The results showed the cell division and growth of ppGpp0 increased by measurement of gene transcription and DCWs. The utilization of nitrogen was affected depend on the nitrogen type, and a significantly higher DCW of the ppGpp0 in medium supplied with the yeast extract, and a lower growth rate in the inorganic nitrogen ammonium salt, while the ppGpp-mediated stringent response could not affect the usage of carbon resources. More importantly, the ppGpp0 inhibited the expression of antibiotic clusters, production of toyocamycin and tetremycin P. The antibiotic resistance was also significantly downregulated in the ppGpp0 mutant. In conclusion, this study shows detailed changes in ppGpp-mediated stringent responses on *S. diastatochromogenes* 1628 cell growth, nutrient utilization, morphological characteristics, antibiotic production and resistance, which will provide insights into the role of ppGpp in Streptomyces.

W26 - The diversity of biogeochemical cycles in hypersaline environments

Presenting Author - *Michael C. Macey, The Open University, United Kingdom*

Author/s – *Ermias Balcha, Susanne P. Schwenzer, Hagos Miruts, Felipe Gomez, Barbara Cavalazzi, Karen Olsson-Francis*

Abstract Content

Studying extremophiles allows the characterisation of the boundaries of life on Earth and the identification of metabolic processes that fuel biogeochemical cycling under extreme conditions. Here we present an analysis of the microbiomes of globally distributed hypersaline environments.

We screened published metagenomes produced from a range of hypersaline environments (Marine salterns in Spain, hypersaline lakes in Chile and Antarctica, and soda lakes in Egypt and Mongolia for the presence, diversity, and abundance of shared functional genes that encode for the enzymes relevant to biogeochemical cycling. The study was expanded by generating metagenomes from DNA extracted from the salt and water of an Ethiopian hypersaline lake in the Dallol Depression. Analysis was performed to compare the functional gene profiles between the hypersaline environments.

The microbial community within the Ethiopian Lake was comprised of Cyanobacteria, Candidate Phyla, and halophilic bacteria and archaea. Screening of the metagenomes identified that phototrophs in hypersaline environments typically possessed the majority of the genes relating to carbon dioxide and nitrogen fixation, indicating that they play a major role in driving both the carbon and nitrogen cycles. High abundances of genes involved in denitrification, methylamine utilisation, and carbon monoxide oxidation classified as Halobacterial were also identified in all the metagenomes, supporting that these taxa are potentially major players in biogeochemical cycling in hypersaline environments. Cultivation efforts are required to further define the interactions between the distinct functional clades identified in the hypersaline environments.

W27 - Exploring nitrogen-induced changes in barley microbial communities through genome-resolved metagenomics

Presenting Author - *Abondance Kalonji Tshisekedi, University Of The Witwatersrand, Johannesburg, South Africa*

Author/s – *Pieter De Maayer, Angela Botes*

Abstract Content

This study investigates the differences in microbial communities of malting barley (*Hordeum vulgare* L.) samples with varying nitrogen concentrations and their impact on germination. Nitrogen-Induced Susceptibility (NIS) is a known phenomenon in which high nitrogen levels in crops increase pathogenicity. However, the precise role of NIS in barley germination and microbial contamination remains unclear. We employed genome-resolved metagenomics to examine microbial communities in barley samples with high and low nitrogen concentrations. The samples were sequenced using the Illumina NovaSeq 6000 platform. We recovered 85 high-quality metagenome-assembled genomes (MAGs) using a combination of metagenome assembly, binning, and functional annotation tools. Comparative genomics revealed that high nitrogen MAGs have a higher copy number of putative genes associated with the Nitrogen Metabolism pathway such as glnK; which encodes a response regulator of the two-component system that senses changes in nitrogen availability and regulates the expression of genes involved in nitrogen metabolism and hmp; which encodes a flavohemoglobin that detoxifies nitric oxide, a reactive nitrogen species. Furthermore, the high nitrogen group also encoded a higher copy number of bacterial genes that are associated with plant pathogenicity. This includes genes encoding type III secretion systems as well as quorum sensing and biofilm formation mediators (i.e., luxS). This study provides new insights into the genomic content of microbial communities found in barley, as well as the genes that play a role in pathogenicity and nitrogen related stress response.

W28 - Sponge-associated *Bacillus* strains as potential reservoirs of antimicrobial and biosurfactant substances

Presenting Author - *Jéssyca de Freitas Silva, Federal University Of Rio De Janeiro, Brazil*

Author/s – *Bruno Francesco Rodrigues de Oliveira, Walter Martin Roland Oelemann, Marinella Laport,*

Abstract Content

Sponge-associated bacteria are prolific sources of biotechnologically relevant substances. In our previous work, two coastal sponge-derived strains of *Bacillus pumilus* and *Bacillus* subtilis showed noteworthy antibacterial and biosurfactant activities, respectively. Therefore, this study aimed to deepen our understanding of both bioactivities in these strains by a coupled strategy using (in vitro) bioassays and genome mining. The methanolic extract of *Bacillus pumilus* exhibited bacteriostatic activity against *Staphylococcus aureus* cells at a minimum inhibitory concentration of 29.6 µg/mL. A total of 12 biosynthetic gene clusters (BGCs), including type I and III polyketide synthases (PKS), non-ribosomal peptide synthases (NRPS), bacteriocins, betalactones, sactipeptides, terpenes, and siderophores were found in the *B. pumilus* genome (3.6 Mbp). Particularly, a potential NRPS product showed 85% similarity with bacilysin, with high homology to the cluster present in other *Bacillus* strains. The investigation of the potential biosurfactant activity of *B. subtilis* revealed a surface tension of 26.91 ± 0.29 mN/m, with emulsifying activity on both mineral oil and n-hexadecane. Eleven BGCs were predicted, including type I and III PKS, bacteriocins, terpenes, thiopeptides, and NRPS in the *B. subtilis* genome (4.1 Mbp). Especially one of the BGC sequences showed 82% similarity with a surfactin from *Bacillus* velezensis FZB42. These results are promising for the application of marine *Bacillus* in the development of new treatments against (multi)drug-resistant bacteria and green alternatives for bioremediation processes and other biomedical applications.

W29 - Genome-resolved metagenomics reveals distinct phosphorus acquisition strategies between soil microbiomes

Presenting Author - *Jingjing Peng, China Agricultural University, China*

Abstract Content

Background: Soil microbiome is the key player regulating phosphorus cycling processes. Identifying phosphate-solubilizing bacteria (PSB) and utilizing them for release of recalcitrant phosphate have implications for improving crop nutrient acquisition and crop productivity.

Objectives: We aimed to investigate thr functional profiles of P cycling in agricultural and reforestation soils, to determine the driving factors for microbial phosphate solubilization, and identify the genetic traits of PSB.

Methods and Results: A combination of a meta-analysis, genome-resolved metagenomics, and amplicon sequencing was applied. We found soil available phosphorus (AP) was the key factor shaping microbial community composition and function across our agricultural and reforestation sites. Membrane-bound quinoprotein glucose dehydrogenase (PQQGDH) and exopolyphosphatases governed microbial phosphate solubilization in agroecosystems. In contrast, genes encoding glycerol-3-phosphate transporters displayed a significantly greater abundance in the reforestation soils. A microbial phosphorus solubilization strategy dominated in the agricultural soils, while a microbial phosphate transporter strategy was observed in the reforestation soils. The gcd gene encoding PQQGDH was found to be the best determinant for AP. Furthermore, we reconstructed 472 MAGs covering agricultural soils from six long-term field trials across China and found large genome size, a high ratio of glycosyl hydrolase genes, and increased capacity for carbohydrate utilization were specific traits of GCD-MAGs. Notably, the gcd copy number showed a significant and positive correlation with genome size. Our study demonstrates that knowledge of distinct microbial phosphorus acquisition strategies and particular genetic traits of PSB in the soils, which may accelerate targeted engineering and improve management practices for sustainable agriculture.

W30 - Preliminary study of shark microbiota at a unique mix-species shark aggregation site, in the Eastern Mediterranean Sea.

Presenting Author - *Dalit Meron, University Of Haifa, Israel*

Author/s – *Goni Bregman, Maya Lalzar, Aviad Scheinin, Eyal Bigal, Dan Tchernov, Leigh Livne, Ziv Zemah Shamir, Danny Morick, Morris Kahn Marine Research Station*

Abstract Content

Sharks play an essential ecological role in shaping the marine food web and maintaining healthy and balanced marine ecosystems. Sharks are sensitive to environmental changes and anthropogenic pressure and demonstrate a clear and rapid response. As a meta-organism, sharks offer selective niches (organs) for microorganisms that can provide benefits for their hosts. However, changes in the microbiota can turn the symbiosis into a dysbiosis and may affect the host's physiology, immunity and ecology. Our study was conducted at a unique coastal site (Israel), where a mixed-species shark aggregation is observed. The aggregation includes two shark species, segregating by sex. In order to characterize the bacterial profile and examine the physiological and ecological aspects, microbiome samples were collected from different organs (gills, skin, and cloaca) from both shark species over three years. The bacterial composition was significantly different between the shark individuals and the surrounding seawater and between the shark species. The most dominant groups for both shark species were Flavobacteriaceae, Moraxellaceae, and Rhodobacteraceae. However, specific microbial biomarkers were also identified for each shark. An unexpected difference in the microbiome profile and diversity between the sampling seasons revealed an increase in the potential pathogen *Streptococcus*. The fluctuations in the relative abundance of *Streptococcus* between the months were also reflected in the seawater. Our study provides initial information on shark microbiome in the Eastern Mediterranean Sea. In addition, we demonstrated that these methods were also able to describe environmental episodes and the microbiome is a robust measure for LTER.

W32 - Flying microbes – Can microbes survive extreme conditions during a stratospheric balloon flight experiment?

Presenting Author - *Katharina Kujala, University of Oulu, Finland*

Author/s – *Tim Heitkämper, Vincent Vonderbank, Mike Lutz, Raphael Roth, Felix Berger, Stephan Harteneck,*

Abstract Content

Earth's stratosphere is characterized by hypobaric conditions, low temperatures, high intensities of UV and cosmic radiation as well as low water and nutrient availability. While it is not considered a permanent habitat for microorganisms, these can be transported to the stratosphere by storms, volcanic action or human activity.

The impact of those extreme conditions on microorganisms and their survival were tested by sending a sample gondola to the stratosphere. The sample gondola was built to allow exposure of *Bacillus* subtilis endospores at different angles to the sun. It moreover had holders for three environmental samples to test the effect of stratospheric conditions on complex microbial communities. The gondola attached to a stratospheric balloon was launched near Kiruna, Sweden, ascended to ~25km and drifted eastwards for ~200km. Samples were exposed to pressures as low as 2 kPa and temperatures as low as -50ºC as well as high UV radiation.

Survival rates of *B. subtilis* were determined by comparing the numbers of colony forming units for the different exposure angles. Survival was negatively correlated with exposure angle, indicating significant impact of UV radiation. The effect of stratospheric conditions on environmental samples was assessed by comparing most probable numbers and microbial community composition to controls that had stayed on the ground. Cultivation was possible from all samples with survival rates of at least 1% and differences in community composition were observed. Survival of environmental microorganisms might have been supported by the sample matrix which provided protection from radiation and desiccation.

W34 - The importance of generalists during the disturbance of gastrointestinal bacterial community in a model system

Presenting Author - *Daniel Herlemann, Estonian University of Life Sciences, Estonia*

Author/s – *Carmen Kivistik, Helen Tammert, Veljo Kisand, Daniel Herlemann*

Abstract Content

The gut microbiome is one of the most important sites of host-microbe interactions, however, mechanisms governing the responses of host-associated microbes to changing environmental conditions are poorly understood. To address this, we investigated individual and combined effects of dietary changes and increase in salinity (from freshwater to salinity 3) or antibiotic concentration on the gastrointestinal bacterial community of the aquatic snail *Ampullaceana balthica*. In parallel, energy reserves of the host were calculated by transforming the measured protein, lipid and carbohydrate content into energy equivalent. A change of natural food source as well as the combined treatment of salinity and food source decreased the richness and changed the composition of the A. balthica gastrointestinal bacterial community. In these treatments *Pseudomonas* became the dominant bacterium. However, energy reserves of the host were higher in these treatments compared to the reference aquaria specimens and the combined treatment of antibiotics with *S. obliquus*. Obviously the presence of antibiotics inhibited the dominance of *Pseudomonas*, and resulted in lower energy reserves despite *S. obliquus* feeding. Therefore the host seems to be able to adapt and replace its bacterial community composition to respond to mild changes in salinity and food source. Antibiotics in the water can disturb this self-regulating mechanism. Therefore, our study underlines the ability of aquatic macroinvertebrates to respond to sudden changes in food source and mild shifts in salinity. Moreover, it emphasizes the strong impact of the food source on the gastrointestinal microbiome and the importance of generalists during disturbance.

W35 - Mitigating salinity stress in tomato by rhizosphere engineering

Presenting Author - *Salila Pradhan, Indian Institute Of Technology Delhi, India*

Author/s – *Shubham Dubey, Shilpi Sharma*

Abstract Content

Salinization of soil is a matter of concern for agricultural sector because it impairs physiology and development of plants. The conventional approach to mitigate salinity stress include application of chemicals which may conversely lead to more soil salinization. An eco-friendly approach is application of plant beneficial microbes as bioinoculants. But indigenous soil microbes outcompete them thereby leading to a reduction in survival and efficacy of bioinoculants. Therefore, to overcome these limitations, one of the effective sustainable approaches is rhizosphere engineering. It involves modification of rhizosphere microbiome to enhance plant growth promotion and mitigate stresses encountered by plants. The objective of the current study was to mitigate salinity stress in tomato plants by a top-down approach through acclimatisation of the microbiome to salt stress over successive plant growth cycles. The rhizosphere microbiome from the best performing plant was used as inoculum for subsequent plant growth cycles. Acclimatisation of rhizosphere microbiome to salt stress has been done across ten plant growth cycles. The results in salinity-stressed plants treated with acclimatised soil microbiome showed an increase in root length, shoot length, chlorophyll and carotenoid contents as well as gradual decrease in the levels of stress indicators like proline, malondialdehyde relative to control plants. Our study brings forth the potential of plant-mediated rhizosphere engineering approach as a novel tool to improve crop productivity in a sustainable way.

W36 - Searching for microbes in mining waste

Presenting Author - *Francisca Prieto Fernández, University of Oulu, Finland*

Author/s – *Katharina Kujala, Stefan L*

Abstract Content

Mining activities generate waste materials. Tailings are one example of mine waste, and its storage represents an environmental challenge. On the one hand, tailings have an impact on the environment due to the potential release of metal(loid)s (e.g., antimony (Sb)) to natural ecosystems. On the other hand, they might still contain small amounts of valuable metals (e.g., copper (Cu)) that were not extracted in the first place. The main goal of this project was to identify, isolate and characterize microbes from mining-impacted sites in (sub-)Arctic regions. Soil samples from tailings were collected in winter and summer. Laboratory enrichment experiments have been carried out at 5°C to isolate microbes that can tolerate and/or utilize the metal(loid)s of interest in cold, in-situ relevant temperatures. Microbial communities in tailings samples and enrichments have been assessed via 16S rRNA gene sequencing. Results of the sequencing indicate diverse microbial communities, containing microbes known for metal tolerance/utilization, enrichment of (novel) Cu/Sb tolerant/utilizing microbes from the study sites, and impact of environmental conditions on microbial community composition. Moreover, isolates of aerobic and anaerobic Cu/Sb tolerators and anaerobic Cu/Sb respiring microbes have recently been obtained. The project's next stages will focus on characterizing these isolates, identifying their genomic potential for Cu/Sb turnover and resolving the expression of the identified pathways during active Cu/Sb turnover. The isolated strains may in future be used in alternative bioengineered solutions for the removal of pollutants (bioremediation) and extraction of mineral raw materials (biomining) under cold climate conditions.

W38 - Casting the infant gut microbiome: more than one leading roles

Presenting Author - *Athanasia Ioannou, Wageningen University & Research, Netherlands*

Author/s – *Bernadet Blijenberg, Marko Mank, Jan Knol, Clara Belzer,*

Abstract Content

The infant gut hosts a variety of bacteria that comprise the microbiota pioneers. These bacteria are highly important for infant health but also imprint the development to adulthood. Breast milk contains more than 200 structures of certain non-digestible carbohydrates, called Human Milk Oligosaccharides (HMOs) that feed and shape the early gut microbiota. It is known that members of the Bifidobacterium and *Bacteroides* species can degrade HMOs and thrive in this environment. However, the mechanism how this leads to the formation of microbial communities in the infant gut is not known. With our research we investigate how different HMO structures can sustain microbial communities as well as the function of each bacterial member within them.

To study this process, we created a synthetic community comprising of 13 bacterial strains that are normally found in the gut of breastfed vaginally born infants. Our synthetic community was subjected to continuous and sequential batch fermentations in basal medium and HMO mixes as carbon source.

The results show that HMOs are fully degraded by the synthetic infant gut microbiome. HMO degrading bacteria dominate the community in presence and activity. The growth of strains that do not possess an HMO degrading capacity from our community suggests cross-feeding of simple carbohydrates, organic acids and gases that are produced by the aforementioned strains. Our study offers not only a viable model for complex microbial interactions, but also an insight into how the infant gut microbiota collaboratively degrades the unique HMOs of human milk and adds to healthy growth.

W39 - Deciphering the interactions between lupin and its root-associated bacteria

Presenting Author - *Maite Ortúzar, Microbiology and Genetics Department, Spain*

Author/s – *Víctor Formariz, Magdalena Slawinska, Pengfan Zhang, Qi Wang, Raúl Riesco, Ruben Garrido-Oter, Martha E Trujillo*

Abstract Content

The European Union highly depends on soy imports (>70%) as a protein source since local production barely covers 5% of its internal demand. Thus, it is necessary to explore alternative sources to reduce this dependence. Among legumes, *Lupinus angustifolius* is as an important alternative given its high protein value and use for animal and human nutrition. This legume is a native plant of Europe, well adapted to the climatic conditions of many countries. It also thrives in poor soils due to its capacity to fix nitrogen. Plant adaptation may be partly due to the microorganisms associated with its roots, providing stability and resilience, in addition to plant growth promoting molecules and nutrients.

This work was designed to study the bacterial microbiota isolated from *L. angustifolius*. Bacteria were isolated from different plant compartments using various isolation media designed to target the most abundant groups detected by 16S rRNA metagenomic profiling. After screening and removing pathogenic strains, we designed and inoculated several bacterial synthetic communities (SynCom), under different cultivation conditions to study the association of these bacteria to the host plant.

Plants grown in a natural soil and in a gnotobiotic system were harvested after 8 weeks. All SynComs improved lupin growth when compared to the un-inoculated plants. In addition, we observed that all SynComs where *Micromonospora* was included, had a stronger interaction with the plant. RNA-seq gene expression was then used to determine the reaction of the plant when inoculated with the SynComs.

W40 - Multiple greenhouse gases are part of the gourmand menu of a thermoacidophilic methanotroph

Presenting Author - *Samuel Imisi Awala, Chungbuk National University, Republic of Korea*

Author/s – *Joo-Han Gwak, Yong-Man Kim, Sung-Keun Rhee,*

Abstract Content

Carbon dioxide (CO2), methane (CH4), and nitrous oxide (N2O) are the three major greenhouse gases (GHGs), and geothermal areas represent one of Earth's major natural sources of these gases. In this study, we isolated strain IT6, a thermoacidophilic and facultative CH4-oxidizing bacterium, from an acidic geothermal sample. It conserves energy by aerobically oxidizing CH4 and hydrogen (H2) while obtaining carbon via autotrophic CO2 fixation. The strain unexpectedly grows on C3 intermediates of propane as a source of additional energy and carbon, and the full biochemical pathway for C3 metabolism was predicted by genomic and transcriptomic analyses and supported by substrate specificity experiments. The genome of strain IT6 contains genes for a sec-dependent N2O reductase, which is similar to those found in *Hydrogenobacter* species, enhancing its ecophysiological adaptability even further. This enzyme allows it to respire N2O and thus continue to function in anoxic environments. Surprisingly, N2O reduction is reportedly inhibited at acidic pH, resulting in N2O accumulation in an acidic environment; however, physiological experiments revealed that this strain could grow on methanol, C3 compounds, and H2 while reducing N2O in an extremely acidic environment (near pH 2). As a result, it is the first extremely acidophilic isolate that reduces N2O while also consuming other GHGs. These findings identify an extremely thermoacidophilic methanotroph's adaptation to life in anoxic geothermal environments, improve our understanding of N2O reduction in acidic environments, and provide clues for the use of this strain in studies mitigating the three most important GHG emissions.

W41 - Cultivation and genomic analysis of a heterotrophic archaeon of Nitrososphaerota from a geothermal spring

Presenting Author - *Joo-Han Gwak, Chungbuk National University, Republic of Korea*

Author/s – *Samuel Imisi Awala, Sung-Keun Rhee*

Abstract Content

Little is known about the physiology and metabolism of deep-branching clades of Nitrososphaerota due to the lack of cultured representatives. *Conexivisphaera calida* NAS-02, a thermoacidophilic sulfur- and iron-reducing organotroph, is the sole representative isolate of the clade *Conexivisphaera*. Here, we report a thermoacidophilic archaeon (named Bin2) belonging to the Nitrososphaerota Beowolf clade enriched at pH 5.5 at 64 ℃ from a geothermal hot spring in Naples, Italy. In contrast to *C. calida* NAS-02, which was unable to grow at 1% or higher O2 concentrations, Bin2 grew aerobically. Bin2 didn't harbor any gene related to ammonia monooxygenase and showed no growth with ammonia oxidation. The microbial consortium in this enrichment was stabilized and successfully transferred to liquid media in polypropylene tubes without providing any organic carbon and energy source; thus, the growth of this consortium might be supported by additives used in polypropylene manufacturing that might be released into the media during incubation. Based on the qPCR analysis, an exponential growth phase was only observed after the bacterial cells entered a death phase indicating cells of Bin2 might graze on the bacterial cell debris. Bin2 cells were grown to 2–4 × 107 cells/ml and comprised up to ~87% of the total prokaryotes in the final stage of a batch culture. It can be corroborated by the results of genomic analysis: the Bin2 genome contains numerous genes encoding for assimilation of complex or simple organic compounds for chemoorganotrophic growth. Together, our results provide additional metabolic insights into the early diverging Nitrososphaerota clades.

W42 - Rumen microbiome of holstein dairy cows associated with greenhouse gas emissions

Presenting Author - *Gi Beom Keum, Dankook University, Republic of Korea*

Author/s – *Hyunok Doo, Sumin Ryu, Eun Sol Kim, Jinok Kwak, Sriniwas Pandey, Hyeun Bum Kim,*

Abstract Content

Background: Greenhouse gases have harmful effects on environments and public health. One of the main contributors to greenhouse gases is methane that can be generated by microbial fermentation in the ruminant intestinal tracts. Therefore, the first step to understanding the methane production by ruminants is to identify and describe microbial communities in the gut.

Objectives: The aim of this study wat to evaluate the rumen microbiome of Holstein dairy cows related with greenhouse gas emissions.

Methods: Ruminal contents were collected from the Holstein dairy cows through rumen cannula, and the solid and liquid contents were separated by centrifugation. Total DNA from each solid and liquid ruminal content was extracted and the V5-6 hypervariable regions of 16S rRNA genes were amplified. 16S rRNA gene sequencing was conducted using the Illumina MiSeq platform. QIIME2 and Mothur pipelines were used for the16S rRNA gene sequence analysis. Statistical analysis was performed using the two-sided Welch's t test in Statistical Analysis of Metagenomic Profiles v2.1.3.

Results: Alpha-diversity indices showed no significant differences between the solid and liquid ruminal contents. Even though Bacteroidetes and Firmicutes were the most abundant phyla in both types of ruminal contents, distinct differences in the microbial compositions of the ruminal contents were observed at the genus level. The relative abundance of the genus *Methanobrevibacter*, methane producing bacteria, was significantly higher in solid than liquid ruminal contents. However, the relative abundance of the genus Selenomonas, that competes with methanogens for H2, was significantly higher in liquid than solid ruminal contents.

W43 - A homogeneous microbial transfer between surfaces from fibre-based food packaging materials to a contact surface

Presenting Author - *Stephanie Maitz, Medical University Of Graz, Austria*

Author/s – *Herwig Friedl, Paul Jakob Schmid, Clemens Kittinger*

Abstract Content

The microbial transfer from food packaging materials to a contact surface is not sufficiently researched until today. In fact, bacterial transfer from fibre-based materials has been studied from laboratory produced packaging products spiked with spores on agar surfaces at low levels (0.03–0.10%). In contrast, a transfer of <0.01–2.48% of the total microbial load of fibre-based materials after the production to a contact agar was observed for industrially produced packaging materials.

Correspondingly, this study focuses on the detailed investigation of the transfer of microorganisms from both outer sides (front, back) of different food packaging materials to a contact surface.

We established a simple model to observe the microbial transfer from packaging materials to agar plates. Images were taken from the colony forming units (CFU) on the agar plates. For detailed analysis, the agar plate was divided into seven equal sectors and all CFU in the corresponding sectors were counted.

If the samples were considered individually, a sample-specific behaviour in the transferred CFU between front and back was determined for the tested samples. The cyclic function showed that the back transferred twice the number of transferred CFU compared to the front for all samples tested. The calculation of the quotient (produced by the division of transferred CFU of the back by the front) reinforces the previous results, demonstrating a different microbial transfer between back and front. In contrast, the ratio of the transferred CFU from the back/front remained the same within the examined sectors for all samples.

W44 - Diverse compost microbiomes suggest plastic degrading capabilities

Presenting Author - *Tobias Spanner, Leibniz University Hannover, Germany*

Author/s – *Nadine Rüppel, Daria Frohloff, Tim Börner, Hannah Kleyer, Marcus A. Horn*

Abstract Content

Composting is a thousand-year-old practice that was industrialized during the last century. Biological waste and its microbiome encounter dynamic temperature and pH variations within the composting process. Contrasting industrial composting practices and various input materials including biodegradable plastics spark interest in which way microbial communities develop during composting, associated degradation potentials, and potential key taxa involved. Thus, we sampled compost and biological waste in early and late stage of composting from 9 different facilities. Environmental parameters like pH, temperature, C/N ratio and moisture were measured. 16S rRNA gene and ITS amplicon sequencing was conducted to analyze microbial communities. Microbial communities were compared with plasticDB database to check for possible plastic degradation potentials. Furthermore, bacteria and fungi were isolated from three facilities and tested for hydrolytic potentials on media supplemented with Impranil®. Temperature increased during early stages of composting prior to a decline towards later time points. pH increased, and C/N as well as moisture decreased during composting. OTUs from Bacillaceae, Thermaceae and Thermoactinomycetaceae as well as unknown families of Saccharomycetales and Eurotiales were parts of the core microbiome. Most abundant bacterial OTUs in compost hosting potential plastic degraders were *Bacillus* and *Pseudomonas*. For fungi, OTUs of *Aspergillus* and *Morteriella* were the most abundant hosting potential plastic degraders. Isolates belonging to *Cupriavidus*, *Sphingobacterium* and *Aspergillus* showed hydrolyzed Impranil®, likewise suggesting plastic degrading potentials. Thus, our combined data suggests the existence of a compost core microbiome despite a broad range of composting plant operation conditions and plastic degrading capabilities.

W45 - Role of the oxidative stress regulator OxyR in an entomopathogenic bacterium

Presenting Author - *Victoria BIENTZ, Montpellier University, France*

Author/s – *Victoria Bientz, Sylvie Pagès, Anne Lanois, Raphaël Bousquet, Nadège Ginibre, Marie Gislard, Emeric Dubois, MGX-Montpellier GenomiX, Institute of Functional Genomics, Montpellier, France*

David Roche, CEA, Genoscope, Paris-Saclay University, Paris, France

Stéphane Cruveiller, CEA, Genoscope, Paris-Saclay University, Paris, France

Julien Brillard

Abstract Content

Xenorhabdus nematophila is a Gram-negative entomopathogenic bacterium, mutually associated with the soil nematode Steinernema carpocapsae. The nematobacterial complex is parasitic for a broad spectrum of insects, and is used as biological control agent. After entering insect larvae, the nematodes release their bacteria, which produce various virulence factors. The insect dies in a few days. Nematodes multiply until all nutrients are used, then re-associate with the bacteria before leaving the cadaver. The transcriptional regulator OxyR is widely conserved in bacteria. It is one of the main actors in the defense against oxidative stress. It activates the transcription of a set of genes that influence cellular defense against oxidative stress. In addition, OxyR has been shown to be involved in the virulence of several bacterial pathogens. We constructed OxyR-mutant in *X. nematophila* and phenotypically characterized it in vitro and in vivo during bacterial interactions with eukaryotic hosts. Results revealed that OxyR played a major role during the bacterial resistance to oxidative stress, as already shown in many other bacterial species. In vivo, compared to a control strain, our OxyR mutant displayed a slight delay in killing insect larvae, revealing its involvement in bacterial virulence. In contrast, the mutant seemed to improve the reproductive success of its mutualistic nematode, suggesting that OxyR can also contribute to the symbiotic stage of the bacterial life-cycle. Our study illustrates the broad range of phenotypes for which the OxyR transcriptional regulator is important.

W46 - Natural existing arbuscular mycorrhizal-bacterial biofilm associations and their functional behavior

Presenting Author - *Aditi Pandit, Teri Deakin Nanobiotechnology Centre, India*

Author/s – *Leena Johny, David Cahill, Lambert Brau, Mandira Kochar*

Abstract Content

In symbiosis with plant, arbuscular mycorrhizal fungi (AMF) access the carbon stored in the roots in exchange for uptake of nutrients and water. In the mycorrhizal-plant symbiosis, AMF–associated bacteria (AAB) serve as a third partner and are tightly linked to AMF. AAB are involved in mycorrhizal activity and nutrient uptake enhancement and have impact on plant development. In order to create biofertilizer for sustainable crop production, it is important to understand the function and process of this inter-kingdom natural coexistence. In our research, we used in vitro and in situ co-cultures to screen 33 AMF species, and we characterized 231 AAB using 16S rDNA analysis. 109 selected AABs were examined for ten functional qualities that promote plant growth, and it was found that different bacterial strains had a variety of advantageous traits. The association of AAB was seen as biofilm and endobacteria using microscopic methods. Further, by using an in vitro assay system, an association recreation of 12 AAB-*Rhizophagus irregularis* was investigated to look at the impact on mycorrhization and functional capabilities. It was observed that AABs moved along the developing R. irregularis hyphae and spores. Different AAB had an impact on AMF development as well as its capacity to solubilize phosphate and potassium and fix nitrogen. We discovered both the synergistic interactions and partnerships between the two cross-kingdom microbial partners. Understanding the molecular elements of these fungal-bacterial connections, which will enable their later use and modification for sustainable agriculture practices, is another area of focus.

W47 - HPr prevents FruR-mediated facilitation of RNA polymerase binding to the fru promoter in *Vibrio cholerae*

Presenting Author - *Seunghwan Lee, Seoul National University, Republic of Korea*

Author/s – *Chang-kyu Yoon, Zhang Jing, Hye-Young Lee, Min-Kyu Kim, Yeong-Jae Seok,*

Abstract Content

Phosphorylation state-dependent interactions of the phosphoenolpyruvate (PEP):carbohydrate phosphotransferase system (PTS) components with transcription factors play a key role in carbon catabolite repression (CCR) by glucose in bacteria. Glucose inhibits the PTS-dependent transport of fructose and is preferred over fructose in *Vibrio cholerae*, but the mechanism is unknown. We have recently shown that, contrary to *Escherichia coli*, the fructose-dependent transcriptional regulator FruR acts as an activator of the fru operon in *V. cholerae* and binding of the FruR-fructose 1-phosphate (F1P) complex to an operator facilitates RNA polymerase (RNAP) binding to the fru promoter. Here we show that, in the presence of glucose, dephosphorylated HPr, a general PTS component, binds to FruR. Whereas HPr does not affect DNA-binding affinity of FruR, regardless of the presence of F1P, it prevents the FruR-F1P complex from facilitating the binding of RNAP to the fru promoter. Structural and biochemical analyses of the FruR-HPr complex identify key residues responsible for the *V. cholerae*-specific FruR-HPr interaction not observed in *E. coli*. Finally, we reveal how the dephosphorylated HPr interacts with FruR in *V. cholerae*, whereas the phosphorylated HPr binds to CcpA, which is a global regulator of CCR in *Bacillus* subtilis and shows structural similarity to FruR.

W48 - Characterization of aeromonas from a recreational estuary reveals the carriage and dissemination of resistance genes

Presenting Author - *Anna Luiza Bauer Canellas, Federal University Of Rio De Janeiro, Brazil*

Author/s – *Bruno Francesco Rodrigues de Oliveira, Marinella Silva Laport*

Abstract Content

From a One Health perspective, investigating antimicrobial resistance in the environment is crucial to predict implications for public health. Our aim was to characterize *Aeromonas* strains isolated from a polluted recreational estuary, focusing on the characterization of 13 strains [1;2]. The presence of antimicrobial (ARG) and heavy metal resistance genes (HMRG), along with the class 1 integron-integrase gene (intI1), were evaluated by PCR. Six of the 13 tested strains (46.1%) were blaTEM-positive, four (30.7%) were blaKPC-positive, and two (15.4%) were mcr-3-positive. The intI1 gene was detected in seven (53.8%) strains. The merA gene (mercury resistance) was detected in 10 strains (76.9%) and, out of 11 strains carrying at least one HMRG, six (54.5%) also harboured at least one ARG. One strain, identified a*s Aeromonas hydrophila*, was selected for whole genome sequencing. Genomic analyses of *A. hydrophila* strain 34SFC-3 revealed ARGs related to ten different antimicrobial classes. Mobile genetic elements were found, including insertion sequences and a plasmid. A highly mobilizable region harboring ARGs, including blaKPC, was discovered. Genes involved in the resistance to several heavy metals were present, along with virulence genes. *A. hydrophila* 34SFC-3 harbours features that contribute to its survival in a deeply polluted environment, such as enzymes involved in the reduction of nitroaromatic and azo compounds. Overall, *Aeromonas* strains isolated from a recreational estuary can harbour ARGs, HMRGs, and virulence factors, thus providing insights into risks to public health by having Aeromonas as a paramount model in that framework.

W49 - Prevalence of domestic animals infecting *Sarcocystis* parasites in samples from sediment of water bodies in the Baltic States and Poland

Presenting Author - *Agnė Baranauskaitė, Nature Research Centre, Lithuania*

Author/s – *Živilė Strazdaitė-Žielienė, Petras Prakas, Dalius Butkauskas, Elena Servienė*

Abstract Content

Sarcocystis spp. are unicellular protozoan parasites having a two-host life cycle and infecting livestock. To date, little research has been conducted on the detection of *Sarcocystis* parasites in environmental samples. The aim of this work was to determine whether sporocysts of *Sarcocystis* spp. settle at the bottom of water bodies and compare their prevalence in the water sediment in the Baltic States and Poland.

Equal amount (n = 20) of water sediment samples were collected from each of the Baltic States and Poland during the summer of 2021 and 2022. Primer pairs that were specific for identification of *S. arieticanis, S. bertrami, S. capracanis, S. cruzi, S. miescheriana*, infecting sheep, horses, goats, cattle, and pigs/wild boar, respectively were selected for PCR targeting cox1 gene. Based on molecular methods, the highest prevalence of *Sarcocystis* was confirmed in Poland, Latvia and Lithuania ranging from 90% to 100%, while occurrence of *Sarcocystis* was seemingly lower in samples from Estonia (55%). Usually three (25-60%) or two (15-45%) different species were identified in an individual sample. Considering whole region, detection rates were: 83.8% *S. cruzi*, 73.8% *S. arieticanis*, 31.3% *S. capracanis*, 26.3% *S. miescheriana* and 18.8% *S. bertrami*. After comparing the obtained results with the amount of corresponding farm animals raised in the countries, it was concluded that *Sarcocystis* spp. prevalence depends not only on the number of animals per 1 km2, but also on the type of farming, free-range or mixed, that is applied.

W50 - In-depth microbiome analysis of the rumen of Ovis Aries under microalgae dietary supplementation. Towards an eco-friendly and nutritional value end-products

Presenting Author - *Dimitrios Skliros, Laboratory Of Molecular Biology, Agricultural University Of Athens, Greece*

Author/s – *Alexandros Mavrommatis, Panagiota Kyriakaki, Chrisanthi Kalloniati, Eleni Tsiplakou, Emmanouil Flemetakis,*

Abstract Content

Background: Rumen is a highly specialized organ of ruminant animals that promotes a community of mutualistic microbial species contributing to the digestion of plant fiber and cellular material. The rumen microbiome is directly related to energy and microbial protein biosynthesis, playing also an important role in milk production and composition. In addition, methanogenic archaea metabolize rumen substances such as carbon dioxide to methane, subsequently released in the atmosphere via ruminants' eructations. Fat-rich microorganisms can be utilized for the modulation of rumen microbiome towards eco-friendly and nutritional value end-products.

Objectives: The aim of this study was to contribute to the knowledge of the effect of microalgae on the methanogenic species and bacteriome structure in the rumen digest of sheep.

Methods: Microalgae were supplemented into dairy sheep diets. The concentrate of the control group (CON) contained no microalgae, while those of the treated group were supplemented daily with 30 g of Schizochytrium spp./sheep. Using 16S and shotgun sequencing, in-depth metagenomics analysis enabled us to identify Amplicon Sequence Variants (ASVs) and quantify ruminal microbial communities.

Results: The relative abundances of total Archaea and methanogens were decreased in microalgae-fed sheep compared to the CON group. Bacteriome analysis unveiled imbalances between proteolytic, amylolytic and cellulolytic bacteria in sheep's rumen digesta indicating a severe swift of rumen habitat and an exciting expansion of niches. Metagenomics can prove a powerful tool for understanding animal physiology towards developing novel dietary supplementations, aiming to orchestrate the biochemistry of rumen favoring methane mitigation and nutrient availability.

W51 - Effect of agricultural management on soil biodiversity is context dependent

Presenting Author - *Lucie Jiraska, University of Auckland, New Zealand*

Author/s – *Paulina Giraldo-Perez, Sarah Knight, Beatrix Jones, Matthew Goddard*

Abstract Content

Soils underpin productivity in all agroecosystems and host a diverse community of organisms that support and regulate ecosystems services and functions. Agricultural intensification threatens soil biodiversity and potentially ecosystem productivity putting future food security at risk. We investigated the effects of agricultural management and different commercial fungicides on soil biodiversity using both a large multi-year, multi-region field-based study and mesocosm experiments with New Zealand vineyards.

Soil samples were collected from 24 commercially managed vineyards in two regions and across three seasons per year for five years to investigate the effect of management on broad biodiversity using 16S, ITS2 and COI amplicon sequencing. The soil mesocosm experimentally tested the effect of commonly applied fungicides on a subset of soils from the large field study in a controlled environment using both amplicon sequencing and RNAseq.

The field-based study revealed a weak and inconsistent effect of management on broad-scale soil biodiversity. In the mesocosm experiment there was also no significant difference in community composition due to fungicide application; however, transcriptional analysis indicated significant differential changes in expression after the application of fungicides, notably in the oxidation of phenols, stress related genes and viral transcripts. Together these data suggest fungicides have a greater effect on community function than community composition. These findings advance our understanding of management regime effects on biodiversity and have implications for the sustainable management of agroecosystems.

W52 - Introducing the novel species of the genus *Roseateles*, isolated from freshwater

Presenting Author - *Darya Guliayeva, Institute of Microbiology of the National Academy of Sciences of Belarus, Belarus*

Author/s – *Anastasiya Sidarenka, Leonid Valentovich*

Abstract Content

Background: The genus *Roseateles*, a member of the family Comamonadaceae, class Betaproteobacteria, was originally discovered in 1999. At the time of writing, the genus contains only 3 validly published species: *Roseateles aquatilis, Roseateles terrae, and Roseateles depolymerans*. Bacteria of this genus are aerobic phototrophs containing bacteriochlorophyll a and capable of degrading aliphatic polycarbonates, which makes them promising for use in plastic recycling.

Objectives: Characterization of biological properties and genome annotation of strain bsSlp3-1, belonging to genus *Roseateles*.

Methods: Microbiological, biochemical, and molecular-genetic methods were used in this study.

Results: Strain bsSlp3-1 was isolated from freshwater of Slepian water system (Minsk, Belarus). Cells were gram-negative rods. On R2A medium strain forms colonies 3–4 mm in diameter, creamy-white and mucous appearance. The biosynthesis of pink pigment and the presence of photosynthetic gene operon in the genome of the strain prove the presence of bacteriochlorophyll a, similar to R. depolymerans. Strain bsSlp3-1 hydrolyzes starch, gelatin, casein, tween-20, and tween-80, is resistant to ampicillin, penicillin, vancomycin, and lincomycin. The whole genome analysis of strain bsSlp3-1 supported the hypothesis that it could be a new species of the genus *Roseateles*. The highest values of average nucleotide identity (81.18%) and digital DNA–DNA hybridization (25.6%) were obtained with the genome of the *R. aquatilis* CCUG 48205T, which is below the cutoff values to be considered the same species. The genome of strain bsSlp3-1 contains genes related to the degradation of biodegradable plastics, such as cutinase and polyhydroxyalkanoate depolymerase, that can be useful for plastic disposing.

W53 - A novel framework to monitor the role of plastic debris in horizontal gene transfer

Presenting Author - *Ifra Ferheern, University Of Camerino, Italy*

Author/s – *Lucia Cimarelli, Roberto Spurio*

Abstract Content

Mobile elements like plasmids, transposons, and integron can mediate the spread of Antibiotic Resistance Genes (ARGs) among bacteria, a common event occurring in natural environments and in clinical settings. Plastic debris provide a new 'hotspot' for the colonization of bacteria, thereby accelerating the horizontal gene transfer phenomena. This study is focused on a detailed analysis of the ability of a microbial community to acquire plasmid DNA under controlled laboratory conditions. Transformation efficiency was investigated by using a novel microcosm model system consisting of a microbial consortium of five environmental strains isolated from fresh water and two plasmids (pACYC:Hyg and pBAV-1k carrying hygromycin and kanamycin resistance genes, respectively). Five variables, which simulate possible conditions experienced by bacteria in the natural environment, were assayed: a) sterile soil, b) CaCl2 solution, c) combination of sterile soil and CaCl2, d) *E. coli* cell-free extract, e) plastic debris. Plastic debris proved to be the most efficient means of transforming naked plasmid into the microbial consortium as compared to the other four variables. Furthermore, we compared the performance of four plastic polymers (Polypropylene, Polystyrene, Polyethylene terephthalate, Polyethylene) in facilitating plasmid uptake by the microbial community. Polypropylene and polystyrene polymers were found to increase preferentially the exchange of ARGs between different bacterial taxa in as short as 4hr time interval. Our results depict the risk of plastic litter present in soil and water environments in promoting the distribution and spread of plasmid DNAs and their associated ARGs.

W54 - The influence of genetic variability of different sugar beet hybrids on the diversity of endophytic bacteria in seeds

Presenting Author - *Marija Petrović, University of Belgrade, Serbia*

Author/s – *Tamara Janakiev, Slavoljub Vukićević, Ivica Dimkić*

Abstract Content

Background: Sugar beet is the most important crop for sugar production in Serbia, and its bacteriobiota is of crucial importance for the study of host-microbiome interaction.

Objectives: For the first time, the non-cultivable seed bacteriobiota of different sugar beet hybrids and their correlation network per hybrid were studied.

Methods: Seeds from five different sugar beet hybrids known as Eduarda (ED), Tibor (T), Tajfun (TF), Koala (KO), and Cercospora-resistant (C) were used. 16S rRNA metabarcoding analysis was performed using the NovaSeq 6000 sequencing platform and bioinformatic strategies.

Results: Higher alpha diversity was observed in ED, KO and T at all taxa levels compared to C and TF hybrid, except at the ASV level. The distance of KO and ED from other hybrids was found at ASV and genus levels. The phylum Proteobacteria was most represented in all hybrids, followed by Cyanobacteria and Actinobacteriota. Firmicutes was strongly represented in the TF hybrid, while Bacteroidota was found in all hybrids except the T hybrid. Low abundance of Acidobacteriota and Chloroflexi were detected only in the ED, KO, and T hybrids. The predominant genus in all hybrids was *Pantoea*, followed by *Pseudomonas*, *Acinetobacter*, *Chalicogloea*, *Corynebacterium*, *Enterobacter*, *Enterococcus*, *Glutamicibacter*, *Kosakonia*, and *Marinilactibacillus*. Unique genera in the hybrids were *Pleurocapsa* and *Arthrobacter* (T), *Klebsiella* (TF), *Apibacter* (ED), and *Alloscardovia* (KO). The genera that were most abundant in one hybrid while only trace or absent in the others were: *Weissella* and *Staphylococcus* (TF), *Streptococcus* (T), *Gardnerella*, *Prevotella*, and *Rothia* (KO), *Gilliamella*, *Lactobacillus*, and *Snodgrassella* (ED).

W55 - *Pseudomonas* isolates from the 4th Joint Danube Survey – an assessment of species distribution and antibiotic resistances

Presenting Author - *Astrid Paulitsch-Fuchs, University Of Applied Sciences Kärnten, Austria*

Author/s – *Michael Koller, Gernot Zarfel, Carina Konstantinovic, Eric Olsacher, Nina Lackner, Astrid Paulitsch-Fuchs,*

Abstract Content

The Joint Danube Survey, the world's largest river expedition, took place for the 4th time in 2019. The aim was to test the water quality of the entire Danube using various parameters. Among these, the identification and resistance testing of *Pseudomonas* from the Danube was carried out to investigate the human influence on this species. An assessment of antibiotic resistance of clinically-relevant and non-relevant species is important to monitor the spread of antibiotic resistance.

The aim of the study was to identify isolates from the Danube river using MALDI-TOF MS and assess the resistance pattern using agar diffusion tests. Therefor twelve different antibiotics, which are commonly used in hospitals to treat infections with *Pseudomonas* were tested.

In total 326 *Pseudomonas* isolates could be detected. The most common species was *P. putida* with 93 isolates (29.2%). *P. aeruginosa*, the most clinically relevant species, has been identified 16 times (5%). 82 isolates (25.9%) could not be assigned to any known species with MALDI-TOF. Resistance testing of *Pseudomonas* revealed a strikingly frequent resistance to aztreonam with 86.3%. Multidrug resistance to 3 or more classes of antibiotics was detected in 5.8% of isolates.

To identify all *Pseudomonas* relevant to this study we further plan to sequence the rpoD gene. In the future, water will have to be increasingly protected. A more conscious use of antibiotics, especially in agriculture and industry, will be needed to further contain the development of resistances.

W57 - Metatranscriptomics shows that *Fomes fomentarius* fruiting body decomposition is driven by Tenebrionidae and Opiidae

Presenting Author - *Jason Bosch, Institute of Microbiology of the Czech Academy of Sciences, Czech Republic*

Author/s – *Priscila Dobbler, Tomáš Větrovský, Petr Baldrian, Vendula Brabcová*

Abstract Content

Background: *Fomes fomentarius* is a white-rot fungus, with a long-lasting fruiting body, that plays a major role in the decomposition of deadwood in Northern Hemisphere forests. It is of scientific interest for its ecological role, potential as a structural material and use as a medicinal supplement. However, little is know about the metatranscriptome of the actively-growing fungus and how this changes during fruiting body decomposition.

Objectives: We hypothesised that the fresh fruiting body metatranscriptome would show the expression of deadwood decomposition-related enzymes, whereas the rotten fruiting bodies would serve as a growth substrate for fungivorous microbes.

Methods: Fresh and rotten fruiting bodies of *F. fomentarius* growing on beech trees (Fagus sylvatica) in Žofínský Prales National Nature Reserve in the Czech Republic were harvested in 2017. RNA was extracted using a NucleoSpin RNA plant kit and sequenced on an Illumina HiSeq 2500. Transcriptomes were assembled with Trinity and analysed in R. The gene expression of six fresh and three rotten transcriptomes was assessed with a focus on Carbohydrate Active enZymes (CAZymes).

Results: In fresh samples, 60-70% of the transcripts belonged to *F. fomentarius* with high expression of short, secreted proteins of unknown function. In rotten samples, fewer than 1% of the transcripts came from *F. fomentarius* and chitinases were produced by Tenebrionidae. Tenebrionidae and Opiidae both produced CAZymes targetting beta-glucans and were present in all rotten samples. We conclude that decomposition of *F. fomentarius* fruiting bodies is primarily due to insects, particularly Tenebrionidae beetles and Opiidae mites.

W58 - Tracking antibiotic resistance genes along the trophic gradient in the central Adriatic Sea

Presenting Author - *Mia Dzelalija, University Of Split, Croatia*

Author/s – *Željana Fredotović, Slaven Jozić, Ivica Šamanić, Marin Ordulj, Ana Maravić*

Abstract Content

Background: Aquatic environment is critical for understanding the evolution and global spread of antibiotic resistance genes (ARGs) because it serves as an endpoint for effluent from wastewater treatment plants and other anthropogenic factors that contribute to the spread of antibiotic resistance. ARGs among major driving factors in regulating microbiome diversity are core issue for understanding the role of bacterial communities as their carriers and in predicting ecosystem responses.

Objectives: Our objective was to quantify the relative abundance of ARGs using RT-qPCR in the marine environment along a trophic gradient in the central Adriatic Sea.

Methods: Total DNA was extracted from 20 seawater samples at the surface and bottom of six sites along the trophic gradient using 0.22 um filters and DNeasy PowerWater kit. RT-qPCR was used to quantify five resistance genes (tetA, sul2, blaTEM, blaVIM, mphA) previously identified in this study area by functional metagenomics, as well as the class 1 integron integrase gene (intl1). 16S rRNA gene copy numbers (rrn) were determined to assess the total bacterial load and calculate the relative abundance of resistance genes in the bacterial community of each sample.

Results: TetA (6.63 copies/rrn) was significantly more abundant in winter at the nearshore sites as well at the sea surface of the offshore sites, demonstrating the persistence of these resistance genes even in the autochthonous marine community. Overall, the ARGs studied were significantly more abundant in seawater at all sites in winter than in summer.

W59 - Decreasing biotite particle size increases siderophore-mediated weathering of biotite mineral by strain PML1(12)

Presenting Author - *Cintia Blanco Nouche, Inrae, France*

Author/s – *Cédric Paris, Tiphaine Dhalleine, Philippe Oger, Marie-Pierre Turpault, Stéphane Uroz*

Abstract Content

In nutrient-poor ecosystems such as forests, the flux of nutrients coming from mineral weathering plays an essential role in the replenishment of soil fertility and in tree nutrition. Consequently, minerals represent important reserves of nutrients, which reactivity depends on their chemistry, size and age. Among the nutrients present in minerals, iron is usually poorly available. Iron can be found as the main constituent of iron oxides as hematite (Fe_2O_3) or in smaller amounts in primary minerals such as biotite ($K(Mg,Fe)_3(AlSi_3O_{10})(OH)_2$). To deal with iron limiting conditions, bacteria have evolved low-molecular weight compounds with high affinities for iron (i.e., siderophores). The question is to determine whether siderophore production plays a role in the mineral weathering ability of bacteria and how the physico-chemical properties of the minerals condition this function. In this work, the model strain PML1(12) Caballeronia mineralivorans, effective at weathering and known to produce a siderophore, was considered. Here we describe the iron acquisition system of this strain (i.e., rhizobactin) and its effectiveness at mobilizing iron from hematite and biotite. To do it, we used a combination of microcosm experiments, using different sizes of biotite as unique source of iron incubated in presence of the wild-type strain and its siderophore-deficient mutant. The quantification of the nutrients released in solution was done by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). Our analyses revealed that rhizobactin production significantly contributes to iron mobilization from biotite, and that the effectiveness of this siderophore depends on the size of the biotite particles.

W60 - Mineral weathering by heterotrophic bacteria as a function of mineral properties, solution chemistry and carbon substrate

Presenting Author - *Stephane Uroz, INRAE, France*

Author/s – *Stephane Uroz, Laura Picard, Cintia Blanco-Nouche, Marie-Pierre Turpault, Philippe Oger*

Abstract Content

Soil is composed of a mosaic of different rocks and minerals, which chemical composition and reactivity strongly condition soil fertility, organo-mineral associations and biological processes. Minerals do not only represent reactive interfaces where nutrients are made available, but also real habitats for microorganisms. However, the nutrients entrapped in these minerals are not directly available to the biosphere and require a process termed mineral weathering. Based on abiotic and biotic reactions, mineral weathering is partly attributed to soil microorganisms (i.e., fungi, bacteria). While the presence of effective mineral weathering bacteria has been reported in different compartments of the soil (mainly the rhizosphere), the molecular mechanisms engaged, the genes and the factors of regulation remain poorly characterized or unknown, making them challenging area of research. In our work, we considered as a model, an effective mineral weathering bacterial strain from the genus *Collimonas* isolated from nutrient poor forest soil. Through a combination of genomics, genetics, microbiology, mass spectrometry, mineralogy and geochemistry, we identified the genes and metabolites explaining its effectiveness at weathering different types of mineral. We also questioned the conservation of the identified genes among bacteria and the potential relations between the physico-chemical properties of minerals, the solution chemistry and the molecular mechanisms engaged. Our results offer a new view on how heterotrophic bacteria weather minerals and provide a better understanding of the sequence of events and regulations involved.

W61 - Degradation of sulfonated polyethylene by Bio-Fenton reaction supported by *Desemzia* sp. strain C1

Presenting Author - *Yongseok Ko, Gwangju Institute Of Science And Technology (GIST), Republic of Korea*

Author/s – *Sunil Ghatge, Sanghyeok Lee, Seunggyun Han, Raan Shin, Hor-Gil Hur,*

Abstract Content

Hydrogen peroxide (H2O2) mediated advanced oxidation process such as Fenton reaction has been studied for degrading recalcitrant organic contaminants. Recently, Bio-Fenton reaction mediated with hydrogen peroxide (H2O2) produced by microbes and its enzymes has been applied to degrade recalcitrant organic substance such as polyethylene, and herbicide.

In the present study, we isolated *Desemzia* sp. strain C1, producing high amount of H2O2 from oil-contaminated soil based on Prussian-blue agar plate forming a zone by reaction with ferric cyanide and H2O2. *Desemzia* sp. strain C1 is classified as *Desemzia* genus based on phylogeny analysis of 16S rRNA gene sequences. *Desemzia* sp. strain C1 produced notably high amount of H2O2 in minimal media in the presence of 10 mM lactate compared with glucose, pyruvate, and oxalate in the resting cell experiment (O.D.600 = 1). *Desemzia* sp. strain C1 produce 0.75 mM H2O2 in the resting cell experiment (O.D.600 = 1) with 10 mM lactate, which was 3.5 times higher than *Streptococcus oralis* KACC 13048T well known as H2O2 producer.

Sulfonated polyethylene was degraded by Bio-Fenton reaction supported by *Desemzia* sp. strain C1 generate degradation metabolites such as sulfoformic acid, sulfoacetic acid, and acetic acid. It indicates radicals produced by bacteria can involved in degradation of polyethylene plastics.

W62 - Catch me if you conjugate! Plasmid recovery from complex ecosystems using secreted pilus machinery as a hook

Presenting Author - *Vuong Van Hung Le, University of Copenhagen, Denmark*

Author/s – *Søren J. Sørensen, Joseph Nesme*

Abstract Content

Background: Plasmid conjugation plays an essential role in facilitating bacterial genomic plasticity and adaptation, including the acquisition and transfer of antimicrobial resistance (AMR) determinants amongst clinical pathogens from pre-existent environmental reservoirs. Hence, knowledge on the diversity and dynamics of conjugative plasmids in complex ecosystems is instrumental to develop new strategies to tackle the global AMR crisis.

Objectives: The current study aims to develop a workflow to enrich and capture bacterial cells containing a conjugative plasmid from environmental samples, followed by genomic sequencing and bioinformatic analyses to investigate the diversity of conjugative plasmids and identify the native hosts of these recovered plasmids.

Methods: GFP-displaying MS2 bacteriophage and SybrGold-stained Pf3 bacteriophage were employed to tag the bacterial cells expressing conjugative pili and sort the tagged cells from a complex microbial mixture using flow cytometry. The genomic DNAs of the sorted cells would be processed with genomic sequencing of single cells, PacBio long-read sequencing of pooled samples and DNA-methylation-profile-based binning.

Results: The GFP-displaying MS2 phage particles tagged a significantly higher proportion of the F'-plasmid-containing *Escherichia coli* population than the plasmid-free *E. coli* population and was able to differentiate the former from the latter in an artificial mixed population containing the two strains in flow cytometry assays. Similar results were achieved with the SybrGold-stained Pf3 phage particles in distinguishing between *Pseudomonas aeruginosa* PAO1/RP1 and the plasmid-free counterpart in a mixed culture. We are conducting proof-of-concept experiments using these two plasmid-dependent phages to 'fish' and sequence the plasmid-containing bacterial cells from wastewater samples.

W65 - A synthetic mucin-degrading community that can be used to model the ecological interactions in the human gut mucus layer

Presenting Author - *Maryse Berkhout, Wageningen University & Research, Netherlands*

Author/s – *Clara Belzer, Caroline Plugge*

Abstract Content

Background: A mucus layer protects the intestinal epithelium from contact with microbes. The outer mucus layer attracts specific gut microbiota of which several bacteria can degrade mucus. Mucin glycan degradation by commensal bacteria is part of the normal turnover and results in production of beneficial short-chain fatty acids (SCFAs) near the host epithelium. Microbial mucin glycan degradation is complex and requires a range of extracellular glycan degrading enzymes, as mucin glycans are intricate and diverse. Consequently, it is hypothesised that mucin degradation requires concerted action of various enzymes in a network of mucosal residents and that this initiates cross-feeding.

Objectives: The objective was to assemble and study an in vitro synthetic mucin-degrading community in anaerobic bioreactors.

Methods: We designed a synthetic community based on reported residents of the human mucosal layer. We selected mucin degraders, butyrate producers and hydrogen consumers. This community was grown in an anaerobic bioreactor with continuous mucin supply. Community dynamics, enzyme expression and metabolite production were monitored.

Results: All members of our synthetic mucin-degrading community grew and were active. The community was dominated by mucin degraders *Akkermansia muciniphila*, *Bacteroides* spp. and *Ruminococcus* spp. Butyrate producers and hydrogen consumers cross-fed on the products of mucin degradation. The main metabolites produced by the community were acetate, propionate and butyrate. Overall, we established a synthetic mucin-degrading community that can be used to model ecological interactions in the mucosal layer. This will lead to new insights in microbial dynamics in the human gut and host-microbial interactions at the mucosal layer.

W67 - Lead is a pain in the brass

Presenting Author - *Claire Hayward, Flinders University, Australia*

Author/s – *Harriet Whiley, Kirstin Ross, Melissa Brown, Richard Bentham, Giles Best, Dr. Sarah Harmer, Jason Hinds*

Abstract Content

Lead contamination of drinking water is a significant public health threat. Recently, there have calls for regulatory changes to ban the use of plumbing materials containing lead, such as brass, in favour of low-lead alternatives such as stainless steel. These alternatives may lower the risk of lead exposure; however there are limited studies investigating the impact of these alternatives on microbial water quality, particularly regarding opportunistic premise plumbing pathogens.

This study aimed to compare the public health risks of lead leaching and microbial growth associated with brass versus stainless steel plumbing fixtures using a model plumbing bioreactor under stagnant conditions.

The model bioreactors were filled with potable water spiked with OPPPs *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Mycobacterium avium* complex and *Acanthamoeba polyphaga*. A combination of culture, molecular and viability flow cytometry techniques were used to characterise alive, injured, and dead OPPPs in response to pipe material and stagnation time.

Overall, this study demonstrated that extended stagnation resulted in lead concentrations and OPPP persistence that is considered a risk to public health. Both brass and stainless-steel bioreactors had elevated lead levels that exceeded World Health Organisation water quality guidelines, despite stainless steel being marketed as a low lead alternative. Stainless steel materials supported the growth of OPPPs to a greater extent than brass. These findings demonstrate that an evidence-based approach should be the foundation for all public policy development and more research is needed prior to regulatory change.

W69 - Immune recognition of the secreted serine protease ChpG restricts the host range of *Clavibacter michiganensis* from eggplants

Presenting Author - *Raj Verma, Agricultural Research Organization, Volcani Center, Israel*

Author/s – *Doron Teper*

Abstract Content

Bacterial wilt and canker caused by *Clavibacter michiganensis* (Cm) inflict considerable damage in tomato-growing regions around the world. Cm has a narrow host range and can cause disease in tomato but not in many eggplant varieties. The pathogenicity of Cm is dependent on secreted serine proteases, encoded by the chp/tomA pathogenicity island (PI), and the pCM2 plasmid. Screening combinations of PI deletion mutants and plasmid-cured strains found that Cm-mediated hypersensitive response (HR) in the Cm resistant eggplant variety Black Queen is dependent on the chp/tomA PI. Singular reintroduction of PI-encoded serine proteases into CmΔPI identified that the HR is elicited by the protease ChpG. Eggplant leaves infiltrated with a chpG marker exchange mutant (CmΩchpG) did not display an HR, and infiltration of purified ChpG protein elicited immune responses in eggplant but not in Cm-susceptible tomato. Virulence assays found that while wild-type Cm and the CmΩchpG complemented strain were nonpathogenic on eggplant, CmΩchpG caused wilt and canker symptoms. Additionally, bacterial populations in CmΩchpG-inoculated eggplant stems were ~1000-fold higher than wild-type and CmΩchpG-complemented strains. Pathogenicity tests conducted in multiple Cm-resistance eggplant varieties demonstrated that immunity to Cm is dependent on ChpG in all tested varieties, indicating that ChpG-recognition is conserved in eggplant. ChpG-mediated avirulence interactions were disabled by alanine substitution of serine231 of the serine protease catalytic triad, suggesting that protease activity is required for immune recognition of ChpG. Our study identified ChpG as a novel avirulence protein that is recognized in resistant eggplant varieties and restricts the host range of Cm.

W70 - Metagenome mining reveals how anaerobic and aerobic integrated treatments shape the resistome profile of municipal solid wastes

Presenting Author - *Alessandra Fontana, Università Cattolica Del Sacro Cuore, Italy*

Abstract Content

Nowadays, the management of municipal solid wastes has become a challenging issue. Anaerobic digestion and aerobic composting processes could be considered the best options for the treatment of the organic fraction of municipal solid waste. These two processes are carried out by a complex microbial consortium, resulting in the final production of biogas and compostable substances that can be used in the agronomic field. Moreover, these integrated approaches can also be exploited to reduce the spreading of antibiotic resistance genes, as municipal solid wastes represent a significant source of such genes of concern.

The objective of this study was an in-depth assessment of the microbiome in an integrated plant based on the anaerobic digestion of the organic fraction of municipal solid waste, followed by the composting of the digestate solid fraction and green wastes.

This aim was achieved by means of DNA shotgun sequencing technique to unravel both the taxonomic and functional profiles of the microbial community, with a particular focus on the antibiotic resistance genes content (i.e., resistome). It was evidenced that the integrated treatment significantly shaped the microbiome, showing that Proteobacteria and Actinobacteria phyla, along with nitrogen-related metabolisms, were the main discriminant features of the composting phase. Moreover, the resistome underwent compositional changes at different stages of the plant. Specifically, composting was the step that mostly affected the content of antibiotic resistance genes for some drug classes, such as tetracycline and fluoroquinolone resistance genes.

W72 - Promotion of cyanobloom forming *Microcystis aeruginosa* by extracellular catalase-producing *Pseudoduganella aquatica* HC52

Presenting Author - *Yerim Park, Korea University, Republic of Korea*

Author/s – *Yeji Cha, Minkyung Kim, Wonjae Kim, Woojun Park*

Abstract Content

Presence of environmental H2O2 leads to a prevalent challenge for catalase-less freshwater bacteria including toxic bloom-forming *Microcystis aeruginosa* to survive particularly under high-light conditions. Although symbiotic interactions of bacterial communities in freshwater have been considered to be important for maintaining freshwater ecosystems, how *Microcystis aeruginosa* copes with hostile environments and underlying mechanisms of its symbiotic bacteria remain unclear. The highest extracellular catalase activity-prossessing *Pseudoduganella aquatica* HC52 was chosen among 36 symbiotic isolates recovered from 0.22 μM-filtered *Microcystis aeruginosa* cells in freshly collective cyanobloom samples. Whole genome sequencing of *P. aquatica* HC52 obtained using the PacBio® sequencing technique generated one contig having ~6.8 Mb size chromosome with four genes (katA1, katA2, katE, srpA) encoding a monofunctional catalase. Secreted proteins showing catalase activity in zymogram gels were analyzed using a LC-MS/MS to identify a target protein in the HC52 strain. Our peptide analysis suggested that KatA1 with no signal peptide was responsible for extracellular catalase activities and its production outside cells was higher at 50 μM H2O2 treatment conditions than at non-treatment conditions. Consequently, more O2 production, a decayed product of H2O2, was observed, which might assist the growth of other neighboring bacteria. Secreted catalases from the supernatants of the HC52 cells-grown cultures promoted the growth of catalase-less axenic M. aeruginosa in the laboratory culture system. Extracellular catalase, KatA, from *P. aquatica* HC52 might play an important role for detoxifying H2O2 in freshwater ecosystem, which can enhance the growth of catalase-less M. aeruginosa under high light-inducing oxidative stress.

W73 - Influence of temperature in the fate of antibiotic resistant bacteria and related genes during conventional activated sludge was

Presenting Author - *Sara Ribeirinho-Soares, Faculty of Pharmacy, Portugal*

Author/s – *Vasco Braga, Sofia Cunha, Vítor Vilar, Célia Manaia, Olga Nunes,*

Abstract Content

Predictive statistical models estimated 1.27 million deaths attributable to bacterial antimicrobial resistance in 2019. Simultaneously, water scarcity is anticipated to affect over half of the world's population by 2050 being wastewater reuse one of the most promising solutions to cope with the increasing global water shortage. However, it has been observed that treated wastewater, despite compliant to current reuse legislation, still may include biological contaminants including antibiotic resistant bacteria and related genes (ARB&ARGs), which can cause resistance accumulation and further proliferation, increasing human health risks.

Therefore, it is urgent to understand the factors influencing wastewater treatment regarding bacteria turnover and associated ARB&ARGs reduction. This study aims at assessing the effect of temperature on the reduction of bacteria, including ARB&ARGs, during conventional activated sludge (CAS) wastewater treatment. To achieve this, laboratorial CAS installations operating in a UWWTP at temperatures of 10°C or 28°C or atmospheric temperature, as control system, have been operated. The other operating parameters were set equal in all the counterparts and values of temperature, pH and dissolved oxygen monitored continuously. Abundance and prevalence of ARB&ARGs as well as bacterial community structure and composition has been accessed in the final treated wastewater and in the surplus sludge.

Regardless the operating temperature, concentration values of chemical and biological oxygen demand, total phosphorus, total nitrogen and total suspended solids in the CAS effluents were in agreement with the European council directive concerning urban wastewater treatment (Directive 91/271/EEC).

W74 - The impact of wooded areas on yeast populations vectored by social wasps

Presenting Author - *Beatrice Valentini, University of Turin, Italy*

Author/s – *Francesca Barbero, Luca Pietro Casacci, Anna Luganini, Irene Stefanini*

Abstract Content

Yeasts are widespread in nature. Since most of them are not airborne, they must rely on natural vectors to spread and colonize new environments. Nevertheless, vectors have been identified only for a limited range of yeast species. Among the known vectors, social wasps have been proven to carry and maintain yeasts all year long. This study aimed to assess the impact of wooded areas, known sources of yeasts, on the yeast communities vectored by social wasps towards vineyards. Adult Vespa crabro and Polistes wasps were captured over two vintages (2020 and 2021) in vineyards far and close to woods in three areas in the Piedmont region (Italy). Culturomics approach of the yeast communities present in the captured insects highlighted that the presence of woods in the proximity of vineyards does influence the insect mycobiota. In fact, wasps caught in vineyards near wooded areas bear in their intestines a higher number of yeast cells and higher species diversity than insects caught in vineyards far from woods. Furthermore, yeasts found only in insects caught in vineyards close to woods belong to species oenologically relevant, first and foremost Saccharomyces cerevisiae, but also Metschnikowia pulcherrima, Hanseniaspora uvarum, and Lachancea thermotolerans. The results of this project clearly highlight the relevance of social wasps in natural yeast ecology and call for the urgency of promoting the sustainable use of terrestrial ecosystems and preserving wooded areas to halt biodiversity loss.

W76 - Metabolic versatility of a novel psychrophilic methanotroph

Presenting Author - *Ramita Khanongnuch, Tampere University, Finland*

Author/s – *Rahul Mangayil, Mette Marianne Svenning, Antti Juhani Rissanen*

Abstract Content

Aerobic methane-oxidizing bacteria (MOB), particularly the genus *Methylobacter* , are abundant in boreal aquatic ecosystems. Previous research has reported the genetic potential of lake MOB for microaerobic/fermentation metabolisms. However, in-depth studies on how stressed environmental (such as frigid and nutrient- and O2-limiting) conditions influence their cellular metabolic responses are required. This work aims to investigate the transcriptomic changes and soluble metabolite production of boreal lake MOB grown under hypoxic conditions. In this study, a novel psychrophilic *Methylobacter* sp. S3L5C strain isolated from a Finnish freshwater lake was used. The S3L5C was tested under (i) oxic (20%CH4:20%O2), (ii) hypoxic (20%CH4:3%O2), and (iii) hypoxic conditions with amorphous ferric oxyhydroxide (Amp-Fe3+) as an alternative electron acceptor. The cultures were grown in ammonium mineral salts medium (pH ~6.8) at 8.0°C and 120 rpm. Gas composition and soluble metabolites were periodically monitored, and cells were collected for RNA extraction. Interestingly, Amp-Fe3+ addition increased acetate generation compared to other treatments. However, harvesting biomass for RNA extraction from the Amp-Fe3+ test was impractical. Compared to oxic conditions, transcriptome analysis of cells grown in hypoxic conditions showed an upregulated Entner-Doudoroff pathway. Furthermore, genes involved in nitrogen metabolism were upregulated, including nitrogen fixation (nifDKH), nitrite assimilation (nirBD), nitric oxide detoxification (norR), and ammonium assimilation (aspB, glnA and gldh/gdh). Our findings provide new insights into the role of *Methylobacter* spp. in carbon and nitrogen cycles in boreal aquatic ecosystems. The results are also valuable for future research on enhancing the efficiency of methanotroph-driven bioconversion of CH4 into industrial platform chemicals.

W77 - Cold plasma inactivation of single- and mixed-species bacterial biofilms

Presenting Author - *Aleksandra Lavrikova, Comenius University Bratislava, Slovakia*

Author/s – *Helena Bujdáková, Mário Janda, Karol Hensel*

Abstract Content

Healthcare-associated infections (HAIs) also known as hospital-acquired infections are among the major complications of modern medical therapy. Transmission of healthcare-associated pathogens most frequently happens through high-touch clinical surfaces. Common HAIs including *Pseudomonas aeruginosa*, Staphylococcus spp. and *Escherichia coli* were found to cause pneumonia, bloodstream infection, and urinary tract infection, respectively. *S. aureus* and *P. aeruginosa* are also most commonly isolated from co-infected wounds. Improved clinical surface bio-decontamination can reduce the transmission of these pathogens and decrease HAIs. Cold plasma is reported as one of the promising techniques for surface bio-decontamination.

The aim of this work was to investigate the effects of cold plasma on single- and mixed-species biofilms of various ages of clinically relevant bacterial species *S. aureus*, *P. aeruginosa* and *E. coli*. The pulsed streamer corona discharge operated in ambient air was used for direct plasma treatment of biofilms. Plasma-generated gaseous reactive species were identified to establish induced effects on bacteria (bacteria viability, biofilm biomass, intracellular metabolism) and the main inactivation mechanisms were proposed.

The plasma treatment showed a strong immediate bactericidal effect on single- and mixed-species bacterial biofilms. Plasma-generated gaseous O3 and NOx combined with the etching effect induced the damage and removal of superficial biofilm layers and suppression of intracellular bacteria metabolism. The age of the biofilm strongly affected inactivation kinetics. In a mixed biofilm *S. aureus* and *P. aeruginosa* behaved cooperatively and displayed higher resistance to plasma than in respective single-species biofilms.

W78 - Seed-borne endophytes: a potential tool for plant microbiome engineering using Chickpea varieties as a case study

Presenting Author - *Lilach Iasur Kruh, University Of Haifa, Israel*

Author/s – *Maya Lalzar, Omer Frenkel, Shahal Abbo, Abraham Gamliel, Lilach Iasur Kruh*

Abstract Content

Chickpea (*Cicer arietinum*) is the third-largest food legumes worldwide. Chickpea seeds, rich in proteins and nutrients, are considered healthy vegan food. Like many other crops, adaptation and resistance of chickpea to stress is inferior compared with their related wild species. Therefore, transferring tolerance to environmental stress from the wild to domesticated species is desired and attempted by breeding or genetic engineering.

However, certain traits rely on plant-microbe interactions. Those are easily lost under intensive agriculture practices.

Since seed-borne bacteria represent the core microbiome of plants and therefore, may serve as reservoir for endophytic bacteria that will be established in the plant tissues, we examined these populations in wild and domesticated Cicer in Israel.

Indeed, key endophytes of wild Cicer populations (*Cicer judaicum*) were absent from domesticated species (*C. arietinum*) even between sympatric populations. The *C. judaicum* seed-borne endophytic community was characterized by high dominance of either *Bacillus* sp., or *Sphingomonas* sp., while communities of domesticated cultivars were dominated by *Burkholderia* sp. with 100% prevalence. A *Bacillus* isolate, representing the dominant seed-borne population of *C. judaicum*, established successfully in domesticated chickpea as root and stem endophyte and presented beneficial effects: enhancing plant biomass and reducing wilt disease symptoms. Interestingly, this isolate could inhabit other legume species (beans and peas), but its population rapidly declined, suggesting phylosymbiotic interaction between this isolate and host.

We demonstrate that endophytes, from wild Cicer plants (*C. judaicum*), are good candidates for modifying the domesticated chickpea's microbiome, as survival and establishment barriers in related plant species are significantly reduced.

W79 - Promoted occurrence of lysis solution-tolerant cells in an air-solid biofilm of *Escherichia coli* and their memory effect

Presenting Author - *Tsubasa Nasu, Nara Women's University, Japan*

Author/s – *Sumio Maeda, Hirona Ikeda*

Abstract Content

Bacterial biofilm cells are tolerant toward various antibacterial treatments. However, as most studies on it used the whole biofilm as specimens, the tolerance effects often mingled with the protection effects inside the cell aggregate. Therefore, the pure tolerance of individual biofilm cells has been insufficiently elucidated.

Persister cells are a specific cell subpopulation that acquires temporary antibiotic-tolerant phenotypes. Recently, using the suspended cells of *Escherichia coli* after an air-solid (AS) biofilm culture and those after a liquid culture, we found that persister cells were produced more in an AS biofilm culture than in the usual liquid culture. These persister cells from the AS biofilms can be maintained in large numbers for an extended period at 37°C in an antibiotic-containing medium, suggesting a long-retention effect, or “memory effect”, in the persister cell state.

In this study, we aimed to examine different types of persister-like cells, which tolerate alkaline-SDS lysis solution (LS). Similar to the antibiotic experiments described above, LS-tolerant cells were significantly more when prepared from the AS biofilm culture than from the liquid culture. Moreover, the biofilm-derived LS-tolerant cells survived for a minimum of 1 week in an LS-containing medium at 37°C, suggesting "a memory effect” of the LS tolerance state. These results indicate that LS-tolerant cells share certain characteristics with antibiotic-tolerant persister cells.

W81 - Ecological relationships in mixed biofilms of nosocomial pathogens and lactic acid bacteria associated to preterm infants

Presenting Author - *Josué Jara Pérez, Complutense University of Madrid, Spain*

Author/s – *Alberto Aragón Ramírez, Rubén Jurado Escobar, Leónides Fernández Álvarez, Juan Miguel Rodríguez Gómez, Belén Orgaz Martín,*

Abstract Content

Background: Biofilm formation on medical devices is a common source of hospital-acquired infections. Especially concerning those founded inside the nasogastric enteral tubes (NEFTs) used for feeding preterm children (Ogrodzki et al., 2017). The type of biofilms described in such devices are recurrently dominated by staphylococci and members of the Enterobacteriaceae family, leading to neonatal infections. Lactic acid bacteria (LAB) are also present in NEFT-associated biofilms. These beneficial bacteria could be used to compete with nosocomial pathogens in order to create a healthier community inside NEFTs.

Objectives: The objective was to evaluate the ecological relationships in multispecies biofilms of four potentially pathogenic strains of Klebsiella pneumoniae, *Serratia marcescens*, *Staphylococcus aureus* and *Staphylococcus epidermidis* and two LAB isolated from the inner surface of preterm NEFTs.

Methods: For this, mono- and multispecies biofilms of the selected microorganisms were developed in a bath system using glass coupons as adhesion substrate. Attached population and biomass were measured over time. Structural changes due to interspecies association were revealed by confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM).

Results: Positive ecological relationships were observed in *S. aureus* biofilms stimulating his matrix production when they were growing in presence of LAB compared with its monospecie biofilm. However, Enterobacteriaceae biofilms showed negative ecological relationships due to the presence of LAB strains in their biofilms, reducing their thickness and volume compared when they were growing alone. A specific selection of potential probiotic LAB strains may be guide future applications for minimizing pathogen biofilm formation inside NEFTs.

W82 - Environmental effect on airborne microbial community structure in Seoul from winter to spring of 2018

Presenting Author - *Sookyung Kang, Ewha Womans University, Republic of Korea*

Author/s – *Geunhee Kim, Ji Yi Lee, Kyung-Suk Cho,*

Abstract Content

Since human and plant health problem has been severe due to high concentration of particulate matter below 2.5 μm (PM2.5), metagenomic information of PM2.5 is very important. Microbial metabolism is significant for understanding atmosphere ecology because airborne microorganisms affect atmospheric reactions. In this study, bacterial and fungal community structure of PM2.5, was analyzed, and then identified relationship between environmental factors and microbial community. DNA was extracted from quartz-filters sampling PM2.5 during 2018 winter and spring in Seoul, South Korea. DNA samples were amplified using primers; 515F/806R for bacteria, ITS3tagmix3/ITS4 for fungi. NGS was performed to the Illumina Miseq. Diversity indexes (Chao1, Shannon) were calculated and analyzed statistically Spearman's coefficient with environmental factors. Dominant bacteria in the winter were Proteobacteria, Firmicutes and Actinobacteria, however, in the spring, Verrucomicrobia, Cyanobacteria and Candidatus Melainabacteria. In fungal phyla, Chlorophyta and Ascomycota in winter; Ascomycota and Basidiomycota in spring were occupied over 90%. In the both seasons, temperature, relative humidity, PM2.5, NO2 and CO had negative correlation with microbial diversity. Whereas wind speed, pressure, solar radiation and O3 correlated positively with it. The absolute value of Spearman coefficient in the winter was higher, therefore relationship in the winter was stronger. Variable importance in projection (VIP) scores calculated from regression model, were higher with factors have negative relation with diversity indexes in the winter. Fungal VIP scores of NO2, CO and SO2 were higher than bacterial VIP scores in the spring. Consequently, the PM microbial information contributes to the accumulation of ecological knowledge in PM2.5.

W83 - Characterization of hydrogen production and bacterial community in microbial consortia using waste as a substrate

Presenting Author - *Geunhee Kim, Ewha Womans University, Republic of Korea*

Author/s – *Hyoju Yang, Jiho Lee, Yun-Yeong Lee, Sookyung Kang, Kyung-Suk Cho*

Abstract Content

Biohydrogen production by the dark fermentation is one of promising approaches to replace fossil fuels with hydrogen. Using food waste for hydrogen production has the effects of disposing of waste and producing renewable energy. In this study, biohydrogen production performance and bacterial community were characterized in microbial consortia using food waste. H_2-producing consortia were prepared by enrichment culture using anaerobic digestion sludge (AS), freshwater sediment (FWS), wetland (WL), forest soil (FS), forest puddle sediment (FP) as inoculum sources, and food waste as a substrate at 37℃ (mesophilic) and 50℃ (thermophilic). The highest H2 yield was found in the mesophilic WL consortium (4,272 mL-H_2·L^{-1}) and in the thermophilic FP consortium (2,613). In both consortia, the dominant bacteria were *Clostridium* spp. The highest H_2 production rates were found in the mesophilic WL consortium (2,266 mL-H_2·L^{-1}·d^{-1}) and in the thermophilic FWS consortium (3,877). The dominant bacteria in the thermophilic FWS consortium were *Sporanaerobacter* spp. The mesophilic (715 mL-H_2·g-COD^{-1}) and thermophilic (632) WL consortia showed the highest H_2 production per used COD. The bacterial community of the H_2-producing consortia was analyzed using an Illumina Miseq Sequencing platform. The mosophilic FWS consortium had a similar bacterial community structure with the mesophilic WL consortium, consisting of *Clostridium* and *Caproicibacterium*. Among the thermophilic consortia, the FWS and WL consortia showed similar community structures, consisting of *Clostridium* and *Sporanaerobacter*. The results of this study can be used as important information for establishing a biohydrogen production strategy using food waste.

W84 - Predation of antibiotic persister bacteria by the predatory bacterium *BdelloVibrio bacteriovorus*

Presenting Author - *Ofra Matan, Faculty of Dental Medicine, Hebrew University of Jerusalem, Israel*

Author/s – *Edouard Jurkevitch*

Abstract Content

Antibiotic resistance (AR) in bacteria is an urgent and global health issue, encompassing clinical, agricultural, terrestrial and aquatic environments. AR is not only expressed through genetic resistance. It also is found in bacteria in a small fraction of populations exhibiting antibiotic 'persister' states, thereby acting as a reservoir for re-growth. The predatory bacteria *BdelloVibrio* and like organisms (BALOs) can consume AR pathogens, reducing populations by orders of magnitude. However, it is not known if antibiotic persistence shields cells from attack by BALOs. Moreover, BALOs do not eradicate prey populations; rather, a small fraction of the population exhibits plastic phenotypic predation-resistance. In this study, we show that *Escherichia coli* antibiotic persisters, obtained by exposure of a sensitive population to ampicillin are preyed upon by *BdelloVibrio bacteriovorus* as much as control populations. Furthermore, phenotypic predation-resistant *E. coli* populations do not show increased AR. In conclusion, antibiotic persistence and plastic phenotypic predation-resistance do not directly affect one another. This knowledge may be important in the use of BALOs to prevent the spread of AR.

W85 - Biotechnological potential of cultivable bacterial communities associated with cave-dwelling marine sponges

Presenting Author - *Gabriel Rodrigues Dias, Federal University Of Rio De Janeiro, Brazil*

Author/s – *Matheus de Oliveira Nithack Marques, Jéssyca de Freitas Silva, Matheus Vieira Lopes, Michelle Klautau, Bruno Francesco Rodrigues de Oliveira, Marinella Silva Laport,*

Abstract Content

Sponge microbiomes have been extensively recognised as a rich source of novel substances with multiple biomedical and industrial applications. Therefore, this work aims to characterize the production of enzymes, antimicrobials, biosurfactants and bioemulsifiers by cave-dwelling sponge-associated bacteria (1-3). A total of 583 bacterial strains were isolated from twenty-nine specimens of demospongiae and calcareous sponges. Of these, 5,5% (32) bacterial strains were positive for alginate lyase, 5,3% (31) for agarase, 1,2% (7) for amylase and 1% (6) for urease activity, presenting an Enzymatic Index ≥ 2.0. Considering the antimicrobial potential, the cell-free supernatants of 15,3% (89) isolates were able to inhibit the growth of the strain *Staphylococcus aureus* ATCC 29213. Also, 16.2% (13/89) showed antimicrobial activity against multidrug resistant strains including: *Acinetobacter baumannii*, *Citrobacter freundii*, *Escherichia coli* and *Staphylococcus epidermidis*. In terms of biosurfactant and bioemulsifier production, 12.3% (72/583) formed dense and stable emulsion layers, with 41.7% (30/72) exhibiting emulsion indexes above 50%. Nevertheless, none of the strains were positive for the drop collapse or oil displacement assay, indicating that the substances are likely bioemulsifiers. The bioactive isolates were identified by MALDI-TOF MS and 16S rRNA sequencing as belonging mainly to *Pseudomonas*, *Vibrio*, *Bacillus* and *Shewanella* genera. These results favor the harnessing of cave-dwelling sponge-associated bacteria as prolific source of biomolecules with a wide plethora of applications in medicine, bioremediation, agriculture, food, and oil sectors.

W86 - The presence and potential of organophosphonate degrading bacteria in Lake Constance

Presenting Author - *Dzikrina Nur Fatima, University of Konstanz, Germany*

Author/s – *Sabrina Borusak, Adrien Lapointe, Eva Riehle, David Schleheck*

Abstract Content

Re-oligotrophication of Lake Constance might have forced the lake's microorganisms to utilize alternative phosphorus (P) sources, specifically organophosphonates (OPs) such as ciliatine, methylphosphonate (MP) or glyphosate. Glyphosate is used worldwide, however extensive use of it give rise to the occurrence of this herbicide in freshwater environment. Bacteria have been long reported to effectively degrade glyphosate and other OPs species, thus may mobilize such additional P sources for the phytoplankton. The objectives are to understand and characterize OPs bacterial degradation from Lake Constance and how the OPs-phosphorus might help phytoplankton growth. Microalgae and bacterial enrichment cultures, HPLC-MS analysis, 16S rRNA-gene analysis and genome sequencing in combination with proteomics were performed. Twelve OPs degrading bacteria were isolated, and *Brucella cytisi* DNF1 was found to completely degrades 0.2 mM ciliatine, glyphosate, MP and used it as P sources after 24 h in P-limited salt medium. The draft genome assembly (IMG Genome ID: 2963528115) identified a phn gene cluster which might be responsible for OPs degradation. Whereas, differential proteomics revealed proteins which are associated with phn G, H, I, J, L, F, M and phosphate transporter were significantly produced in the *B. cytisi* DNF1 cultures grown with 0.2 mM OPs compared to controls which suggest OP degradation via C-P lyase pathway. Tests with microalgae and cyanobacteria culture collections including many Lake-Constance specific species with OPs as P sources were negative thus far, which corroborates the notion that heterotrophic bacteria may be the main contributors to the mobilization of the OPs-phosphorus in planktonic food webs.

W89 - Isolation and characterization of plant growth-promoting bacteria from the microbiome of *Beta vulgaris*

Presenting Author - *Iva Atanasković, University of Belgrade, Serbia*

Author/s – *Tamara Krstić, Nataša Joković, Ivan Nikolić, Ivan Skadrić, Slaviša Stanković, Jelena Lozo,*

Abstract Content

Plant growth-promoting bacteria (PGPB) are beneficial microorganisms that can enhance plant growth and support plant response to stress. In this study, the microbiome of *Beta vulgaris*, a plant of commercial importance, was explored as a source of PGPB. Samples of the rhizosphere and phyllosphere were collected at different stages of plant development, and the bacterial community was examined using metabarcoding. Samples were also plated on different selective media. Morphologically distinct colonies were identified by 16S rRNA gene sequencing, and strains were tested for plant beneficial traits (exopolysaccharide, siderophore, and HCN production; phosphate solubilization; germination stimulation; activity against *B. vulgaris* pathogens). The objective was to find the optimal conditions for the isolation of PGPB and to study the microbiome of *B. vulgaris* in different plant parts and developmental stages. Both culture-dependent and independent approaches demonstrate that Actinobacteriota and Proteobacteria are the two dominant phyla in all samples. The highest bacterial diversity was observed in the rhizosphere of the early developmental stages sampled after three months of growth. The highest abundance of PGPB in the rhizosphere was observed on tryptic soy medium, where 18% of the isolates had 6 of 8 plant-promoting traits. In the phyllosphere, the highest number of PGPB was obtained on LE medium, a plant-based medium containing a *B. vulgaris* extract, where 25% of the isolates exhibited 7 of 8 plant-beneficial traits. Therefore, these media can be used for the isolation of PGPB associated with *B. vulgaris*, and this study provides a collection of isolates with potential agricultural importance.

W90 - Metataxonomic analysis of endometrial microbiota associated with low fertility in dairy cows

Presenting Author - *Jumpei Uchiyama, Okayama University, Japan*

Author/s – *Takuya Yagisawa, Iyo Takemura-Uchiyama, Hironobu Murakami, Osamu Ichii, Osamu Matsushita, Seiji Katagiri, Ando Shun*

Abstract Content

Background: The deterioration in reproductive performance associated with low fertility leads to significant economic losses in dairy farms. In recent years, uterine microbiota has begun to attract attention as a possible cause of unexplained low fertility.

Objectives: This study analyzed the uterine microbiota associated with fertility in dairy cows.

Methods: Endometrial biopsies were sampled from cows that had passed the voluntary waiting period before the first artificial insemination (AI). DNA was extracted and analyzed by 16S rRNA gene amplicon sequencing.

Results: First, the microbial diversity of 69 cows from four farms was analyzed regarding parity and AI frequency to conception, together with factors including housing style and feeding management, as each farm was managed differently. The significant difference was detected in UniFrac with respect to feeding management and housing style, but not parity and AI frequency. Next, we reanalyzed the microbiota data of 31 cows from one farm in relation to parity and AI frequency. In the microbiota diversity analysis, the weighted UniFrac distance matrices were correlated with respect to AI frequency, but not with parity. Differential abundance analyses of microbiota data and predicted functional profile detected a single bacterial taxon of *Arcobacter* and a few pathways, respectively. The co-occurrence network analysis detected the bacterial associations related with fertility. According to these results, the uterine microbiota in the cows may be used as a biomarker prior to the repeated AI in the reproductive management.

W91 - How to develop an activity-based screen for seeking novel CODH enzymes from uncultured microbes

Presenting Author - *Rebecca Bährle, Geomar Helmholtz Centre for Ocean Research Kiel, Germany*

Author/s – *Stefanie Böhnke, Mirjam Perner*

Abstract Content

Carbon monoxide dehydrogenases (CODHs) are a promising resource for biotechnological and industrial applications as these enzymes catalyze the reversible reaction of carbon monoxide (CO) with water to carbon dioxide (CO2) protons and two electrons. Therefore, these enzymes are helping to convert the greenhouse gas CO2 into valuable commodities. CODHs are used by a variety of phylogenetically diverse aerobic and anaerobic microbial organisms in autotrophic carbon fixation and energy conservation such as in the reductive acetyl-CoA pathway, even though CO has a toxic nature. However, the identification of environmental CODH enzymes is limited as the vast microbial majority is currently not cultured. Thus, the exploration of a large biochemical potential remains inaccessible using culture-dependent and sequence-based methods. Therefore, we have developed a cultivation-independent method using an activity-based colorimetric screening approach that enables us to recover novel CODH enzymes from the environment by detecting the oxidation of CO to CO2. To investigate which CODHs from different microbial phyla can be targeted by this method, the screen was successfully applied to fosmid clones prepared with genomic material from R*hodospirillum rubrum, DesulfoVibrio vulgaris, Moorella thermoacetica* and *Methanosarcina mazei*. To discover highly energetic and novel CODHs from the environment, our screen is currently being applied to a metagenomic fosmid library constructed with anoxic marine sediments from the Eckernförde Bight (Baltic Sea, Germany).

W92 - Host-specific adaptations of *Ligilactobacillus aviarius* to poultry

Presenting Author - *Bibiana Rios Galicia, University of Hohenheim, Germany*

Author/s – *Bibiana Rios Galicia, Johan S. Sáenz, Timur Yergaliyev, Amélia Camarinha-Silva, Jana Seifert*

Abstract Content

The genus *Ligilactobacillus* encompass species that are adapted to vertebrate hosts and fermented food. Their genomes encode adaptations to the host lifestyle. Reports of gut microbiota sequencing and cultivation of bacteria from chicken and turkey gastrointestinal tract, agree on a repeated persistence of *Ligilactobacillus aviarius* along the digestive system and its detection has been exclusively reported on poultry. In this work, the pangenome of *L. aviarius* was explored to describe the functional adaptability to the gastrointestinal environment. The core genome is composed of 857 gene clusters that are present at least in one copy that codify to structural and biogenesis proteins. The rest of the identified regions were classified into three different functional clusters of orthologous groups (COGs) that codify to carbohydrate metabolism, envelop biogenesis, viral defence mechanisms and mobilome inclusions. The pangenome of *L. aviarius* is a closed pangenome, exclusively found in poultry and 100% prevalent across chicken faecal samples. It codifies for different clusters of peptidases and glycosill transeferases that mediate interactions with the host cells. Additionally, some accessory features such as Crispr/Cas mechanism and prophage inclusions, were described. This information might provide hints about the interaction of this species with viral particles and other bacterial species. This work highlights functional adaptability traits present on *L. aviarius* that make it a dominant key member of the poultry gut microbiota and enlightens the convergent ecological relation of this species to the poultry gut environment.

W93 - Prebiotic potential of red ginseng dietary fiber to improve gut health by modulating gut microbiota in dogs

Presenting Author - *Seongbeom Cho, Seoul National University, Republic of Korea*

Author/s – *Hyokeun Song, Junbum Lee, Saehah Yi, Woo-Hyun Kim, Yuna Kim, Min Su Kim,*

Abstract Content

Background: Red ginseng is one of the most well-known plant widely used for traditional medicine that improves human health. However, the impact of red ginseng-derived dietary fiber on the gut microbiota in dogs is yet to be determined.

Objectives: This double-blinded, longitudinal study aimed to examine the impact of red ginseng dietary fiber on the gut microbiota and host response in dogs.

Methods: Forty healthy household dogs, selected through the application of exclusion criteria, were randomly assigned to high-dose, low-dose, and control groups. They were fed a normal diet or normal diet supplemented with low or high dose of red ginseng dietary fiber daily. The gut microbiota of the dogs was analyzed at 4 weeks interval using 16S rRNA sequencing.

Results: The alpha diversity of gut microbiota was significantly increased with all treated doses, but higher-dose resulted in a more rapid increase, indicating a dose-dependent effect of red ginseng dietary fiber. Short-chain fatty acids producers, including *Sarcina*, *Turicibacter*, *Leuconostoc* were significantly enriched, while potential pathogens such as *Helicobacter* significantly decreased indicating the increased gut health and pathogen resistance by red ginseng dietary fiber. Network analysis showed that the complexity and stability of gut microbiota was increased by the intake of red ginseng dietary fiber. These findings indicate that red ginseng-derived dietary fiber could be used as a prebiotics to modulate gut microbiota and improve gut health in dogs.

W94 - Denitrification- and DNRA-performing *Neobacillus* spp. strains Isolated from Rice Paddy Field Soil, Republic of Korea

Presenting Author - *Jeonghwan Jang, Jeonbuk National Univerisity, Republic of Korea*

Author/s – *Seohyun Ahn*

Abstract Content

Background: Dissimilatory nitrate reduction to ammonium (DNRA) refers to the microbial enzymatic reaction in which NO3- / NO2- is reduced to produce NH4+. NO3- can be easily lost from agricultural soil via leaching by rainfall, thus, DNRA can decrease N loss and retain biologically available nitrogen in soil, leading to more sustainable farming without excessive application of N fertilizers.

Objectives: The objective of this study is to find DNRA soil bacterial strains producing NH4+ despite of substantial unreduced NO3-.

Methods: The colorimetric screening method using the Griess reagent and vanadium(III) was used to test whether the isolates are capable of removing nitrate from the culture medium. The PCR primers designed in this study were used to detect denitrification and DNRA functional genes. The Oxford Nanopore NGS platform was used for whole genome sequencing of the isolates.

Results: Seventeen *Neobacillus* spp. strains were isolated from soil collected at the rice paddy field and all of the isolates are capable of removing nitrate from the culture medium. The DNRA functional genes nrfA and/or nirB were amplified from 12 of the 17 isolates and 4 of them were shown to harbor the denitrification functional gene nosZ. The five strains among the 17 were selected for nitrate removal and ammonium production test in time-based manner. Interestingly, *Neobacillus* spp. strains PS3-34 and PS3-40 produced ammonium despite of substantial nitrate remained. Whole genome investigation showed that PS3-34 and PS3-40 strains does not contain the known DNRA genes in their genomes.

W95 - Solutions for microbial RNA isolation from inhibitor rich samples

Presenting Author - *Helena Block, Qiagen Gmbh, Germany*

Author/s – *Dominic O'Neil, Silvia Magyar, Stefanie Schroeer*

Abstract Content

Microbiome analysis is of high interest for human biomedical and environmental research. This burgeoning field needs reliable methods for RNA isolation without distorting the real picture of the sample composition. Disruption of all microbes and thorough inhibitor removal are both crucial to receive unbiased insights into the sample. If the chosen method is not able to crack all the different microbes or the selected downstream assay (RT-PCR, digital PCR, Next Generation Sequencing) is inhibited you will receive a wrong message and would not even know it. We have developed processes which overcomes these difficulties and offer dependable results.

Here, we apply the developed procedure to different soil and fecal samples, disrupt the microorganisms, including bacteria, fungi and archaea using a combination of a novel bead beating homogenization and verified chemical lysis. Inhibitors (e.g. humic and fulvic acids in soil, and bile acids and proteoglycans in stool) are removed within the inhibitor removal step. Thereafter, inhibitor-free RNA is bound to a silica membrane, washed and eluted in a convenient straight-forward spin based protocol. Extracted RNA quality was assessed for purity, yield and integrity, as well as tested in RT-qPCR and RNA sequencing experiments.

The optimized method was able to efficiently extract microbial RNA in high yield, quality and purity without inhibition in RT-qPCR with internal control. RNA sequencing revealed highly complex communities, measured by alpha diversity (observed operational taxonomic units (OTUs)), and functional profiling diversity.

This approach can be easily adapted to other inhibitor-rich samples like wastewater or sludge.

W96 - Carbapenemase-producing Enterobacterales - what's the story with Croatian coastal waters?

Presenting Author - *Ana Maravic, University Of Split, Croatia*

Author/s – *Mia Dželalija, Anita Novak, Željana Fredotović, Marija Tonkić, Ivana Goić-Barišić, Ivica Šamanić, Slaven Jozić, Marin Ordulj*

Abstract Content

Background: The rapid spread of carbapenemase-producing Enterobacterales (CRE) in hospitals and natural environment is a global health threat. Extensively and multidrug-resistant KPC- and OXA-48-producing Enterobacterales were only recently detected for the first time in coastal waters in Croatia adjacent to submarine wastewater outfalls (Kvesić et al. 2022).

Objectives: We conducted a comprehensive survey of CRE in central Adriatic (Mediterranean Sea), seasonally following the trophic gradient from the human-impacted river mouth and coastal beach waters to open marine area.

Methods: Isolates were recovered on selective media, identified using MALDI-TOF MS and tested for antibiotic susceptibility. Carbapenem-resistant isolates were PCR screened for genes encoding for extended-spectrum beta-lactamases, plasmid AmpC, carbapenemases, integrases and sul genes. After pulsed-field gel electrophoresis (PFGE) isolate genotyping, selected strains undergone S1 nuclease PFGE and Southern blotting to detect the carbapenemase-encoding plasmids.

Results: Total of 37 KPC-2-producing isolates were recovered from river, public beach and surface bay waters. Isolates belonged to 9 Enterobacterales species, mostly *Klebsiella pnemoniae*, and carried up to nine resistance genes with the highest prevalence of blaSHV and int1 (89.2%) and sul1 (75.7%). BlaKPC-2-bearing plasmids ranged from 33 kb to 160 kb, among which some were of approximately same size as those previously identified as vehicles of this gene in hospitals and wastewaters in Croatia, indicating their further dissemination into environment. Notably, this is the first report of KPC-producing strains in public beach waters in Croatia, pointing to a new reservoir of this infectious threat in Croatian marine environment and a necessity of further monitoring.

W97 - Development of a 3D-printed chamber to assess *E. coli* survival on non-porous surfaces under controlled environmental conditions

Presenting Author - *Alex Cunliffe, Manchester Metropolitan University, United Kingdom*

Author/s – *James Redfern, Joanna Verran*

Abstract Content

The environmental conditions (e.g. temperature, humidity and airflow) used for efficacy testing of antimicrobial materials in standardised methods differ greatly from end-use scenarios, particularly regarding the presence of moisture (which may be limited at point of use). Thus, a 3D-printed chamber has been developed to test potential antimicrobial materials at a range of temperature, humidity, and airflow values, enabling conditions more appropriate to end use to be simulated. Development involved prototyping 3D printed chambers coupled with Arduino-based electronics to maximise the usability and reliability of the chamber. The chamber was used to assess bacterial survival on test surfaces using an adapted version of ISO22196. The effects of airflow were tested via assessment of microbial survival rates at different positions within the chamber, and mapping airflow using computational fluid dynamics modelling. The chamber maintained 15%, 40%, and 95% relative humidity (RH) at 24oC and 15% and 75% RH at 37oC (maximum operatable temperature). An increase in temperature/airflow and a decrease in relative humidity have each been demonstrated to affect the survival of *E. coli* on inert stainless steel 316L by influencing the evaporation rate of the inoculum, the determinant factor for survival of bacteria on a surface, with the effects of these interacting factors highlighting the complexity of what might initially appear to be a relatively simple system. Further work is required to understand these relationships, which will help us to develop a reliable, reproducible standardised test method for antimicrobial non-porous materials that is applicable to point-of-use.

W98 - Bottled drinking water available in Saudi Arabia: chemical and microbiological assessment of 19 (inter)national produced brands

Presenting Author - *Yasmeen Nadreen, King Abdullah University of Science and Technology, Saudi Arabia*

Author/s – *Johannes Vrouwenvelder, Graciela Gonzalez-Gil*

Abstract Content

Bottled drinking water has grown in popularity. However, extracting and transporting it from distant locations carries environmental burdens and may not guarantee better quality than local options. Previous studies mainly examined chemical quality of bottled water types, but not microbial quality. The limited studies that examined microbial quality used the plate-count method, which underestimates bacterial content. A comprehensive understanding of chemical and microbial quality is needed to determine if certain bottled water types provide benefits over others and whether imported mineral water's quality justify its environmental impact.

Therefore, we assessed the chemical and microbiological quality of diverse imported and local bottled waters and explored microbial communities of certain mineral waters. Mineral, artesian, sparkling, and purified bottled water types were investigated. Anions and metals were analyzed by chromatographic and spectroscopic methods, respectively, and bacterial contents were determined by flow cytometry. Microbial communities were assessed by 16S rRNA gene sequencing.

Bottled waters had distinctive chemical compositions with significant variations based on their types, but generally followed health guidelines. While the microbial quality of bottled waters meet health guideline limits, bacterial contents were significantly different among all water types, with natural mineral waters containing the highest cell concentrations and treated purified waters containing the lowest. Based on DNA analysis, mineral water samples may have specific microbial genera based on origin. Ultimately, comprehensive analysis show that all bottled water types available satisfy health regulations. Choosing locally produced purified waters reduces reliance on foreign natural waters, alleviates environmental impact and may improve local economy.

W100 - Periphyton regulates cyanobacterial blooms by altering particle-attached and free-living bacterial communities

Presenting Author - *So-Ra Ko, Korea Research Institute of Bioscience and Biotechnology, Republic of Korea*

Author/s – *Ve Van Le, Mingyeong Kang, Hee-Mock Oh, Chi-Yong Ahn*

Abstract Content

Cyanobacterial harmful algal blooms (CyanoHABs) pose adverse hazards to the ecosystem and humans. Periphyton which consists of autotrophic and heterotrophic organisms can mitigate CyanoHABs. Yet, gaps remain in our understanding of how periphyton affects particle-attached (PA) and free-living (FL) bacteria during CyanoHABs. Using high-throughput sequencing of 16S rRNA genes, we characterized the response of PA and FL bacterial communities to periphyton in an outdoor mesocosm (1000 L). The results indicated that pre-incubation of periphyton before inoculation enhances its efficiency of HABs control. Periphyton reduced chlorophyll concentration and mitigated CyanoHABs on day 11. Cell densities of *Microcystis* and *Anabaena* decreased by approximately 70.8% and 94.8%, respectively. The dissimilarity of control and treatment networks was 92.2%, indicating periphyton changed the bacterial interactions. In response to periphyton, PA bacterial community exhibited higher stability than that of FL bacterial community. Periphyton treatment likely favored the growth of organic matter-degrading bacteria such as Bacteroidetes, Comamonadaceae, and Limnohabitans. Intriguingly, Saprospiraceae, *Aeromonas*, Rhodobacteraceae, and *Brevundimonas*, which have potential inhibitory effects on cyanobacteria, were enriched in the treatment mesocosm. Altogether, our findings suggested that periphyton may mitigate CyanoHABs through interactions with bacterial communities.

W101 - Impact of sample preparation methods on metagenomic analysis of soil and stool using third-generation sequencing technology

Presenting Author - *Tanya Sperling, Qiagen Gmbh, Germany*

Author/s – *Dominic ONeil, Dagmar Herold, Lisa Mahler de Sanchez, Markus Sprenger-Haussels*

Abstract Content

Third generation sequencing technologies are of high interest for metagenomic analysis of many sample types. Low costs of entry and the ability to improve assembly of DNA give good advantages for many studies.

Especially analysis of microbial communities in environmental and human gut samples are of great interest for agricultural and health-related studies. However, these samples are particularly challenging to analyse as they contain inhibitory substances that are often co-isolated with nucleic acids. Additionally, as high amounts of DNA are needed for library preparation for Oxford Nanopore sequencing, yield is another critical factor. To achieve sufficient DNA yields and a realistic representation of the microbial community in the sequencing results, the lysis step is of particular interest.

In this study, we compared the compatibility of several commonly used sample preparation methods involving microbial lysis and inhibitor removal techniques on the MinIon device using soil and stool samples.

Methods involving thorough bead beating and active inhibitor removal showed by far the best yield, whereas methods involving gentle lysis yielded hardly enough DNA for library preparation. Read lengths in MinIon sequencing were comparable for most methods tested. Microbial diversity was also shown to be comparable for methods using thorough lysis. However, methods involving gentle lysis showed a loss in alpha diversity.

Our results show, that sample materials soil and stool can be sequenced using the MinIon device. DNA yield and microbial diversity are dependent on the lysis step and the use of inhibitor removal technologies during sample preparation.

W102 - Creating a standardized system for naming and classifying uncultivated prokaryotes: the development of the seqcode

Presenting Author - *Pushp Lata, Acharya Narendra Dev College (University of Delhi), India*

Author/s – *Rup Lal*

Abstract Content

SeqCode is a Nomenclatural Code for Naming Prokaryotes Based on Genetic Information. With the majority of prokaryotes being inaccessible as pure cultures, they are not eligible for naming under the International Code of Nomenclature of Prokaryotes (ICNP). To address this challenge, a new concept that is SeqCode - a code of nomenclature that uses genome sequences as the basis for assigning names to prokaryotes has been announced in 2022.

The SeqCode enables the valid publication of names for prokaryotes based on isolate genome, metagenome-assembled genome, or single-amplified genome sequences. It operates through the SeqCode Registry, a registration portal that links names and nomenclatural types to metadata. This code provides a framework for reproducible and objective nomenclature for all prokaryotes, regardless of cultivability and facilitates communication across all microbiological disciplines. Additionally, the SeqCode includes provisions for updating and revising names as new data becomes available. The development of the SeqCode is a significant step forward in the study of uncultivated prokaryotes. By providing a standardized system for naming and classifying these microorganisms based on their genetic information, the SeqCode will facilitate the discovery, understanding and comparison of these microorganisms, helping us to understand their role in the environment and how they contribute to the functioning of the Earth. Since SeqCode has been announced only in 2022, we will present the advantages and limitations of this approach by providing specific examples.

W104 - Characteristics of *Acinetobacter baumannii* strains isolated from local community hospitals

Presenting Author - *Jae Hong Jeong, Jeonbuk National Univerisity, Republic of Korea*

Author/s – *Jae-Young Oh, Su Min Kwak, Dokyun Kim, Seok Hoon Jeong, Jong-Chan Chae*

Abstract Content

Carbapenem-resistant *Acinetobacter baumannii* (CRAB) has been involved in numerous nosocomial outbreaks around the world. The resistance is mainly classified with OXA-23, OXA-40, OXA58 and metallo-beta-lactamases (MBLs). This study was to investigate the molecular epidemiological characteristics of Acinetobacter strains obtained from local community hospitals. *Acinetobacter* isolates were identified by MALDI-TOF, OXA-51, and 16S rRNA gene sequence analysis. Phenotypic and genotypic characteristics were revealed by antimicrobial susceptibility tests, carbapenemase gene PCR (MBLs and oxacillinase genes), and Pasteur MLST. A total of 126 *Acinetobacter* isolates were obtained and classified into 6 genospecies. One hundred fourteen (90.5%) *A. baumannii* strains were the most dominant, followed by 6 (4.8%) of *A. pittii* and 3 (2.4%) of *A. junii*. Acinetobacter was most frequently detected in sputum with 74.6% (n=94), followed by urine with 8.7% (n=11), pus with 5.6% (n=7), and wounds with 4.0% (n=5). Unlike other species, *A. baumannii* showed resistance to most antibiotics except colistin and possessed the blaOXA-23 adjacent to the ISAb1 gene. *A. baumannii* was classified into two major genotypes, ST2 (69.3%) and ST2125 (30.7%).

W106 - Systematic exploration into plant-microbe antagonism using the unicellular algae *Chlamydomonas reinhardtii*.

Presenting Author - *Tzila Vikniansky, The Hebrew University, Israel*

Author/s – *Naomie Alon, Elad Meilin, Hadasa Kaufman, Michal Breker, Omri Finkel,*

Abstract Content

Microorganisms' interactions with the host dramatically influence plant growth, development and resilience. Research of negative microbial effects on plants is largely focused on acute plant diseases, but many pathogenic microbes in the soil can cause more subtle, chronic effects on plants. In order to comprehensively delve into the complexity of the plant-microbiota interaction network, we established unique genome-wide approaches utilizing the unicellular green algae *Chlamydomonas reinhardtii*. We developed both agar-based and soil-based assays and screened ~150 bacterial strains isolated from *Arabidopsis thaliana* roots for effects on *Chlamydomonas* growth. Interestingly, we found eight strains that exhibited antagonism towards *Chlamydomonas*, seven of which were pathogenic to *Arabidopsis* as well. The growth-inhibiting strains are divided into three genera - *Burkholderia*, *Paenibacillus* and *Pseudomonas*. To test whether the pathogenicity was specific to photosynthetic organisms, we tested their effects on several non-photosynthetic microorganisms: *E. coli*, *B. subtilis* and *S. cerevisiae*, and found that the two *Burkholderia* strains inhibited *Arabidopsis* and *Chlamydomonas*, but not any of the non-photosynthetic organisms, and did so in a contact-dependent manner. Next, we utilized large null mutant collections as well as UV-mutagenized *Chlamydomonas* strains to hunt for colonies resistant to bacterial growth inhibition, and detected several candidates. Genetic characterization to follow in both algae and bacteria should be a promising path to elucidate pathogenic mechanisms. Target genes identified can be potential subjects for genetic engineering to improve crop yield and fitness.

W107 - Whole genome sequencing of *Listeria* spp. isolated from dairy ruminants' and farm environments

Presenting Author - *Carla Palacios Gorba, Universidad Ceu Cardenal Herrera, Spain*

Author/s – *Carla Palacios-Gorba, Alexandra Moura, Jesús Gomis, Alexandre Leclercq, Ángel Gómez-Martín, Hélène Bracq-Dieye, Yuval Markovich, Nathalie Tessaud-Rita, María Pastor Martín, Guillaume Vales, Carles Escrig, Pierre Thouvenot, Marc Lecuit, Juan José Quereda*

Abstract Content

Listeriosis is a zoonotic disease that can cause, septicemia, meningitis, encephalitis and abortions. Two species of *Listeria* are considered pathogenic, *L. monocytogenes* and *L. ivanovii*. Hypervirulent *L. monocytogenes* clones implicated in human clinical cases are associated with dairy products. The association of hypervirulent clones to dairy products is not well understood. The aim of this study was to unravel the prevalence, ecology and genomic characteristics of *Listeria* spp. in the gastrointestinal tract of individual dairy ruminants and the farm environment. We carried out a large-scale longitudinal study to monitor *Listeria* spp. in 19 ruminant farms during three consecutive seasons. In addition, we also monitored the presence of *Listeria* spp.in feces, tonsils, and udders of 316 dairy ruminants to know if ruminants could carry asymptomatically *Listeria* spp. in organs without fecal shedding. All isolates were sequenced using whole-genome sequencing. *L. monocytogenes* and *L. innocua* were the most common *Listeria* spp. in both dairy ruminant feces and farm-associated settings in the longitudinal study in dairy farms. CC1 and CC4 *L. monocytogenes* clones, which are hypervirulent, accounted for 30% of the isolates and were mostly recovered from host-associated samples (feces) rather than farm environments. Furthermore, we show that in the absence of fecal shedding, ruminants can be asymptomatic carriers of pathogenic *L. ivanovii* in udders and *L. monocytogenes* and *L. ivanovii* in tonsils. *L. innocua*, a non-pathogenic species, was only found in feces samples. These findings underline that dairy ruminants and farm environments are a reservoir for hypervirulent *L. monocytogenes*.

W108 - Calcium carbonate formation and antibacterial activity of *Bacillus altitudinis* B6 for repairing concrete cracks

Presenting Author - *Jihyeon Min, Korea University, Republic of Korea*

Author/s – *Yongjun Son, Woojun Park*

Abstract Content

Maintenance of concrete structures by repairing microcracks using self-healing bacterial agents is a promising strategy for maximizing the lifespan of concrete structures. A non-ureolytic and alkali-tolerant B6 strain, newly isolated from paddy soil, was tested for microbially-induced calcium carbonate precipitation (MICP) performance along with its antibacterial activity for pathogen removals. Whole genome and bioinformatic analyses showed that our B6 strain could be named as *Bacillus altitudinis* with its 16S rDNA and average nucleotide identity. Energy-dispersive X-ray spectroscopy and field emission scanning electron microscope (FE-SEM) revealed that the B6 strain could form vaterite and produce extracellular polymeric substances, contributing to excellent biofilm formation even under high pH conditions. The MICP of the B6 cells inoculated on cracked mortars could repair microcracks (0.3 mm) within 14 days. The presence of vaterite and survivability of the B6 cells inside the healed area was verified using the live/dead viability assay and FE-SEM. Antibacterial activity using the supernatant of the B6 cells grown in rich media at pH 8 showed that cell-free extract could kill only Gram-positive bacteria, including *Staphylococcus aureus* and *Enterococcus faecalis*, by damaging their cellular membranes. Both biosynthetic gene cluster analyses for possible secondary metabolites and chemical analysis using high-performance liquid chromatography and mass spectrometry identified thermostable thiocillin as a putative antibacterial compound in the cell-free extract. Our data demonstrated that the B6 strain's antibacterial and MICP activities could be promising for repairing microcracks and controlling pathogen contamination on self-healing concretes.

W109 - Community structure of indigenous microorganisms in landfill buried 30 years ago

Presenting Author - *So-Jeong Kim, Korea Institute Of Geoscience And Mineral Resources, Republic of Korea*

Author/s – *Wook-Hyun Nahm, Min Han, Gi-Yong Jung, In-Hyun Nam*

Abstract Content

Recently established landfills are well managed but previous landfill were randomly buried with wastes. Previous landfill-related studies have mainly focused on monitoring microorganisms in leachate from landfills. In this study, the current microbial community of a landfill dumped 30 years ago (landfill period, 1987 - 1992) was monitored. Samples by depth were obtained through drilling in the landfill. The amount of microorganisms by depth was observed as 1.7 × 106 - 3.7 × 109 copies/g by quantitative PCR. The microbial community composition was investigated using Illumina Miseq sequencing to understand which species of microorganisms were related to the decomposition of buried garbage. Two bacterial family, Thermoanaerobacteraceae (including genus *Thermacetogenium*) and Thermoanaerobacterales Family III. Incertae Sedis (including genus *Syntrophaceticus*) were dominant members in this landfill. Four archaeal genera were abundant (*Methanoculleus*, *Methanothrix*, *Methanobacterium*, and *Methanoregula*) among Archaea. These microorganisms are known to be associated with syntrophic and methanogenic metabolism, respectively. A metagenome analysis will be additionally conducted to understand the function of the microbial community in the landfill, and this study will provide important insights related to the operation and control of the landfill in the future.

W110 - Passive samplers in wastewater: Virus load could reach its maximum in just a few hours

Presenting Author - *Andreana Shakallis, Flinders University, Australia*

Author/s – *Harriet Whiley, Howard Fallowfield, Kirstin Ross*

Abstract Content

In response to the COVID-19 pandemic, wastewater-based epidemiology has become a promising public health tool. One of the biggest challenges and limiting factors with wastewater-based epidemiology is sample collection. In Australia, passive sampling using 3D printed passive samplers containing electronegative membrane filters has become the standard approach to sampling wastewater for SARS-CoV-2 detection. Due to the urgency of implementing monitoring programs, there are a limited number of studies investigating the efficacy of viral adsorption and recovery from passive samplers. This study aimed to address this gap in knowledge before this methodology is adopted more broadly for routine wastewater monitoring. In this laboratory based study, Bacteriophage MS2 was used as a model virus for SARS-CoV-2 and the effect of different aqueous matrices, virus concentrations, pH, and deployment time on viral adsorption onto electronegative membrane filters was investigated. Virus recovery was shown to be dependent on the virus concentration present in the matrix with an increase in virus adsorption and recovery associated with an increase in virus concentration. Adsorption was at its lowest in alkaline matrices, and its highest in acidic matrices. Time was found to have no statistically significant effect on viral adsorption, with maximum adsorption reached within 3 hours. This research suggests that short deployment of passive samplers may not satisfy the goal of time integrated sampling to provide samples that represent the virus load over a longer period of time.

W111 - Host microbiome-associated phenotypes are the remedies for nitrogen loss at the field scale

Presenting Author - *Mitra Ghotbi, University of Illinois at Chicago, United States*

Author/s – *Mitra Ghotbi, Martin Bohn, Alonso Favela, Angela Kent*

Abstract Content

Nitrification carried out by Archaea and bacteria is a source of N loss from the soil system. Specific maize genotypes have displayed the microbial-associated phenotypes for inhibiting biological nitrification (BNI), which contributes to leaching losses. In this study, we used N transformation assays as the actual functions of microbes and DNA amplicon sequence analysis to evaluate the soil microbiome N-cycling genes and dynamics to BNI. We compared N-cycling microbial assembly, structure, and the synergy between BNI and N fixing inoculants in modern maize (B73) versus teosinte-maize near-isogenic line (BNI NIL) rhizospheres. Our goal was estimating the capacity of N loss mitigation by introgression of teosinte genes. The assembly of N cycling microbial community was distinct between B73 and the BNI NIL. Estimating the distribution of genes highlighted the significance of growth stage in adjusting abundance of prokaryotic nitrifiers and denitrifiers in both genotypes. Higher abundance of nitrifiers and denitrifiers genes in the rhizosphere of B73 compared with the BNI NIL was evident. This highlights the potential of the microbiome-associated phenotypes of the BNI genotype to decline N losses at the field scale. Taxon co-existence pattern of BNI NIL differed from that of B73, suggested the genotype impacts on microbiome species associations. N accumulation particularly in presence of the N-fixing inoculant was enhanced by the BNI phenotype, supporting a role for BNI in N retention. All in all, the consortium of genotype microbiome-associated phenotypes by recruitment of specific N cycling microbes increases soil N retention which eventually favors sustainable agriculture.

W112 - Exopolysaccharide amendment in bioformulation for improved shelf life and functional efficacy

Presenting Author - *Sonal Srivastava, Indian Institute Of Technology Delhi, India*

Author/s – *Sonal Srivastava, Shilpi Sharma*

Abstract Content

Soil salinization is a global issue and 20% of irrigated lands around the globe are salt-affected. It is estimated that by 2050, approximately 50% of arable land globally will get salinized. Salinity stress adversely affects the growth and productivity of leguminous crop pigeon pea (*Cajanus cajan*). Exopolysaccharide (EPS) producing microbes have a profound effect on plant growth under salinity stress. Non-toxic, structural versatility and nutrient-rich attribute make EPS a potential amendment in bio-formulations to improve their shelf life and efficiency, however, these studies are still to be explored. Present study involved the screening of high EPS producing bacteria. Among different bacteria, strain identified as *Bacillus haynessi* showed higher EPS production under salinity stress. This strain exhibited increased biofilm formation, auxin production and phosphate solubilization ability at high salt concentrations. Fourier transform infrared spectroscopy revealed the presence of hydroxyl, carbonyl, and ether groups in EPS. Additionally, it displayed high water holding capacity and stability up to 60 °C temperature. Inoculation of *B. haynessi* in pigeon pea plants subjected to saline stress improved biometric and physiological parameters along with reduced stress markers viz. proline, electrolyte leakage and malondialdehyde. Moreover, the amendment of EPS in bioformulation improved its shelf life, and its functional efficacy was also sustained. Biplot analysis showed a positive correlation between plant growth and physiological parameters with treatments including bioformulation amendment, whereas a negative correlation was found with salinity stress. Thus, the present study opens new avenues for wide application of EPS-amended bioformulation to maintain crop productivity in saline soil.

113 - Biodegradation of commercial monomers used for polyester synthesis under soil condition

Presenting Author - *Woo Yeon Cho, Ajou University, Republic of Korea*

Author/s – *Pyung Cheon Lee*

Abstract Content

The biodegradation of polymers has garnered worldwide attention due to its potential in fostering sustainable development. Despite the multitude of studies conducted on this topic, the underlying mechanism of biodegradation remains largely unclear. Environmental microorganisms play a critical role in the polymer biodegradation process: biodeterioration, biofragmentation, assimilation, and mineralization. These microorganisms eventually decompose and metabolize biodegradable polymers to produce water and carbon dioxide (or methane) as final metabolic products.

The biodegradation of monomers commonly used in the manufacture of polyester polymers was investigated in soils by analyzing the evolution rate of carbon dioxide and the population change of the microorganisms during the biodegradation of the monomers. Biodegradation experiments were performed under natural soil conditions in accordance with ISO 17556:2019. During the biodegradation process, total metagenomic DNAs were extracted from soil samples and subjected to 16S rDNA amplicon sequencing and metagenomic shotgun sequencing. Metagenomic sequence data were analyzed by using QIIME2, DADA2, and/or SqueezeMeta2 programs to obtain crucial information on microbial and gene populations in soils undergoing biodegradation. The information on unique characteristics of each monomer biodegradation could contribute to a better understanding of the biodegradation mechanism of biodegradable polymers and highlight the role of environmental microorganisms in the process.

W114 - A large diversity of spoilage bacteria in the tuna necrobiome

Presenting Author - *Yvan Bettarel, Montpellier University, France*

Author/s – *Elsa Gadoin, Antoinette Adingra, Christelle Desnues,*

Abstract Content

Like other seafood products, tuna is highly perishable and sensitive to microbial spoilage. Its consumption, whether fresh or canned, can lead to severe food poisoning due to the activity of specific microorganisms, including histamine-producing bacteria. Yet many grey areas persist regarding their ecology, conditions of emergence and proliferation in fish. In this study, we used 16S rDNA barcoding to investigate post-mortem changes in the bacteriome of fresh and brine-frozen yellowfin tuna (*Thunnus albacares*), until late stages of decomposition (i.e. 120 h). The results revealed that despite standard refrigeration storage conditions (i.e. 4°C), a diverse and complex spoilage bacteriome developed in the gut and liver. The relative abundance of spoilage bacterial taxa increased rapidly in both organs, representing 82% of the bacterial communities in fresh yellowfin tuna, and less than 30% in brine-frozen tuna. Photobacterium was identified as one of the dominant bacterial genera, and its temporal dynamics were positively correlated with histamine concentration in both gut and liver samples, which ultimately exceeded the recommended sanitary threshold of 50 ppm in edible parts of tuna. The results from this study show that the sanitary risks associated with the consumption of this widely eaten fish are strongly influenced by post-capture storage conditions.

W115 - *Vibrio* sp. are abundant members of the seagrass meadow ecosystem

Presenting Author - *Rebecca Gebbe, University of Greifswald, Germany*

Author/s – *Katharina Kesy, Dorothea Hallier, Anne Brauer, Mia M. Bengtsson*

Abstract Content

Seagrass meadows ecosystems offer several valuable ecosystem services in coastal regions around the world. One such important service recently discussed is reduction of pathogenic bacteria and specifically *Vibrio* sp. in the surrounding water. However, the mechanisms of pathogen reduction remain unclear. Whether pathogenic bacteria subsequently persist in the sediment or in other compartments of the seagrass meadow is currently poorly understood.

In this study, we investigated the relative abundance and community ecology of *Vibrio* sp. bacteria in Baltic Sea seagrass meadows using both culturing and culture-independent methods.

We detected high relative abundances of *Vibrio* sp. on young seagrass leaves and superficial roots, supporting previous observations that new surfaces are selectively colonized by *Vibrio* sp. bacteria and suggesting that these habitats are important for the persistence and possibly release of *Vibrio* sp. into the water column. Sediments and older leaves displayed lower relative abundances of *Vibrio* sp, which could be due to antagonistic interactions with other biofilm microbial inhabitants in these environments.

Our findings highlight the need to understand the ecology of potentially pathogenic bacteria in marine environments, including interactions with host organisms such as seagrass and with microbial predators.

The removal, persistence and release of *Vibrio* sp. is a dynamic process likely influenced both by abiotic factors such as temperature and salinity and biotic factors such as biodiversity on multiple trophic levels within the seagrass meadow ecosystem.

W117 - Cysteine-degrading bacteria as hydrogen sulfide source in recirculating aquaculture systems

Presenting Author - *Alexandre Nguyen-tiet, Technical University of Denmark, Denmark*

Author/s – *Stefan Bertilsson, Fernando Puente-Sanchez, Sanni-Leea Aalto*

Abstract Content

Hydrogen sulfide (H2S) is a major challenge in marine land-based recirculating aquaculture systems (RAS), leading to massive fish mortality even at very low concentrations. One source for H2S could be sulfur amino acids that are available as e.g. uneaten fish food and feces, and could be degraded into ammonia, pyruvate, and hydrogen sulfide by cysteine degrading bacteria. However, identity, ecology, and physiology of cysteine degrading and H2S producing bacteria is not known in aquaculture yet. In this study, we examined the role of cysteine degradation pathway in H2S production in different compartments of marine RAS (biofilter backwash (BW), activated sludge (AS), and biofilter biofilms (BF)). In the anaerobic mixed reactors with cysteine as the only sulfur source, H2S concentrations reached 77-180 mg H2S/L already after ten days, the H2S production rate being 9.04mg H2S/L/h. When further examining the bacterial community raised with cysteine, we found that was it incapable of producing H2S with other sulfur sources, such as sulfate (traditional H2S source) or methionine (another sulfur rich amino acid present in RAS). The microbial community analysis showed the presence of three interesting families that were more abundant in the reactors than in the original samples: Fusobacteriaceae, Dethiosulfatibacteraceae, and Vibrionaceae. The potential and the possible enzyme involved in the H2S-production through cysteine degradation were further examined with metagenomic and metatranscriptomic analysis and the results will be discussed in the poster.

W118 - Characterization of pigmented areas on a coating surface at different scales

Presenting Author - *Clotilde MAESTRI, Cergy Paris University, France*

Author/s – *Thibault Harlé, Ronan L. Hébert, Patrick Di Martino*

Abstract Content

Microbial contamination of surfaces may cause economic and sanitary problems in various industrial sectors. This microbial contamination can lead to aesthetic disorders occurring as colored spots that can be pigmented in black, green or red. The investigation of these pigmented areas at different scales, allows to understand the origin of the color change due to the implication of microorganisms. In this study, surface coatings with different colour and intensity were characterized in situ on a macroscopic scale, before being sampled for laboratory analysis. The first analysis was done with a spectrophotometer in order to quantify the colorimetric features of the different types of spots, before being analysed on a microscopic scale with a binocular loupe. The samples were further analysed by confocal microscopy based on natural fluorescence of photosynthetic pigments and after staining with DAPI (4', 6-diamidino-2-phénylindole), a DNA intercalating dye. Scanning electron microscopy observations were also made in order to identify the changes at the surface topography of the material, as well as the presence and localisation of microbial cells. The colorimetric results, analysed by statistical method, confirm the colour differences observed macroscopically, and allow to distinguish 3 main spots colours. Microscopic observations showed the presence of microorganisms in the different stained areas, some of them being autofluorescent. The microbial colonization is always located where the coating layer is damaged suggesting that a physical alteration of the material surface is a prerequisite to the colonisation by pigmented microorganisms that conduct to pigment accumulation.

W119 - Hydrogen sulfide producing microbes in aquaculture systems

Presenting Author - *Sanni-Leea Aalto, Technical University of Denmark, Denmark*

Author/s – *Alexandre Nguyen-tiêt*

Abstract Content

Currently, sudden outbreaks of microbial-produced hydrogen sulfide (H2S) cause massive fish mortality events, human security risks, and economic losses in the marine land-based aquaculture systems. While the traditional H2S producers, sulfate reducing microbes (SRM), are expected to thrive under sulfate- and organic matter rich conditions offered by marine aquaculture systems, their activity does not explain the observed H2S incidents. In this project, we aimed to gain more knowledge on H2S producing microbes in aquaculture systems. We collected samples in aquaculture systems and conducted a series of experiments using anaerobic reactors operated with different enrichment media and/or aquaculture-realistic conditions. We measured H2S production and substrate consumption in the reactors and examined microbial community composition with high-throughput sequencing of 16S rRNA gene both in reactors and in situ samples. We found the high H2S production to be related to the presence of potential cysteine degraders (e.g. family Fusobacteriaceae) rather than to SRM (e.g. phylum Delsulfobacterota). In the enrichment cultures, cysteine-based H2S production began almost immediately after reaching the favorable conditions, while sulfate-based H2S production required twice as much time to reach the same H2S concentration, being probably more dependent on the other microbial processes present. Furthermore, cysteine degrading H2S-producing communities were also capable of switching to sulfate reduction. Our future work will focus gaining more knowledge on the metabolic pathways involved and on genomic and transcriptomic profiling of the found H2S producers.

W120 - Biodiversity and antimicrobial potential of Actinobacteria from Nepalese soil

Presenting Author - *Sagar Aryal, Tribhuvan University, Nepal*

Author/s – *Sagar Aryal, Ronald Garcia, F.P. Jake Haeckl, Rameshwar Adhikari, Balmukunda Regmi, Rolf Müller, Dev Raj Joshi*

Central Department of Microbiology, Tribhuvan University, Kathmandu, Nepal

Abstract Content

Background: Actinobacteria are gram-positive filamentous bacteria having high G+C content. Multidrug resistance is currently one of the major health problems globally. To overcome this problem, the world urgently needs a novel candidate for the discovery of new antimicrobials.

Objective: The objective of this study is to isolate soil Actinobacteria and perform antimicrobial activities of the strains against pathogens.

Methods: Fifty-six (56) soil samples were collected from different parts of Nepal. Actinobacteria were isolated using an Actinomycete Isolation Agar (AIA) and further cultivated on International Streptomyces Project 2 (ISP-2) liquid medium. DNA was isolated using a QIAGEN kit followed by PCR (Polymerase Chain Reaction) using universal primers (27F and 1492R). The 16S rRNA gene sequencing was done at the LGC Biosearch Technologies using the same universal primers (27F and 1492R). The crude extracts were tested in sterile 96 well plate against two Gram-negative bacteria (*E. coli* BW25113 and *E. coli* JW0451-2), one Gram-positive bacteria (*B. subtilis* DSM 10) and two fungi (*Pichia anomalis* DSM 6766 and Mucor hiemalis DSM 2656).

Results: Based on cultural, morphological and 16S rRNA gene sequencing, a total of 59 Actinobacteria strains was obtained from 56 soil samples. Streptomyces was found in the highest number with 74.58% of total isolates. Among 59 strains of Actinobacteria, extracts from 29 strains were found to have some antimicrobial properties against tested organisms while one of the extracts was active against all 5 tested organisms. Most of the extracts showed antifungal activity followed by Gram-negative activity.

W121 - Untapped diversity: Adaptations traits of a new *Sphingomonas* species (strains So64.6) to the harsh Antarctic environment.

Presenting Author - *Kattia Núñez-Montero, Universidad de la Frontera, Chile*

Author/s – *Dorian Rojas-Villalta, Leticia Barrientos,*

Abstract Content

An Antarctic *Sphingomonas* strain So64.6b was previously reported by our research group as an antibiotic-producer in response to different elicitation treatments. It was hypothesized that adaptation to the Antarctic might drive a new diversity of specialized metabolites, in accordance with multiple other studies that recommend bioprospection for new natural products of bacteria from extreme environments. Nonetheless, there is no evidence of a correlation between the bioactivity potential with the adaptation of *Sphingomonas* species. Therefore, in this work, we aimed to determine the genetic evolutive traits of *Sphingomonas* sp. So64.6b that might be associated with adaptation to the Antarctic environment, and that could lead to its diversity of antibiotic metabolites. To do this, the complete genome sequence of the Antarctic strain was obtained. Comparative genome analysis based on multi-locus phylogenomics, Biosynthetic Gene Clusters (BGCs) phylogeny, and pangenomics were conducted within the closest species. We found that the Antarctic strain showed the closest identity with *Sphingomonas alpina*, however containing a significant genomic difference of ortholog clusters related to resistance and pollutants degradation. It also has three BGCs associated with potentially novel antibiotic compounds. Analysis of a common BGC showed great diversity between the *Sphingomonas* genus, but grouped in clades according to similar isolation environments, suggesting an evolution of BGCs that could be linked to the specific ecosystems. Altogether, our results showed the unique genetic content of the Antarctic strain *Sphingomonas* sp. So64.6, −a probable new species–, which might produce novel antibiotic compounds because of the adaptation to Antarctic poly-extreme conditions.

W122 - Prevalence and molecular characteristics of pathogenic *Escherichia coli* isolated from humans, pets, and wild animals

Presenting Author - *Jong-Chan Chae, Jeonbuk National Univerisity, Republic of Korea*

Author/s – *Jae Hong Jeong, Su Min Kwak, Kwang Won Seo, Dokyun Kim, Jae Young Oh*

Abstract Content

In a view of epidemiological perspective of disease, it is generally accepted that many zoonotic pathogens from wild animals spread to humans through livestock or companion animals. Therefore, early monitoring and control efforts for zoonotic pathogens can reduce disease rates in humans. This study is to monitor the prevalence and molecular epidemiological characteristics of diarrheal toxin-producing *Escherichia coli* in humans, pets, and wild animals in urban area. *E. coli* was isolated from 150 fecal samples from patients with enteritis, 464 feces from pets with diarrhea, and feces from 113 healthy wild animals. Among the 516 *E. coli* strains (120 from humans, 283 from companion animals, and 113 from wild animals), resistance rates to beta-lactams, cephalosporins, and fluoroquinolones were significantly higher in those from pets. Phylogenetic group B2 (extra-intestinal strains) classified by virulence markers was 20.8% in humans, 31.1% in pets, and 21.2% in wild animals. *E. coli* harboring the diarrheal toxin gene was 13 (12.7%) in humans, followed by 9 (3.2%) in pets, and 5 (4.4%) in wild animals. The most was atypical EPEC with eaeA. Atypical EPEC ST517 clones were found from human and pet with high genetic similarities. Whereas the patient, human origin of the strain, had diarrhea, the companion dog, pet origin of the strain, showed no symptom. This suggests that pathogenic *E. coli* possibly causes virulence depending on hosts and thus companion animals could harbor and transfer a causative agent of gastrointestinal infection in the community.

W123 - Diverging risk preference of marine bacterial foragers

Presenting Author - *Katsuki Hara, University of Tsukuba, Japan*

Author/s – *Yiyun Zhang, Tomohiro Hirayama, Nobuhiko Nomura, Nozomu Obana, Kyosuke Takabe, Yutaka Yawata, Yuka Iwai, Ryosuke Fukuda*

Abstract Content –

Active search for particulate organic matter (POM) by marine bacteria can be considered as a gambit, where bacteria first invest in the production and maintenance of locomotion appendages, for the rearward to access vital nutrients stored in POM in nutrient depleted water column. As the distance between POM increases, this gambit becomes riskier. How pervasive is this risk prone strategy among marine bacteria, and the variability in risk preferences, however, remained elusive. Here, we systematically quantified the maximum duration for which bacteria can sustain active swimming, as a proxy for risk preference, across the marine bacterial taxa. The genetic basis of the discovered cell-cell and species-species variability in the endurance capabilities was also analyzed. Our microfluidic experiment that tracked bacterial swimming for up to 10 days revealed the striking diversity in risk preferences among bacterial taxa. Notably, while some species stopped swimming within a few hours, part of the *Vibrio* oldarii population kept swimming for >10 days. Analysis of kinetic parameters suggested that the long swimmer adjusts their sensory behavior over time, with gradually increasing the frequency of reorientation. The comparative genomics and gene expression analysis suggested key genes for differential risk preferences, that are upregulated with the cells that sustained swimming over an extended duration. This study provides new insights into the diverse risk preference among marine bacteria, the important drivers of marine microbial loop.

W125 - Visualization of bacterial communication network

Presenting Author - *Yuka Iwai, University of Tsukuba, Japan*

Author/s – *Chikaho Sano, Kyosuke Takabe, Masanori Toyofuku, Nobuhiko Nomura, Yutaka Yawata*

Abstract Content

Microorganisms form multispecies communities across environments including hydrosphere, soil, and other environments. Many of the member of such communities are known to either secrete or response to extracellular signaling substances. This implies that the existence complex network of information mediated by signaling substances within the microbial communities. However, the little known on is such information network in microbial community, due mainly to technical challenge detect cellular response to chemical signals. Here, we developed a method to detect signal reception using cellular autofluorescence as an indicator and comprehensively cataloged signal-responsive bacteria in soil and hydrospheric microbial communities. We also comprehensively analyzed the AHL productivity of these bacterial species. Based on these results, we visualized the bi- directional information network as a connected graph. The results showed that approximately 17% of the cells were responsive to AHLs, of which 31% were Gram-negative bacteria and 69% were Gram-positive bacteria. Individual nodes in the network had an average of 8 linkages. On the other hand, species with a maximum connectivity of about 26 were also observed. This suggests that a hub species in the information network within the community. This study provides new insights into exists the structure of communication networks among microorganisms and the diverging roles of microorganisms in the network.

W126 - Simple and robust workflows for wastewater surveillance

Presenting Author - *Lena Sundermann, Promega Corporation, France*

Author/s – *Melanie Preston, Laura Alexander, Emma Fischer, Laurence Delaurière, Brigitta Saul*

Abstract Content

Recent public health concerns, such as corona and monkeypox viruses, have put an emphasis on effective monitoring of virus prevalence in the population at large. Virus spread in a community can be tracked by assaying wastewater samples for the presence of virus that has been shed from infected individuals. Such assays present certain challenges, notably a low concentration of target in the sample, the need to process large volumes, as well as the presence of inhibitors that can affect downstream detection assays. This work demonstrates several strategies for effective concentration and purification of virus nucleic acids from wastewater samples followed by sensitive and robust downstream detection via qPCR. Membrane- or bead-based capture methods were used for concentration of wastewater samples. Nucleic acid purification was then performed in different formats corresponding to different throughput needs in a manual or automated manner. From the obtained nucleic acid, SARS CoV-2 or monkeypox virus could be detected by amplification in qPCR assays. These multiplexed assays allowed for amplification of several targets using CDC-recommended primer/probe sets and internal controls in a single reaction. The assay was optimized for inhibitor rich samples, increased reliability, and sensitivity. Overall, this work offers ready-to-use solutions for viral detection in wastewater - from sample preparation to viral nucleic acid amplification.

W127 - Rhizosphere microorganisms, *Bacillus megaterium* GEB3 and GEB13 as potential agent for reducing drought stress

Presenting Author - *Jung-Ae Kim, Jeonbuk National Univerisity, Korea, Republic of*

Author/s – *Jung-Ae Kim, Seonbong Choe, Dae-Hyuk Kim, Yangseon Kim*

Abstract Content

Abiotic stress is an important factor that seriously hinders crop growth and productivity, and it is predicted that the frequency and intensity of abiotic stress will increase due to global climate change. Therefore, research is needed to improve the abiotic stress resistance of crops, and in this regard, the utilization of rhizosphere microorganisms and the possibility of industrialization are receiving high attention around the world. We aimed to analyze the specificity and functionality of improving the abiotic stress resistance of crops and promoting crop growth by isolated rhizosphere microorganisms. *Bacillus megaterium* GEB3 and GEB13 were isolated from the rhizosphere of ginseng and identified by sequencing 16S rRNA. Rice seeds were treated with GEB3 and GEB13 culture solutions, and seed germination rates were measured by culturing the seeds under drought conditions of 0 Mpa, -0.15 Mpa, and -0.49 Mpa. In drought conditions, the seed germination rate was higher in the GEB3 and GEB13 treatment groups than in the microbial-free control group. In greenhouse, GEB3 and GEB13 were treated to rice seedlings under drought conditions and the chlorophyll content and dry weight were measured. Although there was no difference in dry weight in all test groups after 4weeks, the chlorophyll content was 34.1 SPAD for GEB3 treatment and 33.4 SPAD for GEB13 treatment, which was significantly increased compared to the control value of 21.6 SPAD without microbial treatment. These results indicate that *B. megaterium* GEB3 and GEB13 can be used as biostimulants with abiotic stress resistance in crop cultivation.

W128 - Comparative analysis of the bacteriobiome in epilithic and epiphytic lichen species

Presenting Author - *Razmik Sargsyan, Department of Biochemistry, Microbiology, and Biotechnology, Yerevan State University, Armenia*

Author/s – *Hovik Panosyan*

Abstract Content

Background: The study of microbial community structure and taxonomic composition of the endolichenic microbes is important to gain insight into their potential functional roles

Objectives: Objects of the study were the endolichenic microbial communities associated with *Ramalina polymorpha* and *Ramalina sinensis*, two lichen species widely distributed on the territory of Armenia.

Methods: Total DNA was extracted from each lichen, and sequenced using Illumina HiSeq, followed by quality control, adapter trimming, and microbial community analysis using Kaiju. Contig assembly was performed using three different assemblers, followed by binning using three different binning tools and optimization with DAS tool. The resulting bins were checked for quality and annotated using RAST.

Result: In total 7 bins from *R. polymorpha* and 1 bin from *R. sinensis* were obtained. Two of these MAGs did not phylogenetically classify within any existing species (Rhodopila sp. RP MAG and *Mucilaginibacter* sp. RP MAG). The *Rhodopila* sp. RP MAG consisted of 5,251,751bp, 274 contigs and 5572 predicted coding sequences with a completeness of 93.53% and contamination at 1.09%. The *Mucilaginibacter* sp. RP MAG consisted of 3,857,500 bp, 73 contigs and 4093 predicted coding sequences with a completeness of 97.14% and contamination at 0.0%. Taxonomic classification revealed that Proteobacteria was dominating phylum in both R. sinensis and R. polymorpha, as well as both communities shared phyla of Actinobacteria, Bacteroidetes, and Acidobacteria. The high abundance of Proteobacteria may be based on nitrogen fixation, sulfur oxidation, and carbon cycling which are key metabolic characteristics of lichen communities

W129 - Gene transfer agents in paracoccus species: a new bacterial model

Presenting Author - *Camille Tinguely, Université De Neuchâtel, Switzerland*

Author/s – *Valentin Voegeli, Pilar Junier, Diego Gonzalez*

Abstract Content

Gene transfer agents (GTA) are domesticated bacteriophages that bacteria use to transfer their DNA to recipient cells as a means of horizontal gene transfer (HGT). Only few GTA systems have been investigated experimentally besides the GTA of Rhodobacter capsulatus (RcGTA), discovered in 1973. In this study, we present a new bacterial model to study GTA-mediated HGT, Paracoccus versutus, which readily produces GTA particles (PvGTA) under standard laboratory conditions. While Paracoccus and Rhodobacter are quite closely related, their GTA differ in regulation and chromosome coverage. We find that the proportion of cells inducing PvGTA production is highest during exponential phase and in the absence of physiological stress, while RcGTA expression is maximal in stationary phase; consistent with this, the expression and transfer rate of PvGTA are not dependent on GafA, one of the main regulators of RcGTA. The content of PvGTA, unlike RcGTA, is strongly biased towards loci around the origin of replication, which suggests that PvGTA production could be synchronized with the initiation of DNA replication. PvGTA production and reception seem to be very specific, since none among three new strains of P. versutus and six other Paracoccus species demonstrated any ability for self-transfer or PvGTA reception. Thus, unlike other HGT mechanisms like natural transformation, GTA systems might have evolved to enforce gene exchange within clonal populations, either to spread beneficial alleles or to help counter the accumulation of deleterious mutations. This study illustrates the importance of researching alternative models of known systems to identify both invariants and context-dependent phenotypes.

W131 - Analysis of microbial communities along the Salinity Gradient in Tengiz-Korgalzhyn Lakes using 16S Nanopore sequencing

Presenting Author - *Polina Len, Nazarbayev University, Kazakhstan*

Author/s – *Ayagoz Meirkhanova, Galina Nagumanova, Ivan A. Vorobjev, Erik Jeppesen, Alessandro Cestaro, Claudio Donati, Natasha S. Barteneva*

Abstract Content

Tengiz-Korgalzhyn Lakes system, designated under the Ramsar Convention and UNESCO World Heritage Sites, is a unique ecosystem of wetlands inhabited by more than a hundred protected and endemic species. In the context of the constant ecological pressure in the area, it is critical to investigate the lake bacterioplankton species and their relationship with abiotic factors, especially since microbiome studies of the region are practically absent in the literature.

This study aims to investigate the role of salinity gradient in shaping bacterial communities in lake ecosystems, as well as the extent to which the overall abiotic factor explains the heterogeneity of microbiome composition across the region.

Data on microbial communities is based on the full-length 16S amplicons obtained with the MinION mk1c. Species-level classification and analysis are performed in Emu, R, and R Studio, using packages 'phyloseq' and 'vegan'.

Our research has confirmed the importance of the salinity gradient in shaping the microbiome composition in limnetic and oligohaline lakes. We have shown that out of all abiotic factors, salinity exerts the most influence on the composition of microbial communities. The abundance of Beta- and Gammaproteobacteria classes changed in parallel with raising salinity levels across all sampling sites: decreasing and increasing, respectively. Moreover, salinity negatively correlated with the community evenness index across distinct small lakes, implying the presence of dominant species. The high degree of variability between isolated water bodies was mainly attributed to the geographical separation.

W132 - 65 years long-term lime application enhanced soil microbial nitrogen cycle processes in acidic grassland meadow

Presenting Author - *Akari Kimura, Hokkaido University, Japan*

Author/s – *Késia Lourenço, Yoshitaka Uchida, Eiko Kuramae*

Abstract Content

The acidity of soil is related to decreasing nutrient availability and plant production. Microorganisms that perform biological nitrogen (N) transformation processes, including nitrifiers, denitrifiers, and N-fixing microorganisms, are influenced by soil pH. Also, acidity is associated with increased N2O emissions from grasslands. Historically, lime application has been used to decrease soil pH in grasslands, and effectively improves plant production. However, still the long-term impact of lime application on microbial N cycling processes is less evaluated. Therefore, we investigated the impact of 65 years of long-term lime application on N cycling processes, including nitrification, denitrification, and N-fixing microbial abundance. Soil sampling was conducted in the grassland meadow in Netherlands in April and July 2022, with seven time points paralleled with gas sampling to measure N2O emissions. The abundances of N cycle genes were determined by quantitative real-time PCR, including ammonia oxidation (amoA-bacteria and archaea), denitrification (nirS, nirK, nosZ), and N-fixation (nifH). Our results show that long-term lime application had a positive impact on N-cycling microbial abundance, which in turn increased plant productivity. Specifically, ammonia oxidation showed stronger positive impact by the liming compared to the denitrification and N-fixation. Although there were more denitrifiers, the N2O emission was not significantly increased by the liming. Our results indicate that the decrease of soil acidity with lime application facilitates microbial N cycling processes without increasing N2O emissions.

W133 - *Rhodoferax lithotrophicus* is a key xylene degrader under microaerobic conditions

Presenting Author - *András Táncsics, Hungarian University of Agriculture And Life Sciences, Hungary*

Author/s – *Sinchan Banerjee, André Soares, Anna Bedics, Balázs Kriszt*

Abstract Content

Background: Among monoaromatic hydrocarbons xylenes are considered one of the most common environmental contaminants. Since many microorganisms can use xylenes as source of carbon and energy under aerobic conditions, biodegradation of the contaminated environments is usually an obvious solution. However, in subsurface environments the availability of oxygen is always restricted. Under oxygen-limited conditions, the degradation rate of para- and ortho-xylenes is low, which makes these compounds persistent in subsurface environments.

Objectives: Enzymes playing crucial role in the microaerobic degradation of aromatic hydrocarbons are the I.2.C-type extradiol dioxygenases, which are mainly harboured by members of the Burkholderiales. Nevertheless, still little is known about bacteria capable of degrading xylenes under microaerobic conditions.

Methods: Aerobic and microaerobic xylene-degrading enrichment cultures were established using groundwater taken from a xylene-contaminated site. The enriched bacterial communities were investigated using a combined omics approach, including 16S rRNA gene amplicon sequencing and genome-resolved metagenomics.

Results: The xylene-degrading bacterial communities were distinctly different between aerobic and microaerobic enrichment conditions. Rhodoferax and Azo*Vibrio* lineages were abundant only under microaerobic conditions. Analysis of a metagenome-assembled genome of a Rhodoferax-related bacterium revealed aromatic hydrocarbon-degrading ability by identifying two catechol 2,3-dioxygenases in the genome. Moreover, phylogenetic analysis indicated that both enzymes belonged to a newly defined subfamily of type I.2 extradiol dioxygenases. It was concluded that the observed differences between the bacterial communities of aerobic and microaerobic xylene-degrading enrichments were driven by the method of aromatic ring-activation, the type of EDO enzymes and the ability of degraders to respire utilizing nitrate.

W134 - Meter-scale soil heterogeneity drives the distribution of fungal and bacterial biomass in an agricultural field

Presenting Author - *Yiqing Zhang, Helmholtz Center For Environmental Research Leipzig - UFZ, China*

Author/s – *Marie Uksa, Lukas Wick*

Abstract Content

Moisture, nutrient availability (C, N, P, K), and pH are known determinants of soil bacterial and fungal biomass. As a first step towards unraveling the interactions between soil fungi, water retention, and microbial pesticide degradation, here we assessed the effect of meter-scale soil heterogeneity on the distribution of fungal and bacterial biomass in an agricultural field of 37ha. Sixty topsoil (0-30 m) and 30 subsoil (30-60 cm) samples were collected and determined for their soil properties including texture, pH, organic carbon, further nutrients, and water-holding capacity (WHC). Bacterial and fungal biomass was assessed by the 16S rRNA gene and ITS fragments and their abundances correlated to the soil properties by partial least squares path modeling (PLSPM). The test site exhibited significant meter-scale soil texture heterogeneity ranging, e.g. from silty clay to loamy sand. PLSPM further revealed that nutrient availability is key to microbial development as was observed by consistently lower bacterial and fungal biomass in the subsoil than topsoil samples. At the horizontal field scale, however, strong effects of soil texture on the microbial biomass were found with higher fungal biomass in sandy areas exhibiting clearly reduced WHC. Further effects of nutrient content on both bacterial and fungal gene abundances were seen, while pH only showed an apparent impact on bacterial biomass development. Ongoing work analyses the effects of field heterogeneity on microbial diversity and effects on the pesticide degradation potential.

W135 - The expanding Diazeniumdiolate signal family

Presenting Author - *María Rodríguez García, University of Zurich, Switzerland*

Author/s – *Anugraha Mathew, Simon Sieber, Leo Eberl*

Abstract Content

Introduction: Bacteria have evolved elaborate means to communicate with each other and coordinate their behaviours. Among the huge amount of signalling molecules employed by bacteria for cell-to-cell communication, a novel signal was identified by the collaborative work of the Eberl and Gademann groups. This new class of signalling molecules contains an unusual diazenium diolate group and was characterized for the first time in the opportunistic human pathogen, *Burkholderia cenocepacia* H111. This signal, known as valdiazen regulates the expression of more than 100 genes and is encoded by a cluster of genes that has homologs in many other bacteria.

Objectives: The main aim of this project is to characterise this new class of signalling group, attending to their biosynthesis, biological function, mechanism of action, role in pathogenesis and regulation of phenotypical traits in *Burkholderia glumae* PG1. Additionally, we aim to identify the signal-responsive regulators and the putative receptor of the signal in *B. cenocepacia* H111.

Methods: Mutant deficient in signal production was constructed in PG1 and a comparative metabolomic analysis between the wild-type and mutant strains was performed. Validation of a putative regulator/receptor of valdiazen (cepS, an AraC-like transcription factor which induces the expression of valdiazen) is being carried out by purification of the protein and Electrophoretic Mobility Shift Assay (EMSA) with the promoter region of valdiazen gene cluster in H111.

Results: Our study reveals the diazenium diolate signal controls the production of several bioactive secondary metabolites in PG1 including its main toxin, tropolone, contributing to its virulence.

W136 - The impact of biocompatible hydrogels on soil microbes and soil properties

Presenting Author - *Atif Aziz Chowdhury, Free University of Bozen-Bolzano, Italy*

Author/s – *Silvia Pioli, Federica Piergiacomo, Lorenzo Brusetti*

Abstract Content

Background: Biocompatible hydrogels are useful against soil desiccation and can improve the physicochemical quality. Because of their outstanding absorption properties, a hydrogel comprising xanthan gum (4%, w/v) and cellulose fibers (2%, w/v) was synthesized.

Objective: To check the impact of the hydrogel on soil properties in a controlled setup.

Methods: A microcosm experiment (soil: hydrogel = 3:1; 400 g; hydrated with 100 mL sterile water) was performed (incubation: 60 days at 25 ± 1°C) and soil properties was compared with the control.

Result: The soil treated with the hydrogel had more moisture (~50% higher), and the water holding capacity of the soil increased by more than 22%, which means the soil retained water better. However, the soil pH and oxidation-reduction potential did not change significantly. The hydrogel also increased the carbon and nitrogen content of the soil. Though, the number of bacteria (including phosphate solubilizing bacteria) decreased, while the number of phosphate solubilizing fungi increased. The microbial metabolic activity was higher, as shown by Biolog EcoPlateTM analysis data of average well color development (AWCD), backed by a 69% increase in heterotrophic soil respiration. The treatment also had higher microbial diversity, as indicated by the Simpson and Shannon indices, and a more even distribution of microbial groups, as indicated by Pielou's equitability index. These findings suggest that the hydrogel can help retain water in the soil and may be useful in managing soil drought.

W137 - Deciphering a novel T4BSS involved in interbacterial killing

Presenting Author - *Miguel Angel Lopez Carrasco, University of Zurich, Switzerland*

Author/s – *Miguel Ángel López Carrasco, Gabriela Purtschert Montenegro, Leo Eberl*

Abstract Content

In most environmental niches, bacteria exist as multispecies biofilms, where cells are embedded in an extracellular matrix that protects them from external stresses, like nutrient limitation, predation and the host immune response, and also restricts the entry of biofilm invaders. Therefore, the competition for space and resources within biofilms has triggered the evolution of competitive strategies to persist in multispecies communities.

In this study, we use different experimental approaches to evaluate the competitive behaviour of the plant-associated bacterium *Pseudomonas* sp. strain IsoF in mixed species biofilms. We demonstrate that IsoF is able to outcompete various soil- and plant-associated bacterial species in a contact-dependent manner. Specifically, we show that IsoF is able to invade and displace a pre-established biofilm of *P. putida* strain KT2440, using flow-through chambers. We identified a gene cluster responsible for contact-dependent killing in IsoF through generation and screening of a mini-Tn5 transposon insertion library. Bioinformatic analyses revealed that the locus encodes a novel type IVB secretion system (T4BSS). Inactivation of genes encoded by this cluster resulted in mutants that were no longer able to kill other bacteria nor were they able to invade existing biofilms, in contrast to the wild-type strain. We also demonstrate that IsoF is able to protect tomato plants from *R. solanacearum* employing its T4BSS in vitro and in soil experiments. These results provide evidence that the competition strategy of IsoF is based on a T4BSS, a defensive and offensive system used for the invasion of pre-established biofilms with potential biocontrol applications.

W138 - Membrane Vseicle-mediated release of AFC-BC11 by *Burkholderia cenocepacia* K56-2

Presenting Author - *Ines Tavares, University of Zurich, Switzerland*

Abstract Content

An increasingly relevant role has been attributed to membrane vesicles (MVs), not only for the host bacterium but also in the interactions between the host and its environment. Among other functions MVs can exhibit, MVs can be used by bacteria to release active compounds. AFC-BC11, an antifungal compound thought to be associated with the cell membrane, is present in the MVs of *Burkholderia cenocepacia* K56-2. The MVs from this strain collected by ultracentrifugation display activity against Fusarium solani. However, if the afc gene cluster is deleted, the activity is lost. HPLC and LCMS analysis showed that B. cenocepacia K56-2 produces other compounds closely related to AFC-BC11. Mutations in the afc cluster interfere with MV formation in *B. cenocepacia* K56-2.

W139 - Comparative genomics of heme biosynthesis in prokaryotes

Presenting Author - *Val Karavaeva, University of Vienna, Austria*

Author/s – *Maria Filipa Baltazar de Lima de Sousa*

Abstract Content

Modified metallated tetrapyrroles are large macromolecular cycles that are essential to many biological processes, e.g. electron transfer and microbial energy conservation solutions. Central to these processes are hemes b, which are found across all domains of life. For a long time, it has been assumed that heme biosynthesis exists as a single conserved pathway in all organisms able to produce hemes. However, with time, additional pathways for heme biosynthesis came into light. Currently, there are three known heme biosynthesis pathways, named after the key intermediate compound before the formation of heme b.

Although the biosynthesis of hemes has been studied for over half a century, no large-scale analysis of distribution and evolution of different pathways has been conducted. In addition, the focus of research was placed predominantly on heme biosynthesis and acquisition within eukaryotes or well-studied prokaryotes, leaving these pathways within the less studied and/or newly discovered prokaryotic lineages without resolution. With the advent of metagenomics and sequencing technology in the last decade, it became increasingly necessary to conduct genomic analyses on a large scale to obtain a fuller picture of the metagenomics-derived organismal diversity. This project intends to fill this gap by using methods of comparative genomics on metagenomic data to resolve the distribution, composition, and evolution of hemes biosynthesis pathways in prokaryotes. Here, we present the analysis of taxonomic distribution and composition of heme biosynthesis pathways across 35 000 prokaryotic assemblies. The data is presented in the context of evolution.

W140 - Evaluation of microbial biomarkers to monitor the effect of irrigation with wastewater carrying contaminants of emerging concern

Presenting Author - *Luciano Beneduce, Department of Agriculture, Food, Natural Resources and Engineering (DAFNE), Italy*

Author/s – *Marcella Giuliani, Anna Gagliardi, Carlo Salerno, Giuseppe Gatta*

Abstract Content

Background: Contaminants of emerging concern (CECs) may impact the safe reuse of wastewater for irrigation in agriculture. Microbial communities are affected by the intake of CECs in agricultural soil and may contribute to their fate, including bioaccumulation in the crops. Induced Resistance to antibiotics and other CECs could represent an additional risk.

Objectives: We monitored microbial communities in an agricultural field irrigated with wastewater carrying CECs. The aim was to identify microbial biomarkers associated with the intake of CECs and may aid to determine soil ecosystem perturbations.

Methods: Soil plots were irrigated with tertiary treated wastewater. 15 CECs were monitored in the water, soil and crops. The experiment was conducted along 4 agricultural cycles in which tomato and wheat were alternated. Microbial analyses included qPCR targeting key nitrogen-cycle bacterial populations, NGS targeting bacterial 16S and fungal ITS genes.

Results: Ammonia-oxidisers were affected by wastewater irrigation, while nitrogen-fixing and denitrifiers populations were dynamic according to the cultural crop. Bacterial population shifted with time. Fungi clustered with time and crop type. In both cases there no influence of the irrigation water source was observed. Through LefSe analysis biomarkers associated with CEC rich wastewater were detected: Among bacteria, Phenilobacterium, a genus including species able to degrade the halogenated heterocyclic Chloridazon, was distinctive of treated soil. Oligothropic *Aquabacterium* and uncultured *Rhizobiales* KF_JC30_B3 were biomarkers of conventional water treated soils. Among fungi, Dominikia, an arbuscular mycorrhizal genus, was biomarker for wastewater, while several Sordariomycetes were distinctive of conventional water treated soils.

W141 - Rhizosphere microbial communities of Poaceae species in Hungarian grasslands: screening for plant growth promoting properties

Presenting Author - *Milan Farkas, Hungarian University of Agriculture And Life Sciences, Hungary*

Author/s – *Milán Farkas, Dalma Márton, Gergely Maróti, Neveen Majdi Almalkawi, Balázs Kriszt, Mátyás Cserháti*

Abstract Content

Background: Drought is a major challenge for plant growth especially in dry grassland areas. Bacteria that stimulate plant growth are often isolated from extreme environments such as arid regions and these bacteria are able to increase the survival of other types of plants during the dry period, not just those from which they have been originated.

Objectives: The objective of this study is to reveal arid open and closed sand steppes rhizosphere bacterial communities. We investigate whether the isolated bacteria with plant growth-stimulating properties can improve the growth of maize under normal and drought stress conditions.

Methods: The diversity of bacterial community from the rhizosphere and bulk soil samples were investigated by Illumina metagenome sequencing and culturing methods. Additionally total of 149 strains were isolated from the sandy grasslands and the selected nonpathogenic 48 strains were screened for plant growth promoting (PGP) traits, such as: osmotic stress tolerance, indole-3-acetic acid, exopolysaccharide, siderophore, and 1-aminocyclopropane-1-carboxylate deaminase production and phosphate solubilization. The short-term effect of the strains to maize was tested in a phytotron pot experiment, while the study of drought stress conditions is planned to be investigated in an open field experiment.

Results: Based on metagenome analysis representatives of the Actinobacteria, Proteobacteria and Acidobacteria were the most abundant in both rhizosphere and soil samples. A short-term phytotron study of the isolates with the best PGP properties shows that the growth of maize plants was mostly enhanced by the Brevibacillus, Priestia, Kocuria species. Field trials are currently in progress.

W142 - Metagenomic insights into microbial carbon cycling in an Arctic River influenced by permafrost thaw

Presenting Author - *Lucia Winkler, Friedich Schiller University Jena, Germany*

Author/s – *Lucia Winkler, Will Alan Overholt, Kirsten Küsel, Carl-Eric Wegner, Karel Castro Morales*

Abstract Content

The release of organic carbon and land-formed methane into the Arctic rivers is expected to increase due to the progressive thawing of permafrost. In June (freshet) and August (summer) 2019, we sampled the surface Kolyma River and its tributary Ambolikha in Northeast Siberia, whose watershed is completely underlain with continuous permafrost. We investigated if their microbial communities have the genomic potential to cope with elevated freshet carbon input, by elucidating key genes of methane cycling and organic matter degradation. The microbial community structure differed between sampling sites and the two time points, with a higher alpha-diversity in June than in August. A taxonomically highly diverse fraction of 10-15% of the freshet community belonged to OTUs not present in August samples. Amongst the freshet-specific taxa were up to 0.03% methanogenic Euryarchaeota. We could not detect methanogenesis marker genes though in shot-gun metagenomics data. OTUs associated with methanotrophy/methylotrophy reached relative abundances of up to 4% and were more abundant in June. Based on marker gene abundance, methylotrophy is more common than methanotrophy. The Burkholderiaceae family, known to harbor aromatic compound-degrading genera, was most abundant in all samples. We could not detect genes encoding lignin-depolymerizing peroxidases/laccases but the potential to metabolize aromatic monomers. Our metagenomic data suggest that there is neither an effective biological filter to prevent methane outgassing nor substantial degradation of lignin during the transport of organic matter from the river to the Arctic Ocean during the open water season possibly influenced by the low water retention time.

W143 - Fermentation potential of different types of whey for the production of biodegradable hydrogels

Presenting Author - *Jitka Peroutkova, Dairy Research Institute Ltd., Czech Republic*

Author/s – *Alexandra Šalaková, Markéta Borková, Jan Drbohlav, Ladislav Bár*

Abstract Content

Background: The dry summers negatively affect the soil quality, especially its retention capacity. Hydrogels, are used to retain and release water. Alternative to synthetic hydrogel is a biodegradable one, which can be based on Polylactide (PLA) produced by polycondensation of lactic acid. Whey, a by-product of the dairy industry, is a suitable raw material for obtaining lactic acid.

Objectives: The goal of this work was to determine the fermentation potential of whey by selected lactic acid bacteria (LAB) with high lactic acid production.

Methods: Four different types of whey were inoculated with strains of LAB from The Collection of dairy microorganisms Laktoflora® (CCDM). Homofermentative bacteria from the genus *Lactobacillus*, *Lactococcus* and a mixed yogurt culture were used. During fermentation, active and titratable acidity were measured, and the number of microorganisms was determined at the end of fermentation.

Results: All tested whey samples represented a very good cultivation substrate for the selected microorganisms, as their viable counts after fermentation reached ≥ 107 CFU/ml. The highest titration acidity was found in acid whey fermented by the strain *Lactobacillus helveticus* CCDM 121. The most suitable of the tested strains with a high ability to form lactic acid in whey for subsequent polycondensation and production of hydrogels is the genus *Lactobacillus*, specifically *Lactobacillus helveticus* CCDM 121 and CCDM 98. Based on these results, acid whey seems to have a high fermentation potential.

W144 - Effects of two types of hydroxyl acids on soil bacterial and fungal communities

Presenting Author - *Chunge Li, Research Center for Eco-environmental Sciences, Chinese Academy of Sciences, China*

Author/s – *Jingguo Wang, Ye Deng*

Abstract Content

Background: Soil phenolic acids mainly come from crop residue and root exudates, which were often reported as allelochemicals affecting crop growth and soil microbial community, especially more serious in monoculture soil.

Objectives: Here we studied the effects of phenolic acids [p-hydroxyphenylacetic acid (HPA) and p-hydroxybenzoic acid (HBA)] amendments on soybean and corn soil microbial community under room conditions.

Methods: All soils were incubated for 35 days, the microbial community was determined by PCR-DGGE (polymerase chain reaction-denatured gradient gel electrophoresis) and clone methods, microbial biomass carbon (MBC) was measured based on the fumigation–extraction method.

Results: The result revealed that HPA and HBA had the significant impact on soil microbial community and MBC. We found that bacterial diversity had significantly negative correlation to fungal diversity, and HPA enriched more bacteria than fungi. We inferred the above enriched bacteria were active, positive to against HPA/HBA amendment and beneficial to soil health recovery and plant growth. We speculated that HPA stimulated more microbial growth and the increase of MBC in SP (HPA amended soybean soil) mainly came from bacteria.

W145 - Efficient high throughput sorting of double emulsions for bacterial micro-community analysis

Presenting Author - *Wannes Nauwynck, Center for Microbial Ecology and Technology (CMET), Belgium*

Abstract Content

High-throughput phenotyping of bacterial (co-)cultures combines flow cytometric analysis with compartmentalization of bacteria in double emulsions (DE). Different assays allow for the measurement of phenotypical information regarding substrate utilization, product formation or characterization of bacterial interactions from mixed bacterial communities. Furthermore, the connection between phenotype and genotype can be directly established through sorting.

Despite the promise of these functional assays as a household technique for the analysis of bacterial communities, the application of these assays has, up until now, been limited to specialized labs with access to custom microfluidic chips, requiring extensive in-house knowledge and equipment to fabricate and operate.

In this study, a vortex method is introduced for the production of highly monodisperse DE requiring no prior in-house knowledge or specialized equipment. With the use of imaging flow cytometry, a gating strategy is validated for successful identification of the desired population of DE. Finally, we investigated the effect of DE size on the efficiency of fluorescence activated sorting.

Overall, this study provides a simple and widely applicable blueprint for high-throughput phenotyping of bacterial (co-) cultures, allowing widespread use of this promising technique.

W146 - Screening the aquatic environment for plastic degrading mircoorganisms

Presenting Author - *Valérie Mattelin, Center for Microbial Ecology and Technology (CMET), Belgium*

Author/s – *Kankana Kundu, Lennert Verfaille, Stefaan De Wildeman, Nico Boon*

Abstract Content

Plastic waste accumulation creates new niches for microorganisms in natural environments. In the marine environment, this niche is referred to as the plastisphere. Although it is widely accepted that microorganisms inhabiting the plastisphere are not representing the surrounding water community, it remains difficult to establish actual plastic biodegradation. Furthermore, key species in the different steps of biodegradation, and their specificity for different types of plastic is not well understood.

In this research, we focussed on two different methods to investigate plastic biodegradation. First, marine samples were used to enrich for plastic degrading microorganisms on different types of plastics in a bioreactor trickling filter design. Second, a novel, rapid and easy to apply colorimetric technique was developed to screen, in high throughput, the depolymerisation of plastics in natural environments, under different conditions.

By 16S rRNA gene sequencing of the enrichments, we found that different plastic types can enrich for different species. For example, the genus *Tistrella* was present in the enrichment of a novel polymer with a relative abundance of more than 60%. The colorimetric method, on the other hand, was used to investigate the biodegradation potential in aquatic environmental samples, without enriching. It was, for example, observed that poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) was degrading faster than poly caprolactone in seawater.

In conclusion, both methods offer a valuable way of exploring plastic degrading microbes. Enrichments select for species that have the metabolic capability to live in these conditions, while the colorimetric technique can screen for plastic degraders across different environments.

W147 - Radical-based enzymatic degradation of synthetic dyes

Presenting Author - *Astrid Rombouts, Ghent University, Belgium*

Author/s – *Nico Boon, Tom Desmet*

Abstract Content

Background: Water pollution increases, causing a possible threat to the environment and our health. Pollutants such as organic contaminants and microplastics get up in effluent due to the inefficiency of current water treatment systems. The use of radical-based enzymatic degradation is one of the new promising methods for preserving our water quality. Enzymes are known to catalyse chemically difficult reactions, and radical molecules, due to their high reactivity, induce chain reactions and eventually degrade substrates.

Objectives: The first crucial step is to scan, identify and characterize microorganisms using enzymes with potential radical-based degradation functionalities.

Methods and results: *Bacillus spizizenii* and *Trametes versicolor* are selected as model organisms to degrade methyl orange and methylene blue under different growth conditions. The two dyes serve as model compounds of organic pollutants to be able to verify the degradation by absorbance. After 10 days of incubation, up to 96% of the dyes is degraded in glucose containing growth medium. The observed degradation is explained using flow cytometry in combination with live/dead labelling. In a second experiment, methyl orange and methylene blue are added to microorganisms and communities (bacteria, fungus, or a combination of them) from sludge, waste water, and natural habitats. Additionally, a colony selecting and dilution to extinction-based isolation experiment is set up to find and define the best dye degrading microorganisms, using enzymes with potential radical-based degradation functionalities. Up till now, several species are isolated and ready to identify.

W148 - Single-cell adhesion force mapping of a highly sticky bacterium in liquid

Presenting Author - *Katsutoshi Hori, Nagoya University, Japan*

Author/s – *Shogo Yoshimoto*

Abstract Content

Background: The highly sticky gram-negative bacterium *Acinetobacter* sp. Tol 5 adheres to various material surfaces via its cell surface nanofiber protein, AtaA, a member of the trimeric autotransporter adhesin family and forms a long nanofiber 260 nm in length. The adhesiveness of Tol 5 cells has been evaluated based on the amount of cells adhering to a surface. The adhesiveness measured by this method slightly reflects the adhesion strength through resistance against shear stress caused by washing steps but is significantly affected by attractive and repulsive forces in the initial attachment process as well as autoagglutination of the cells. Therefore, the pure adhesion strength of a bacterial cell cannot be determined by this method.

Objectives: In this study, we aimed to determine the strength and distribution of the adhesion force on Tol 5 cell covered with AtaA fibers in liquids using atomic force microscopy (AFM).

Methods and Results: The adhesion force mapping of a single Tol 5 cell in liquid using the quantitative imaging mode of AFM revealed that the adhesion of Tol 5 was near 2 nN, which was 1–2 orders of magnitude higher than that of other adhesive bacteria. The adhesion force of a Tol 5 cell was drastically reduced in the presence of 1% casamino acids but not in deionized water, although both liquids decrease the adhesiveness of Tol 5 cells, suggesting that deionized water and casamino acids inhibit the cell approaching step and the subsequent direct interaction step of AtaA with surfaces, respectively.

W149 - Resistome and phageome from oxidation lagoons of a First Nation community in Canada

Presenting Author - *Miguel Uyaguari, University of Manitoba, Canada*

Author/s – *Adetola Adeniji*

Abstract Content

Oxidation lagoons (OLs) are the primary wastewater treatment system used in rural and remote areas across Canada1. OLs are essential in maintenance of health and safety of the public. Although these facilities are designed to remove harmful pathogens they may also serve as reservoirs of antibiotic resistance genes (ARGs). The discovery of drug-resistant genes in aquatic environments increases the possibility of these genes being transferred to other microorganism during wastewater treatment. While the performance of the OLs in the reduction of bacteria has been characterized, not many studies have been addressed on the phageome population present in wastewater. Hence, there is very little knowledge about the occurrence and distribution of bacteria and phage resistance genes in OLs.

In this research, we employ a sequence-based metagenomics approach to characterize the total resistome from bacteria and phage populations present in OLs of a First Nation community in Manitoba (MB). The community has less than 5,000 people and is located 2 hours from Winnipeg, MB. Nanopore sequencing in combination with quantitative PCR are used in the identification of the antimicrobial resistance profiles present in the microbial population.

W150 - Exploring the effects of methanol-methane mixtures on microbial communities and polyhydroxybutyrate (PHB) synthesis efficiency

Presenting Author - *Hyerim Eam, Korea Advanced Institute of Science and Technology, Korea, Republic of*

Author/s – *Jaewook Myung*

Abstract Content

The increased amount of plastic pollution has resulted in a growing demand for biodegradable polymers such as polyhydroxybutyrate (PHB). PHB is a biodegradable and biocompatible polymer that can be synthesized inside the cells and degraded in natural environments such as oceans and soils. Aerobic methylotrophs and methanotrophs can oxidize C1 sources (e.g. methanol and methane) and biologically convert them into PHB. Methane and methanol are potential feedstock for PHB production to reduce production costs. Methanol, an intermediate product of methane oxidation, is known to support the growth of methanotrophs, however, the effects of methanol on PHB production have been rarely been investigated. Here, we examined the efficiency of PHB synthesis and microbial communities under different ratios of the methane and methanol. Five different groups were monitored, each with a different ratio of methane and methanol (e.g. methane 1.8 mmol, methane 1.26 mmol and methanol 0.54 mmol, methane 0.9 mmol and methanol 0.9 mmol, methane 0.54 mmol and methanol 1.26 mmol, methanol 1.8 mmol). We assessed how their microbial compositions and diversity changed over time. PHB contents were observed due to vary based on changes in the microbial communities in response to substrate composition.

W151 - Physiological importance and role of Mg2+ in improving bacterial resistance to cesium

Presenting Author - *Masahiro Ito, Toyo University, Japan*

Author/s – *Yoshiki Ishida, Chongkai Zhang, Katsuya Satoh*

Abstract Content

Microbacterium sp. TS-1 (TS-1) is an alkaliphilic bacterium resistant to 1.2 M CsCl (1). In previous studies, we suggested that the high Cs+ resistance of TS-1 is due to the efflux of Cs+ by CshA and the Mg2+ uptake system Mgt (1,2). In this study, we investigated changes in intracellular Cs+, K+, and Mg2+ concentrations in the presence of Cs+ and their effects on oxidative damage to cells and ribosome complexes using TS-1, its Cs+ sensitive strains (CshA and Mgt mutants), and *B. subtilis*. We measured the intracellular concentrations of each ion which showed that Cs+ decreases the intracellular K+ concentrations and simultaneously promotes the uptake of Mg2+ in *B. subtilis*. On the other hand, the intracellular Cs+ concentrations were kept below 250 mM in TS-1. We investigated the effect of Cs+ on ribosomes, indicating that the ribosomes of TS-1 are not affected by Cs+, whereas their Cs+ sensitive strains have degraded 70S ribosomes. These results suggest that Cs+ destabilizes ribosomes in the TS-1, but that the intracellular uptake of Mg2+ stabilizes the ribosome structure and that at Cs+ concentrations of 250 mM or higher, CshA suppresses the increase in the intracellular Cs+ concentration by efflux of Cs+, thereby leading to high Cs+ tolerant.

W152 - DNA-damaging effect of the cyanotoxin β-N-methylamino-L-alanine (BMAA) on neurons at micromolar concentration

Presenting Author – *Seyed Hamed Kazemi Shariat Panahi, Macquarie University, Australia*

Author/s – *Sina Shadfar, Seong Beom Ahn, Julie D. Atkin, Gilles J. Guillemin*

Abstract Content

Background: BMAA, produced by many Cyanobacteria spp. during algal blooms, is a potential environmental etiology factor for some sporadic neurodegenerative diseases. Although mechanisms of BMAA toxicity on protein misincorporation and misfolding are well-studied, there is limited information on the effects of BMAA on DNA.

Objectives: This study for the first time investigates the DNA-damaging effect of BMAA on neurons at micromolar concentration.

Methods: Neuro2A cells were exposed to BMAA (100 µM) in the presence of 20 mM $NaHCO_3$. Etoposide (13.5 µM; treatment duration, 30 min) and culture medium (DMEM+10% FBS) were used as the positive control and the blank, respectively. After 48 h treatment, immunocytochemistry was done sequentially using rabbit anti-γ-H2AX antibody, Alexa488, and HOESHT to detect and visualize γ-H2AX, the phosphorylated form of histone H2AX that functions as a sensitive marker for double-strand breaks, and cell nucleus.

Results: BMAA triggered neuronal DNA damage at a much lower concentration than previously reported (100 µM vs. 1000-3000 µM). The average number of DNA damage foci/50 cells for control, etoposide, and BMAA were 5±0.43, 24±0.74, and 19±0.91, respectively. The exposure to BMAA or etoposide induced the natural DNA damage rate in Neuro2A by almost four times ($P \leq 0.01$) and five times ($P \leq 0.01$), compared to the control. Since the difference between etoposide- and BMAA-treated samples was not statistically significant, it can be concluded that BMAA at 100 µM concentration can cause a relatively similar damaging impact on DNA after 48 h.

W153 - Multi-transcriptome analysis to elucidate the flavobacterium-mediated suppression of bacterial wilt and the causative bacterium

Presenting Author - *Sujin Lee, Gyeong-sang National University, Korea, Republic of*

Author/s – *Hyein Park, Boyoung Lee, Jaehyo Park, Ju Yeon Song, Soon-Kyeong Kwon, Jihyun F Kim*

Abstract Content

The soil-borne pathogen *Ralstonia solanacearum* causes bacterial wilt and thereby crop losses in the Solanaceae plants including tomato, potato, pepper, and eggplant. Although the susceptibility to the wilt disease primarily depends on the plant genotype, the microbial community in the rhizosphere also contributes to the severity. A flavobacterium TRM1, isolated from the wilt-resistant tomato cultivar Hawaii 7996, suppresses Ralstonia wilt in a susceptible tomato cultivar. The antagonistic activity of TRM1 against *R. solanacearum* was also observed from co-cultivation of the two bacteria in mCPG medium. To infer the wilt-suppressing mechanism, a large-scale transcriptional characterization was conducted. The transcriptional changes of TRM1 and *R. solanacearum* under the co-cultivation condition were compared to those in mono-cultivation. The transcriptome data of TRM1, *R. solanacearum*, and tomato were also collected in the plant rhizosphere. Genes for several secretion systems in *R. solanacearum* were inferred to be associated with virulence, while genes encoding some membrane-bound proteins in TRM1 appeared to be associated with virulence suppression. Integrating the results of these transcriptional data helped us systematically understand the wilt-suppressing mechanisms between the plant pathogen, the disease-suppressing microbe, and the host plant.

W154 - *Pseudomonas lini* colonizes the root and enhances the tolerance of osmotic stress in sugar beet

Presenting Author - *Rico Leiser, Rwth Aachen University, Germany*

Author/s – *Jan Paulini, Uwe Conrath*

Abstract Content

Climate change-associated osmotic stresses, such as drought and soil salinization can reduce crop yield. Some plant-beneficial rhizobacteria can activate the innate osmoregulation of crops thus alleviating stress related yield losses. Here we present a novel soil isolated and characterized *Pseudomonas lini* strain that produces siderophores, forms biofilms on sugar beet roots, fixes atmospheric nitrogen, and exhibits 1-aminocyclopropane-deaminase activity. We tested whether the *P. lini* strain enhances the physiological status of sugar beet during continuous and intensive drought and soil salinization.

We demonstrate that *P. lini* colonizes the root of sugar beet within 24 hours and forms stable biofilms on the rhizoplane. This colonization led to improved plant growth under drought (27 % dry mass increase) and salinity stress (86% dry mass increase) compared to untreated control plants. The better growth of colonized plants was attributed to an enhanced status of Photosystem II, resulting in greater photochemical quantum yield (+3 % Fv/Fm-value). In addition, the relative leaf water content was higher (+9 %) in colonized than in uncolonized plants indicating stronger osmotic adjustment in the leaves.

In conclusion, our study suggests that the isolated root-colonizing *P. lini* strain enhances osmotic stress tolerance of sugar beet plants and has the potential to be used as a biostimulant for improving crop yield under adverse climate conditions.

W155 - The transient and residential gut microbiome of the benthic copepod *Platychelipus littoralis*

Presenting Author - *Bram Martin, Ghent University - Marine Biology Department, Belgium*

Author/s – *Marleen De Troch, Nico Boon*

Abstract Content

Background: Copepods comprise an immensely diverse and abundant group of meiofauna in marine sediments. They are the natural food source for fish larvae and juvenile fish, providing large potential for use in aquaculture. However, as they can carry fish-pathogenic *Vibrio* spp., the use of copepods in live aquafeeds is limited. Many copepod species show coprophagic feeding behavior as they reingest their own fecal pellets. Therefore, investigating the gut microbiome of copepods and understanding nutritional host interactions is of large interest.

Objective: Characterisation of the transient (food-dependent) and residential (continuously present) gut community of *Platychelipus littoralis*, a key harpacticoid species in intertidal sediments along West-European shore. This is the first quantitative and qualitative analysis of the gut microbiome of marine benthic copepods.

Methods: The bacterial community on dissected copepod guts was counted and identified with flow cytometry and 16S rDNA amplicon sequencing, respectively. Copepods were dissected within 24h of field collection to analyze filled guts, while other specimens were starved for 48h to obtain fully cleared guts. This allows for a comparison between the transient and residential microbiome.

Results: Large variability between copepod individuals was expected but not observed. Transient and residential gut communities were significantly different. The transient microbiome was more diverse, and two core taxa were identified. The residential microbiome contained an additionial core genus: Colwellia.

W156 - Isolation of biocrust cyanobacteria from contaminated soils for using in soil restoration

Presenting Author - *Carlotta Pagli, University of Rome Tor Vergata, Italy*

Author/s – *Sonia Chamizo, Lorenza Rugnini, Giovanni De Giudici, Giada Migliore, Yolanda Cantón, Laura Bruno*

Abstract Content

Biocrusts are topsoil microbial communities composed of different organisms such as cyanobacteria, algae, fungi, lichens, and mosses associated with soil particles. These soil communities have a fundamental role in ecosystem functioning by stabilizing the soil surface, regulating soil water balance, and providing nutrients. Biocrust-inhabiting cyanobacteria are pioneer organisms, capable of colonizing very different environments thanks to their resistance to stress factors. The ability of these microorganisms to colonize degraded soils and improve soil conditions has encouraged their use as inoculum for restoring soil functions in degraded areas. With this objective, in this study, cyanobacteria species were isolated and identified from biocrusts present in a mining area affected by heavy metal contamination in the southern province of Sardinia (Italy). Biocrust samples were collected from four sites near abandoned mines and their microbial community composition was determined through next-generation sequencing of 16S rRNA and ITS genes. Cyanobacteria strains were isolated and identified with a multiphase approach including the morphological characterization by observing the samples under a light microscope and 16S rRNA gene sequence analysis. The phylogenetic tree revealed the taxonomic positions of the cyanobacterial strains which were also tested for their ability to synthesize exopolysaccharides (EPS). The most efficient EPS-producing cyanobacteria were later tested for their ability to remove Cu, Pb, and Zn from water. The isolation and characterization of these pioneering cyanobacteria species, open the field for future applications in studies of restoration of degraded soils such as lands subjected to erosion, metal-contaminated soils, fire-damaged lands, and exhausted quarries.

W157 - LysR regulates both Ethylene glycol and polyhydroxyalkanoate (PHA) metabolism in *P. umsongensis* GO16

Presenting Author - *Jounghyun Um, University College Dublin, Ireland*

Author/s – *Kevin O'Connor, Tanja Narancic*

Abstract Content

Polyethylene terephthalate (PET) is a plastic mostly used as packaging and synthetic fibres. Its short shelf-life, durability, and recalcitrant nature hugely contribute to plastic pollution. Previously, up-cycling PET into valuable products using micro-organism was demonstrated as a valuable approach to contribute to plastic waste solution. PET can be enzymatically depolymerised into terephthalic acid (TA) and ethylene glycol (EG), followed by the conversion of the hydrolysate into bacterial polyesters polyhydroxyalkanoates (PHAs) using bacteria such as *Pseudomonas umsongensis* GO16.

There are two main types of PHAs depending on the number of carbon atoms in the monomer units of the polymer chain; short chain length PHA (SCL-PHAs; C3-C5), usually hard and brittle with high melting temperature, and medium chain length (MCL-PHAs; C6-C14), which tend to be soft and elastomeric. GO16 can accumulate both types. Furthermore, in GO16, a gene for transcriptional regulator LysR is located 5'- of the gene for glyoxylate carboligase (gcl), involved in EG metabolism. The lysR Knockout strain can't grow on EG as a sole carbon and energy, demonstrating its role in EG metabolism. When lysR KO strain was grown with TA or glucose the growth was comparable to the WT GO16. However, the PHA accumulated by the deletion strain synthesis of SCL-PHA monomer (C4) was increased 5.4-fold and 1.4-fold when TA or glucose were used respectively. This indicates a more complex role of LysR than simply activating EG metabolism in GO16. We demonstrated proteomic analysis to find out which genes are regulated by lysR type regulator in GO16.

W159 - Host and microbiome jointly contribute to adaptation to a complex environment

Presenting Author - *Hinrich Schulenburg, University of Kiel, Germany*

Author/s – *Inga K. Hamerich, Karen L. Adair, Hanne Griem-Krey, Brendan J.M. Bohannan, Hinrich Schulenburg*

Abstract Content

Background: Most animals and plants are host to a community of microbes, their microbiomes, which often provide essential life-history functions. Given these functions, microbiomes have the potential to contribute to adaptation of the host-microbiome assemblage, especially as they can change more rapidly than their hosts. To date, however, it is not well understood to what extent host and microbiome jointly mediate adaptation to a novel environment.

Objectives: The objectives of our study are to disentangle the contributions of hosts and microbiomes to evolutionary adaptation using a novel experimental study system.

Methods: We established a new mesocosm approach, with which we adapted the nematode Caenorhabditis elegans as host with its diverse microbiome to a novel complex environment. After 100 days, we harvested worm populations and associated microbiomes and subjected them to a common garden experiment, to unravel the impacts of microbiome composition and host genetics on adaptation.

Results: We found that adaptation took different trajectories in different mesocosm replicates, where some increased while others decreased their fitness in the novel environment. Interactions between host and microbiome played a critical role in the observed evolutionary paths. By focusing on two exemplary mesocosms, we reconstructed the specific changes in microbiome composition (for both bacteria and fungi) and also host genetics that accounted for the observed change in fitness.

Conclusion: Our study provides experimental evidence that adaptation to a novel environment is jointly influenced by host and microbiome, highlighting that both need to be considered for a full appreciation of the process of evolution.

W160 - The heavy metal resistome of *Cupriavidus metallidurans* CH34

Presenting Author - *Diana Galea, Martin-Luther University Halle-Wittenberg/Institute for Biology/Microbiology, Germany*

Author/s – *Martin Herzberg, Matt Fuszard, Dirk Dobritzsch, Dietrich H. Nies*

Abstract Content

Cupriavidus metallidurans CH34 is a highly metal-resistant betaproteobacterium which serves as a model organism for the study of multiple metal homeostasis pathways. In the natural environment, opposed from the finely controlled laboratory conditions, bacteria are exposed to a variety of stress factors. Among them, abiotic stresses, such as heavy metal ions, require specific survival strategies. *Cupriavidus metallidurans* CH34 is a survivalist in metal-enriched sites, with molecular determinants involved in metal resistance harboured on all four replicons (chromosome, chromid and the 2 megaplasmids, pMOL28 and pMOL30).

We aim at making a comprehensive inventory of the metal resistome of *Cupriavidus metallidurans* CH34 when challenged simultaneously with a mix of metals (Zn, Co, Ni, Cu, Cd, As, Cr, Hg). Therefore, bottom-up proteomics was employed to identify and quantify the set of proteins which confer the outstanding resistance of this bacterium. To complete the resistance profile, physiological and analytical characterization was performed.

A “personalised” multi-toxic solution was made to suit the resistance of this bacterium, based on the IC50 for each distinct metal. Exponentially growing cells were challenged with the same solution to determine the metal accumulation. Tandem mass spectrometry was performed on both the cytoplasmic and membrane proteins and the proteome was identified and quantified through label-free quantification.

In order to reach full resistance, *Cupriavidus metallidurans* CH34 employs several regulatory and transport systems which mediate its survival when exposed to micromolar and millimolar heavy metal concentrations.

W161 - Extracellular enzymes as promising soil health indicators: assessing response to different land uses using long-term experiments

Presenting Author - *Munisath Khandoker, Rothamsted Research, United Kingdom*

Author/s – *Munisath Khandoker, Stephan Haefele, Andrew Gregory*

Abstract Content

Extracellular enzymes play a key role in soil organic carbon (SOC) decomposition and nutrient cycling and are known indicators for soil health; however, it is not understood how these enzymes respond to different land uses and their relationships to other soil properties have not been extensively reviewed.

We examined the relationships among the activities of three soil enzymes: β-glucosaminidase (NAG), phosphomonoesterase (PHO) and β-glucosidase (GLU). The impact of soil organic amendments, soil types and land management on soil enzyme activities were reviewed, and we hypothesised that soils with increased SOC have increased enzyme activity. Long-term experiments at Rothamsted's Woburn and Harpenden sites in the UK, were used to evaluate how different management practices affect enzyme activity involved in carbon (C) and nitrogen (N) cycling in the soil. Samples were collected from soils with different organic treatments such as straw, farmyard manure (FYM), and compost additions, and cover crops and permanent grass cover to assess whether SOC can be linked with increased levels of enzymatic activity, and what influence, if any, enzymatic activity has on total C and N in the soil.

Investigating the interactions of important enzymes with soil characteristics and SOC can help us to better understand the health of our soils. Studies on long-term experiments with known histories and large datasets can help us. SOC tends to decrease during land use changes from natural ecosystems to agricultural systems, therefore it is imperative that agricultural lands find ways to increase and/or maintain SOC in the soil.

W162 - Signal transduction in global marine biofilms and the influence of signal molecules on the development of marine biofilms

Presenting Author - *Ruojun Wang, The Hong Kong University Of Science And Technology, Hong Kong*

Author/s – *Wei Ding, Weipeng Zhang, Lexin Long, Peiyuan Qian*

Abstract Content

Background: Microbes utilize complicated signal transduction systems to respond to environmental stimuli. Due to complex multi-species interactions, the microbial community in natural biofilms enhances intricate conditions. However, signal transduction systems in marine biofilms have hardly been explored.

Objectives:

- Profiling signal transduction systems in global marine biofilms;
- Identifying the influence of different signal molecules on shaping marine biofilm communities.

Methods:

- Biofilms and seawater sampling across oceans
- DNA extraction and metagenomic analyses of marine biofilm and seawater samples
- Signal molecule treatment experiment and metagenomic analyses
- Bacterial isolation, *Pseudomonas* quinolone signal (PQS) treatment experiment, and transcriptomic analyses

Results:

- Metagenomic profile of signal transduction systems in global marine biofilms (n = 101) displayed distinct patterns from worldwide seawater samples (n = 91), indicating the specificity of signal transduction system in marine biofilm communities;
- Most signal transduction genes in marine biofilms were enriched in marine biofilms and showed different taxonomic sources compared to those in seawater, but potential inter-phyla interactions between microorganisms might exist between marine biofilms and surrounding seawater;
- Comparative metagenomics after signal molecule treatments suggested the distinctive influence of these molecules on the microbial structure and function of multi-species biofilm communities, and the biofilms treated with PQS shared the least similarity with the control and initial biofilms;
- The PQS-based signal transduction system may play an important role in regulating microbe-microbe interactions and the assemblage of biofilm communities.

W163 - Discovery of novel lipase and its-secreting microorganisms from anaerobic digester sludge

Presenting Author - *Riku Sakurai, Tohoku University, Japan*

Author/s – *Yasuhiro Fukuda, Chika Tada*

Abstract Content

Lipid hydrolysis is one of the rate-limiting steps in the anaerobic digestion process. It has remained unknown what microorganisms perform lipid hydrolysis in the anaerobic digestion process. In this study, we aimed to identify the cardinal lipase and its-secreting microorganisms in anaerobic digestion sludge. Our zymography, followed by two-dimensional polyacrylamide gel electrophoresis, showed that 4 protein spots possessed lipase activity. We excised the spots from the gel and applied the nanoLC-MS/MS analysis to identify the partial amino-acid sequences of the lipases. To facilitate the protein identification, we generated a metagenomic database using PacBio Sequel lle sequencer and successfully identified 6 hypothetical proteins as candidates for the lipase. To evaluate whether the candidates possess lipase activities, we produced these recombinant proteins by *Escherichia coli* and confirmed that one of these hypothetical proteins contained the catalytic activity for a model substrate. The substrate specificity of this novel lipase was analyzed after purification using para-nitrophenyl esters. The DNA sequence of this protein was a component of a metagenome-assembled genome, which was classified as Oscillospiraceae. This study would become a cornerstone for gaining knowledge on lipolysis during anaerobic digestion.

W164 - Synergistic effects of catalase and ferulic acid esterase or xylanase on lignocellulose degradation.

Presenting Author - *Chao-Hsun YANG, Providence University, Taiwan*

Author/s – *Chao-Hsun YANG, Wan-Yu Liao*

Abstract Content

Background: The thermophilic actinomycete was reported to be able to produce numerous extracellular lignocellulose-degrading enzymes. Previous results showed that enzyme Tfu-1649, secreted from the *T. fusca* BCRC 19214, had catalase activity.

Objectives: This study uses Tfu-1649 alone and synergy with other lignocellulolytic enzymes to degrade lignocellulose.

Methods: The bagasse was used as a lignocellulosic substrate. The phenolic acids were measured by High-performance liquid chromatography.

Results: When the bagasse was treated with Tfu-1649 alone, the total phenolics (28 μg/mL) and p-coumaric acid (16 μM) were accumulated in the broth. When hydrogen peroxide (4 μM) was added to the reaction, the hydroxybenzoic acid (36 μM) accumulated significantly in the broth. When the ferulic acid esterase or xylanase co-operated with catalase to degrade bagasse, the total phenolics and p-coumaric acid were increased significantly. Hence, the cooperative degradation of lignocelluloses by catalase with ferulic acid esterase or xylanase could contribute to biomass decomposition and further applications in the sustainable environment.

W165 - Molecular basis of host adaptation and lifestyle transition in *Plectosphaerella cucumerina*, a widespread root-colonizing fungus

Presenting Author - *Ram Sevak Raja Kumar, Max Planck Institute for Plant Breeding Research, Germany*

Author/s – *Stephane Hacquard, Tak Lee, Arpan Kumar Basak*

Abstract Content

Roots of asymptomatic plants engage in intimate associations with microbes that impact host performance. *Plectosphaerella cucumerina*, an ascomycete fungus often describe as a necrotrophic fungal pathogen, is one of the most prevalent and abundant fungal taxa that associate with roots of *Arabidopsis thaliana* in natural populations across Europe. This suggests that the pathogenic potential of *P. cucumerina* defined based on mono-association experiments with host plants might largely be kept at bay in a community context. Confocal microscopy reveals an endophytic colonization pattern of *P. cucumerina* in roots not only of *Arabidopsis* but also of tomato and barley. However, the molecular mechanisms driving its broad host range, ubiquitous distribution, and robust *A. thaliana* root colonization capabilities remain poorly known. This project aims at understanding host colonization strategies, genetic determinants driving adaptation to different hosts, and dominance in the root microbiome using transcriptomic and experimental evolutionary approaches. Results reveal that there is a unique fungal transcriptional response activated in response to the different plant species. Particularly, genes encoding carbohydrate active enzymes are strongly activated in response to all three-plant species and large subsets display host-specific activation that reflect plant cell wall compositions of the hosts. Experimental evolution of the fungus in response to the three-plant species has been performed and reciprocal inoculation revealed that the strain evolving on barley and microtom became less detrimental on *Arabidopsis*. Candidate genes identified based on these transcriptomic and evolutionary approaches are currently validated experimentally using forward genetics.

W166 - Livestock wastewater treatment and power generation using a semi-wet biocathode electrode microbial fuel cell

Presenting Author - *Chika Tada, Tohoku university, Japan*

Author/s – *Hiroto Nakano, Masaki Umetsu, Yuta Nakayasu, Koji Yokoyama, Hideyuki Takahashi, Chika Tada*

Abstract Content

As a cathode electrocatalyst for a microbial fuel cell (MFC), we investigated methanogenic biocathode electrodes, which utilize methanogens to receive electrons while converting CO2 to methane. We further developed a semi-wet biocathode electrode in which the electrode is placed in the gas phase rather than in water. In the semi-wet biocathode electrode MFC, methanogens grew and produced electrical power at the electrode, comprised of oak white charcoal in gel. However, those studies used artificial medium containing glucose as the carbon source in the anode solution.

In this study, actual cattle wastewater was continuously fed into the anode solution to investigate the capacity of the semi-wet biocathode electrode MFC to remove organic matter and generate electricity. The MFC fed on cattle wastewater had maximum power density of 2.4 mW/m2, which is higher than the value measured on artificial medium.

W167 - Global and local distribution of antibiotic resistance genes and associated phenotypes in marine bacterial communities

Presenting Author - *Javiera Ortiz-Severín, Universidad de Chile, Chile*

Author/s – *Iñaki Hojas, Pamela Aravena, Camila Stuardo, Mauricio González, Alejandro Maass, Verónica Cambiazo, Christian Hodar*

Abstract Content

The environment, other microorganisms, and anthropogenic factors such as aquaculture, influence marine bacterial communities. Here, we investigated the effect of salmon farming industry in the abundance of antibiotic resistance. Using CARD, antibiotic resistance genes (ARG) were predicted for two metagenomes from the prokaryotic fraction of superficial water samples collected in an intensive aquaculture zone (Q site) and in a pristine zone (P site) and compared with 83 publicly available metagenomes of superficial water samples. Regarding the public metagenomes, the most abundant drug families were betalactams, multidrug, tetracyclines and aminoglycosides. Using machine-learning methods, the parameters that best described the distribution of drug families in the metagenomes were temperature, oxygen, UV, NO3, carbon-flux and density (feature selection - Lasso regression). Although betalactams, multidrug and aminoglycosides were also the most abundant families for P and Q samples, rifamycins, diaminopyrimidines and phenicols were highly abundant exclusively in Q site. In addition, the Q and P samples were cultured in a battery of media and susceptibility tests were carried out with antibiotics used by the salmon industry (florfenicol, flumequine and oxytetracycline). Although a higher frequency of resistant colonies was measured in the Q site, ranging from 102 to 106 increase depending on the media, ARG abundance was higher in Q site only for florfenicol resistance genes, whereas ARG abundance for flumequine and oxytetracycline was higher in P site. This suggests that ARGs is not the only factor to evaluate antimicrobial resistant potential in an intervened bacterial community.

W169 - Reconstruction of physiological functions of marine flavobacterial transcriptomes with microbial rhodopsins

Presenting Author - *Yurim Bae, Gyeongsang National University, Korea, Republic of*

Author/s – *Lae-Geun Jang, Jihyun F. Kim, Soon-kyeong Kwon*

Abstract Content

Microbial rhodopsin is commonly found in aquatic microorganisms and enables them to survive in extreme environments simply by using light energy. Proteorhodopsin-bearing microorganisms such as Flavobacteriia play a pivotal role in the biogeochemical cycles of the ocean. Analysis of the genome sequences of the marine flavobacterium Nonlabens dokdonensis DSW-6 and other marine flavobacteria found a gene encoding a sodium-pumping rhodopsin (NaR) containing a unique motif. Previously known proteorhodposin (PR) has been studied to stimulate ATP synthesis by forming a proton gradient through its proton pumping function. NaR moves sodium out of the cell, but the relationship between the activity of this protein and bacteria has not been studied. Inferring physiological functions and related metabolic networks of sodium and proton-pumping rhodopsin in *N. dokdonensis* DSW-6. We generated rhodopsin knockout mutants and investigated the expression levels of rhodopsin and other genes by RNA sequencing when they survived nutrient limitation and salt stress in a light environment. A rhodopsin-specific KEGG metabolic pathway was inferred by comparing expression between the wild-type strain and the rhodopsin knockout strain. Energy metabolism pathways, including ATP production pathways consistent with previous studies, could be identified. Analysis of the rhodopsin mutation-related metabolic network allows us to infer a more precise relationship between rhodopsin and bacteria, which is helpful in understanding the physiological function of bacteria for survival in an oligotrophic marine environment.

W171 - Overlooked majority of groundwater bacteria in carbonate aquifers

Presenting Author - *Alisha Sharma, Friedrich Schiller University Jena, Germany*

Author/s – *Alisha Sharma, Martin Taubert, Olga Carasscal, Robert Lehmann, Thomas Ritschel, Kai Totsche, Assistant Prof. Dr. Cassandre S. Lazar, Prof. Dr. Kirsten Kuesel*

Abstract Content

Most investigations in groundwater ecosystems target planktonic microbes easily obtainable via water sampling. In contrast, little is known about microbes adhering to rock surfaces, which is especially true for carbonate rock-type aquifers.

To investigate bacterial attachment under conditions close to a pristine aquifer, we installed laboratory-scale bioreactors with rock material continuously flushed with groundwater from oxic and anoxic zones with Triassic limestone-mudstone alterations of the Hainich Critical Zone Exploratory. Half of the carbonate rock chips were coated with iron oxide, representing secondary mineralization observed on aquifer rocks.

Bacteria colonized the rock chips in both oxic and anoxic bioreactors within 2 days of incubation, reaching up to 2.5 x 103 cells/mm2. SEM analysis revealed patchy and non-homogenous cell attachment, with rock chips from oxic bioreactors being more densely covered than those from the anoxic bioreactors. Oxic bioreactors featured communities dominated by proteobacterial genera *Aquabacterium* and *Rhodoferax*, while *Rheinheimera* and *Simplicispira* were the key players of anoxic bioreactors. Interestingly, differences in community composition between bare and Fe-coated rock chips decreased towards the end in the oxic experiment, while being consistently low for anoxic experiment. Genes for motility, attachment, and biofilm formation were predicted in all these key players based on groundwater metagenome-assembled genomes or NCBI reference genomes. Based on the cell density observed on the rock chips and previously reported planktonic cell numbers in aquifers, we estimated conservative ratios of 200:1 (oxic) and 1:1 (anoxic) between attached and planktonic cells, indicating a high importance of the attached community for aquifer functioning.

W172 - Sampling and analyzing microbial aerobiome on intensive livestock farms by using microbial traps in drones

Presenting Author - *Marta Nerini, University of Florence, Italy*

Author/s – *Marta Nerini, Marco Merlini, Giuseppe Rossi, Elena Pilli, Massimiliano Marvasi*

Abstract Content

Dispersal of particulates of organic materials carrying microorganisms is commong in presence of large livestock production. Particulate can be released directly from the animals but can also result from feeding, manure storage and other animal management. Microorganisms, mainly enterobacteria, can expose workers to potential health risks, such as particulate PM2.5 and gases of various origins such as ammonia. To prevent diseases due to infections, zoonoses and inflammatory states, it is therefore essential to monitor the dispersion of microorganisms present in the air in the open environment, both in internal areas and in areas adjacent to intensive farming. The aim of the study is to develop a system of microbial traps to be equipped on a drone to sample the microbial aerobiome surrounding farms. Traps for microorganisms were developed: agar plates and petrolatum-coates plates were designed and tested, inserted in a drone with aspiration capability of 1000 L in a few minutes. We developed a DNA extraction method that allowed to extract and amplify very low amount of bacterial DNA, adapting a high sensitivity extraction kit dedicated to viral nucleic acids combined with the use of a specific lysis buffer. In addition, microbial communities were also analyzed using Ecoplate Biolog, resulting in comparative phenomics from a urbanized and a rural area, possibly correlated to the level of pollutants of the two sites. In future we aim to sequence the metagenomes through Next Generation Sequencing, to study the phylogenetic and taxonomic relationships of the microbial aerobioma sourrounding large livestock facilities.

W173 - Spatiotemporal variation in prokaryotic and eukaryotic microbial communities in an estuarine and coastal system

Presenting Author - *Spencer Long, University of Southampton, United Kingdom*

Author/s – *Phyllis Lam, Andy Rees*

Abstract Content

Estuarine and coastal ecosystems host some of the most diverse and productive microbial communities on Earth. Steep physiochemical gradients and various anthropogenic and natural factors are known to dictate the microbial ecology of these environments, yet the key drivers shaping prokaryotic and eukaryotic communities and how they interact with each other over spatiotemporal scales has not yet been fully explored. Here, over a 3-year period, we assess the physiochemical characteristics and variations in microbial community structure along the salinity gradient of the Tamar estuary, Plymouth, and the coastal waters of Western Channel Observatory station L4. Through 16S and 18S rRNA gene sequencing, we show that both prokaryotic and eukaryotic assemblages appear largely influenced by the same environmental variables, with both seasonal and spatial factors such as temperature, nutrient loading and salinity strongly dictating their makeup and diversity. Novel co-occurrence network analysis revealed important clusters of key taxa across both sites, with dominant prokaryotic families such as Rhodobacteraceae and Flavobacteriaceae (14.6% and 11.9% of prokaryotic reads, respectively) exhibiting high degrees of intra- family niche differentiation. Dinoflagellates (36.8% of eukaryotic reads) were found to be central hubs within protist communities, and prevalent spring blooming taxa such as Diatoms and Phaocystsis showed varying spatiotemporal patterns across both locations, matching with known bacterial specialists of algal organic matter degradation such as Verrucomicrobia. Altogether, this study increases our understanding of the drivers and links between prokaryotic and eukaryotic communities within coastal systems, offering fresh insights into what dictates their ecology within complex and dynamic waters.

W174 - Are we in trouble if we travel? – Epidemiological analysis on the impact of tourism on the spread of relevant *Escherichia coli*

Presenting Author - *Alan Ele a, Technische Universität Dresden, Germany*

Author/s – *Alan Elena, Degrâce Batantou, Mélanie Pimenta, Uli Klümper, Sebastien Breurec, Christophe Dagot, Thomas Berendonk*

Abstract Content

Bacterial infections caused by multidrug-resistant pathogens are among the most relevant threats to human health. As commercial travel becomes increasingly accessible, these pathogens regularly hitchhike across long distances within their human hosts. Hence, the impact of tourism on the dissemination of these microorganisms needs to be assessed.

Here, we sought to understand the impact of tourism on the spread of *E. coli*, with relevant epidemiological traits: antimicrobial resistance genes (ARGs), virulence genes (VGs) and replicons.

A total of 709 *E. coli* were isolated and whole-genome sequenced from a partly high-tourism impacted, island, with three different sampling continua defined: Hospitals, low-tourism and high-tourism region. The association of high-risk isolates to different continua was assessed by the comparison of features of epidemiological interest including ARGs, VGs, Replicons, Sequence-types and phylogroups.

ARG and replicon carriage per isolate in touristic locations was significantly higher compared to the non-touristic areas. Despite the lack of differences in the overall VGs per isolate across continua, a cluster of particularly virulent and genotypically diverse *E. coli* was detected. This group was mainly composed of isolates recovered in hospitals or the touristic region. The high-tourism and hospital related continua were the source of the widest clonal diversity and the highest number of high-risk clones.

Findings indicate that the spread of high-risk strains of multidrug-resistant and virulent pathogens is indeed facilitated by tourism, with a risk for the propagation of new highly successful clones introduced in regions with high touristic activities.

W175 - Enrichment of candidate phyla radiation bacteria in groundwater incubation experiment

Presenting Author - *Ekaterine Gabashvili, Friedich Schiller University Jena, Germany*

Author/s – *Kirsten Küsel, Martin Taubert*

Abstract Content

Recently discovered and widespread Candidate Phyla Radiation (CPR) depends metabolically on host microbial species. Consequently, only few isolates are currently available, and most CPR are lost once removed from their natural environment. Incubation experiments with groundwater, where CPR can constitute >50% of the microbiome, typically show drastic decreases in CPR abundance, as suitable incubation conditions and hosts are unknown. Here, we examined 16S rRNA bacterial gene amplicon datasets from 384 groundwater enrichments to determine how CPR diversity and abundance can be retained. These enrichments covered a wide range of conditions stimulating chemolithoautotrophy, methylotrophy or necromass degradation, partly spanning several years.

Following the initial decrease, an increase of CPR abundance was typically observed in batch incubations after several weeks, reaching 11-30%. As CPR usually have less 16S rRNA gene copies than other bacteria, their actual abundance might be even higher. Under conditions stimulating chemolithoautotrophs, *Cand. Parcubacteria* UBA9983 and *Cand. Jorgensenbacteria* showed highest abundances, while on organic carbon, *Cand. Kaiserbacteria* were more prevalent. Additionally, certain CPR were present independently of the enrichment approaches. ASV-based analysis demonstrated a correlation between the composition of the CPR community and the stimulated metabolic process independent of the presence of other bacteria, indicating that CPR bacteria may either contribute to these processes or be co-enriched with multiple host organisms that carry them out. Our research suggests that certain CPR have a broad host range and benefit from conditions where other microbes are starving, and might help to identify potential host partners and optimal conditions for their enrichment.

W176 - Root exudates regulate plant colonization by *Methylobacterium* spp. and *Methylorubrum extorquens*

Presenting Author - *Welington Araújo, University Of Sao Paulo, Brazil*

Author/s – *Maria Alejandra Mantilla-Galindo, Manuela Nóbrega Dourado-Ribeiro, Sarina Tsui*

Abstract Content

The genus *Methylobacterium* can establish mutualistic interaction with different plant species. For the establishment of interaction, chemical communication between plant and bacteria take place, in which the root exudates probably play a key role. In this study, we aimed to determine the role of soybean and corn seedling root exudates in the establishment of interaction with *Methylobacterium* spp. and *Methylorubrum extorquens*. Thus, we quantify three strains of *Methylobacterium* spp. (R16E,SR1.6/6 and MP2-3) and one strain of *Methylorubrum extorquens* (AR1.6/2), by qPCR, in un-sterile soil and in seedlings roots both when inoculated individually and in the consortium. In un-sterile soil, only the AR1.6/2 and MP2-3 strains survived in soil for up to 90 days when inoculated individually, while in the consortium only the MP2-3 strain survived for 90 days. Otherwise, in the interaction experiments the SR1.6/6 strain was the most abundant in soybean roots when inoculated individually but in consortium showed less abundance, suggesting that the competition with other strains present in the rhizosphere is necessary for root colonization. The analysis by GC-MS of root exudates indicated that the abundance of carbohydrates and amino acids increased in both soybean and corn root exudates during interaction with the bacteria, suggesting that plants respond in a specific way to each strain. Finally, we evaluated the influence of root exudates in the bacterial biofilm formation, a significant reduction in soybean exudate was observed, while corn exudates observed an induction on biofilm, suggesting that root exudates are involved in the regulation of biofilm formation.

W177 - Investigating the role bacteriophages in ARG dissemination

Presenting Author - *Praveen Kant, IIT BOMBAY, India*

Author/s – *Kiran Kondabagil*

Abstract Content

The contribution of bacteriophages in the dissemination of antimicrobial resistance genes (ARGs) has been debated over the last decade. Several questions regarding the ARG dissemination potential remain unanswered. For example, do phages have the ability to evolve ARGs de novo? Do phages acquire ARGs from their host? Are phages selective in acquiring the ARGs compared to other host genes? We identified the frequency of antimicrobial resistance proteins in phage proteome using CARD database. And checked for lysogeny markers both using BLASTN and manually. Using GC%, Tetranucleotide frequency-Chi2 index and codon usage we determine the horizontal transfer of ARGs as well as other bacterial genes for comparison. 90 out of 3401 phages RefSeq genomes were found to carry ARGs. All phage ARGs were found to be horizontally acquired. Based on the presence of lysogenic markers and nucleotide compositional difference of core phage genes with ARGs, we suggest that ARGs are acquired from the hosts by temperate phages via the specialized mode of transduction. The close association of the ARGs with lysogenic markers and mobile genetic elements also point towards specialized transduction as a predominant mechanism of acquisition of ARGs by phages. Furthermore, ARG acquisition by phages appears to be a chance event and confer no discernable advantage to phage. Although the frequency of acquisition of ARGs by phages is low, close association of phage ARGs with MGEs and constant evolution of phages suggests phages as an important component of ARG dissemination.

W178 - The microbiome composition of *Lobaria pulmonaria* L. Hoffm. differs between Tanzanian Kilimanjaro and Austrian Alps regions

Presenting Author - *Niclas Kuck, University of Greifswald, Germany*

Author/s – *Niclas Kuck, Anteneh T. Bogale, Daniela Zühlke, Jörg Bernhardt, Katharina Riedel, Mia M. Bengtsson, Maria Braun, Ulf Schiefelbein, Martin Grube*

Abstract Content

Lobaria pulmonaria is a lichen model organism that grows on tree trunks in forests across the northern and southern hemispheres. Apart from fungal and algal partners, *L. pulmonaria* has been shown to have variable and integrated bacterial communities (microbiome) whose functions and community compositions are less explored. Therefore, the aim of this study is to comparatively investigate the composition of the *L. pulmonaria* microbiome in two geographically distinct regions, Tanzanian Kilimanjaro, and Austrian Alps.

We employed 16S rRNA gene amplicon sequencing analysis in order to investigate the structure and community composition of the microbiome of *L. pulmonaria* of specimens from the two regions. We observed that Alphaproteobacteria are the most dominant phyla in both regions. Moreover, our NMDS analysis indicated that the two regions are different in microbiome community composition. However, differences were also observed among some of the Tanzanian Kilimanjaro sampling locations.

W180 - Spatial and temporal variation in freshwater bacterioplankton community structure in Lake Soyang

Presenting Author - *Suhyun Kim, Inha University, Korea, Republic of*

Author/s – *Ilnam Kang, Jang-Cheon Cho*

Abstract Content

Extensive research has been underway on taxonomic and functional analysis of microbial communities based on culture-free method to elucidate the key role of bacterioplankton in freshwater microbial ecosystems. Nevertheless, studies have been focused on specific water bodies or limited geographic areas, highlighting the need to collect data on microbial communities more diverse freshwater habitats in order to obtain a comprehensive picture of freshwater lake ecosystems worldwide. In this study, we investigated the bacterioplankton community composition in Lake Soyang, the deepest lake in South Korea, using high-throughput 16S rRNA gene amplicon sequencing and metagenomic sequencing. Sampling at a pelagic site for 24 months from five depths of the water column revealed that the bacterial community composition in Lake Soyang was influenced by seasonal changes, especially in surface water. Generally, Actinobacteria acI-A7 and B1 were the most dominant groups in bacterial community in in the whole water layer and all seasons, while potential specialists performing a specific role in lake preferred the hypolimnion habitat. At the hypolimnion layers, members of Chloroflexi and Planctomycetes (e.g., CL500-11 and CL500-3) were highly represented, with nitrifiers (e.g., *Nitrospira* and *Nitrosomonas*). In metagenomic analysis, these microbes were assumed to perform nitrifying and carbon degradation based on MAGs analysis. This study identified the microbial community patterns in Lake Soyang, suggesting that the biogeochemical cycling of this lake is driven by the microbial community that are different by spatial and temporal changes.

W181 - Machine learning for predicting biomethane production from microbial communities' composition in full-scale anaerobic digesters

Presenting Author - *Andreia F. Salvador, University of Minho, Portugal*

Author/s – *Georges F. R. Radohery, Giovanni Melandri, Sylvain Prigent, M. Alcina Pereira, Pierre Petriacq*

Abstract Content

Background: Conversion of waste to biomethane is performed by anaerobic communities composed by thousands of species. It is difficult to correlated microbial diversity to biomethane productivity in such complex environments, and to identify the most important microorganisms contributing for the process efficiency.

Objectives: The aim of this work is to predict biomethane production based on 16S rRNA gene sequencing data from anaerobic digesters, by using machine learning algorithms.

Methods: Sludge samples from five different full scale anaerobic digesters were taken for DNA extraction and 16S rRNA gene sequencing. Six time points were collected during a period of approximately one month. The microbial composition profiles were used to train machine learning models for predicting biogas production in the anaerobic digesters. Three different learning algorithms were applied, i.e., support vector machine with linear kernel (SVM), lasso and elastic-net regularized generalized linear models (GLMNET) and random forest (RF). Trained models were then used to identify the microbial species influencing most the biogas production.

Results: We trained three predictive models of biogas production from microbial diversity profiles with a coefficient of determination (R-squared) of 0.9 (95%CI 0.84,0.96), 0.88 (95%CI 0.78, 0.99) and 0.85 (95%CI 0.78,0.92). These models identified 54 microbial species that score at least 60 on a 0-100 importance scale for the biomethane production prediction. Among the microorganisms with higher importance score (IS), were syntrophic bacteria such as *Syntrophobacter* (IS=100 with both RF and SVM), *Syntrophomonas* (IS ranging from 76 to 79), *Pelotomaculum* (IS=77), and the filamentous methanogen *Methanosaeta* (IS=87).

W182 - Inoculation with arbuscular mycorrhizal fungi accelerates the degradation process of pesticides in maize planted soil

Presenting Author - *Anna Manukyan, University of Thessaly, Greece*

Author/s – *Kalliope Papadopoulou, Dimitrios Karpouzas, Panagiotis Karas, Carolin Schneider, Louis Mercy, Myrto Tsiknia*

Abstract Content

The use of Arbuscular mycorrhizal fungi (AMF) as biostimulants in agriculture is becoming a common practice in the sustainable production of horticultural crops and an alternative or addition to plant-protection products. Nevertheless, there is a poor understanding of the possible interaction between pesticides and these organisms in soils and how these affect plant growth.

The main objective of the present work is to investigate the effects of an AMF and pesticide application on the growth parameters of corn, interactions between the fungus and contaminants, and pesticide fate in the soil media. To test our hypothesis with an AM fungal inoculum (*Funneliformis mosseae*) and two pesticides with two application rates (Tembotrione and Pyraclostrobin) a greenhouse pot experiment was performed.

Introduced inoculum did not affect the plant growth in all the treatments, including the control group, although AMF colonization levels were significantly increased, especially at the later sampling point. On the other hand, the pesticides had a negative effect on plant growth, which was ameliorated by the end of the experiment, irrespective of the introduced inoculum. Pyraclostrobin in general increased the colonization percentage of AMF. For Tembotrione, at the recommended dose it had no significant adverse effects on the AM colonization in both natural and introduced AMF inoculum treatments. Mycorrhizal treatment influenced the dissipation of both pesticides at both application rates and the introduced AMF inoculum accelerated the degradation process of the pesticides (irrespective of application rates), indicating a promising potential of AM fungi in the degradation of contaminants in soils.

W183 - Development of database for oral microbial interactions networks (DOMINO)

Presenting Author - *Szymon Szafranski, Medizinische Hochschule Hannover, Germany*

Author/s – *Szymon Piotr Szafranski, Matthias Steglich, Rumjhum Mukherjee, Meike Stiesch*

Abstract Content

Background: Oral biofilms cause prevalent chronic infections, such as periodontal and peri-implant diseases. Physical and metabolic interspecies interactions are extremely common in oral biofilms and they strongly contribute to microbial succession. However, comprehensive descriptions for such interactions are limited.

Objectives: Creation of a database for interspecies interactions in human oral cavity, which meets the principles of findability, accessibility, interoperability, and reusability (FAIR). It is intended to be used for applications in microbiome research, microbial ecology, biomarker discovery and general education.

Methods: The graph Database for Oral Microbial Interactions Networks (DOMINO) was designed to contain and link several types of data: taxa (reference strains of model oral species, all human oral species including allochthonous organisms and higher taxa), enzymes, metabolites, drugs, interactions and references. The Neo4j database platform was used as the implementation base. The nodes were hyperlinked to other databases, such as eHOMD, HMDB, KEGG and PubMed. Information for microbial interactions was retrieved from literature or predicted based on microbial genomes and was manually curated.

Results: The current version of the database contains 4,000 nodes. Unexpected putative relationships between oral microorganisms were discovered, e.g. food chains.

Conclusions: DOMINO is a strong foundation for further expansion, including integration of next generation sequencing data and improving data visualization.

W185 - Diversity patterns of bacteriophages infecting species across clades and niches

Presenting Author - *Matthias Steglich, Medizinische Hochschule Hannover, Germany*

Author/s – *Muqing Niu, Andreas Winkel, Meike Stiesch, Szymon P. Szafranski*

Abstract Content

Background: *Veillonella* species are relevant human commensals and accessory pathogens. Consequently, their bacteriophages may have significant impact on human microbial ecology and pathologies.

Objectives: Uncovering the prevalence and diversity of bacteriophages infecting *Veillonella* species which colonize the human oral cavity.

Methods: Publicly available genome sequences for *Veillonella* strains and human oral metagenomes were retrieved. Genome mining was performed with PHASTER and VirSorter. Data from IMG/VR was integrated. Phage-specific PCRs were established (n = 50). Samples were taken from three different oral sites from 15 individuals. Phage diversity was characterized using PCRs.

Results: Genome mining with comparative genomics, screening of clinical isolates and samples, as well as profiling of metagenomes allowed characterization of fifteen major phage clusters, mostly represented by previously uncharacterized phages. Phage diversity patterns varied significantly for different phage types, host clades, and environmental niches. *Veillonella* phages were prevalent at multiple oral sites. Human tongue hosted the most abundant populations of these phages.

Conclusions: The results support the exploration of the eco-evolutionary forces shaping phage-host interactions in the human microbiome. Furthermore, putative lytic phages may provide new therapeutic options.

W190 - Temporal comparison of archaeal and bacterial communities in hypersaline mats

Presenting Author - *Alejandro Lopez-Cortes, Centro de Investigaciones Biologicas del Noroeste, Mexico*

Author/s – *Jose Q. Garcia-Maldonado, Patricia J. Ramirez-Arenas, Hever Latisnere-Barragan*

Abstract Content

Guerrero Negro hypersaline microbial mats are complex, stratified and highly diverse ecosystems, which have been studied for decades as a model system for microbial ecology and life on the early Earth. However, microbial community stability over the time has been not previously analyzed using next generation sequencing. In this study, temporal changes in the structure and composition of the Bacteria and Archaea in microbial mats from A4N5 and A5 of Exportadora de Sal, S.A. (ESSA) collected in 2019 and 2022, were analyzed by amplicon sequencing of the 16S rRNA gene. Moreover, taxonomic profiles were correlated to environmental parameters to determine their influence on the microbial structure. Bacterial and archaea diversity was similar among the sites. However, differences in community structure and composition were found for the two sites of 2019 and between the years. Samples from 2019 were dominated by Bacteroidia, Alphaproteobacteria, Cyanobacteriia and Spirochaetia, while 12 different archaeal classes were detected, being Nanoarchaeia, Thermoplasmata and Lokiarchaeia the better represented. Samples collected in 2022 were dominated by Gammaproteobacteria, *Desulfovibrionia*, Bacteroidia and *Desulfobacteria*, while Nanoarchaeia and Micrarchaeia were the archaeal taxa better represented from A4N5 and A5, respectively. PERMANOVA analysis revealed that pH and temperature were the environmental conditions better explaining the differences in community structure. This study expanded the understanding of the microbial diversity in hypersaline microbial mats and showed important changes occurring during two different years in ESSA.

W191 - The plant rhizosphere indigenous microbiota modulation and its application and benefits for sustainable agriculture technology

Presenting Author - *Raimonda Mažylytė, Vilnius University Life Sciences Centre, Lithuania*

Author/s – *Justina Kaziūnienė, Audrius Gegeckas*

Abstract Content

Plants in natural ecosystems can host a multitude of microorganisms in the rhizosphere or other tissue compartments, collectively known as the plant microbiota. Manipulation of the plant microbiota can reduce the incidence of plant disease, influence plant physiology, and increase agricultural production and development. The plant rhizosphere is a dynamic environment and the microbiota varies quickly over time and location, mounting data suggest that plants can manipulate the rhizosphere microbiota to their advantage and effectively make use of the microbial functional repertoire. In this study, three ecological farming fields were selected using the same crop rotation and tillage technology (in 2020, Zea mays were planted in agricultural lands; in 2021, *Phaseolus vulgaris* were planted in agricultural lands; and in 2022, Triticum aestivum was planted in agricultural lands). The rhizosphere samples were processed and analyzed with the Zymo-BIOMICS® targeted sequencing service (Zymo Research, Irvine, CA). Microbial composition of the rhizosphere soil samples showed that predominated phyla were Actinobacteria, Proteobacteria, Acidobacteria, Chloroflexi, and Firmicutes. Taxonomy abundance heatmaps with sample clustering and ASV heatmaps methods were allowed to identify the most dominant bacteria at the species level. Furthermore, using the Taxa2ASV decomposer analysis, a taxon of interest was decomposed into its unique amplicon sequences within the most abundant taxa identified in the samples and was organized by taxonomy classifications (family, genus, and species). Our analysis opens new avenues to understanding the complexities of rhizosphere soil microbiota changes and will serve to direct future microbiota research for enhancing soil ecosystem services and functionality.

W192 - Detection of faecal bacteria and antibiotic resistance genes in biofilms attached to plastics from human-impacted coastal area

Presenting Author - *Elisenda Balleste Pau, University of Barcelona, Spain*

Author/s – *Hongxia Liang, William de Haan, Cristina Garcia-Aljaro, Cerdà-Domenech Marc, Sanchez-Vidal Anna, Miriam Pascual-Benito, Paula De Castro-Fernández*

Abstract Content

Plastics act as a substrate to develop biofilms and they have been claimed as vectors of bacterial dispersal. We evaluated the presence of faecal and marine bacteria adhering to the biofilm developed on marine plastics and the abundance of antibiotic resistance genes (ARGs). Thus, we sampled two coastal waters strongly impacted by human faecal pollution (either from rivers and submarine sewage outfalls). Samples of water, sediments and plastics from surface waters and sediments were collected during two campaigns in summer and autumn 2021. The presence of the faecal bacterial and viral indicators, 3 ARGs, potential pathogens like *Pseudomonas* spp. and *Vibrio* spp. and other bacteria was analysed by culture and/or molecular methods.

Results indicate that plastics are covered by a bacterial biofilm mainly of bacteria including *Vibrio* species. Low concentration of viable *E. coli* and Enterococci (42% and 67% of the samples) was detected on floating plastics. The ARGs were detected in 67-88% of the surface plastic samples and 29-57% of sediment plastics with a concentration of up to 6.7x102 gc·mm-2. These results suggest plastic debris may have come from wastewater or been colonised in environments with faecal contamination. The presence of faecal indicators in sediment plastics was null or low. Therefore, although in low concentrations, faecal bacteria and species of *Pseudomonas* and *Vibrio* were identified in marine plastics, suggesting that plastic pollution may be a potential reservoir of human pathogens and ARGs. Considering the abundance of plastic in aquatic environments, its potential risk must be assessed.

W193 - Genomic and biogeographic analysis of the largest group of new crAssphages isolated so far

Presenting Author - *Maria Teresa Muniesa, University of Barcelona, Spain*

Author/s – *Clara Gomez-Gomez, Maria Dolores Ramos-Barbero, Laura Sala-Comorera, Lorena Rodríguez-Rubio, Sara Morales-Cortes, Gloria Vique , Anicet R. Blanch , Elisenda Ballesté , Cristina Garcia-Aljaro*

Abstract Content

CrAssphage was detected *in silico* and is one of the most abundant viruses in the human gut ad infects Bacteroides. Despite its abundance, to date only four virions have been isolated. Here, we report the isolation of new crAssphage virions from wastewater, using enrichment cultures of Bacteroides and successive propagations steps. Among the hosts tested, *B. intestinalis* showed the best results. CrAssphages in the propagated samples were analyzed by qPCR, those showing high numbers were used to isolate virions by plaque blot using a specific probe designed from the first crAssphage isolate (ΦcrAss001). We isolated up to 25 new crAssphage phages, all generating small turbid lytic plaques and showing burst sizes from 59-120 phages/ cell. All phages propagate similarly and reach titers of 109 viruses/ml while the culture does not clear during the propagation and the number of host cells remains constant, suggesting some balance between the crAssphages and the host.

The analysis of their genomes confirmed they are unique, virulent phages with genomes size between 75-103 Kb (101-114 ORFs) and a 35 % GC content. All the genomes studied harbor genes of the former order Caudovirales (Podoviruses). The 25 phages are similar but not identical, belong to the same genus as ΦCrAss001 and to seven species.

To explore the global occurrence of this new group of phages, a biogeographic study, comprising 1,255 human-gut viromes from 14 countries, showed that the new crAssphages are detected in 51 % of the viromes analyzed with a relative abundance higher than ΦCrAss001.

W194 - The predator *Myxococcus xanthus* upregulates the expression of genes to consume PHB granules of the prey *Sinorhizobium meliloti*

Presenting Author - *Jose Muñoz-Dorado, Universidad de Granada, Spain*

Author/s – *Lucía Cabello-Alemán, Francisco Javier Contreras-Moreno, Juana Pérez, Aurelio Moraleda-Muñoz*

Abstract Content

Background: *Myxococcus xanthus* is a soil-dwelling bacterium with ability to prey on several microorganisms, including eukaryotes. Although it is known that this predator utilizes several mechanisms to identify, kill and consume their prey, we are still far from thoroughly understanding its predatory behavior, especially because it uses different weapons against different prey. We have carried out a transcriptomic analysis during predation of *M. xanthus* on *Sinorhizobium meliloti*, and the results obtained have revealed that several genes upregulated during predation exhibit similarities to proteins that may be involved in consumption of the reservoirs of poli-β-hydroxybutyrate (PHB) accumulated in the prey.

Objective: We have focused on the characterization of these genes.

Methods: We have used a variety of techniques, including predation assays with mutants, analyses of gene expression by transcriptomic experiments and lacZ fusions, microscopy and bioinformatic analyses, among others.

Results: The results obtained have revealed that *M. xanthus* encodes an operon that is upregulated during predation on *S. meliloti* and in the presence of crystalline PHB and monomers of hydroxybutyrate. Among the genes contained in this operon we have identified a putative PHB depolymerase, a protein that resembles a porin, and a lipopoprotein located in the outer membrane, which seem to function as transporters, and two proteins related to lipid metabolism, that may be involved in the catabolism of the hydroxybutyrate. Moreover, we have identified a TetR-like repressor, which is responsible for the expression of the operon. More details will be presented at the meeting.

W195 - The *Sinorhizobium meliloti* defensome against *Myxococcus xanthus* predation

Presenting Author - *Juana Pérez, University Of Granada, Spain*

Author/s – *José Muñoz-Dorado, Aurelio Moraleda-Muñoz, Francisco Javier Contreras-Moreno, María José Soto*

Abstract Content

Background: *Myxococcus xanthus* is a soil bacterium that is able to prey on other bacteria sharing the same environment using an epibiotic multicellular hunting strategy. In our laboratory, it has been previously described that this myxobacterium is able to prey on *Sinorhizobium meliloti*, an agronomical and biotechnological important bacterium. During co-cultures of *M. xanthus* with several strains of *S. meliloti* the predator exhibits different predatory strategies depending on the presence of exopolysaccharide. In this interaction copper plays an important role, stimulating the prey biosynthesis of melanin to protect itself against predation. Recently we have elucidated the earlier global transcriptomic changes that the predator uses to attack this prey (predatosome).

Objective: The main goal of this study is to complete a global vision of the metabolic adaptations and strategies used by both bacteria in the first hours after contact by analyzing the transcriptomic changes that this prey undergoes as a consequence of the interaction (defensome), in which *M. xanthus* ends consuming the rhizobia.

Methods: Transcriptome has been obtained by RNA-seq technology.

Results: The global analysis showed an increase in protein synthesis and secretion and fatty acid (FA) biosynthesis. In contrast to what happens with the predator, FA degradation is down-regulated. However, and as observed with the predatosome, mechanisms involved in iron uptake are up-regulated, indicating a strong competition for this metal during interaction. The analysis also showed changes in the cell envelope and membrane and the up-regulation of genes involved in the activation of defense mechanisms and stress responses.

W196 - Co-cultivation of Mortierellaceae with *Pseudomonas helmanticensis* affects both their growth and volatilome

Presenting Author - *Maraike Probst, University of Innsbruck, Austria*

Author/s – *Anusha Telagathoti, Bianka Siewert, Iuliia Khomenko, Emanuela Betta, Franco Biasioli, Ursula Peintner*

Abstract Content – Volatile organic compounds (VOCs) might mediate microbial interactions, especially in spatially structured environments, such as soil. However, the variety and specificity of VOC production are poorly understood. Here, we studied 25 Mortierellaceae strains belonging to the genera Linnemannia and Entomortierella in both pure and co-culture with *Pseudomonas helmanticensis* under laboratory conditions. We analysed both the fungal growth depending on co-cultivation and the cultures' volatilomes applying proton-transfer-reaction time-of-flight and gas chromatography-mass spectrometry (PTR-ToF-MS and GC-MS). In a strain-specific manner, we found the fungi's radial growth rate and colony morphology affected by the presence of *P. helmanticensis*. The fungus seemed to generally reduce the bacterial growth. The volatilomes of the fungal and bacterial pure and co-cultures were diverse. While the fungi frequently consumed VOCs, *P. helmanticensis* produced a higher diversity and amount of VOCs than any fungal strain. Our results support that both the pure and co-culture volatilomes are taxonomically conserved. Taken together, our data supports the relevance of VOCs in Mortierellaceae-*P. helmanticensis* interaction. We also discuss individual VOCs that appear relevant in the interaction.

W197 - Ecotypes of extremely acidophilic bacteria of the Acidithiobacillia class along a natural pH gradient

Presenting Author - *Raquel Quatrini, Universidad San Sebastián, Chile*

Author/s – *Francisco Issotta, Camila Rojas-Villalobos, Ana Moya-Beltrán, Dilanaz Arisan, Juan Carlos Duarte, Raquel Quatrini*

Abstract Content

Rio Agrio (RA) is a natural extreme acidic watercourse located in the Patagonic Andes. Volcanic and glacial in origin, this river features an ample gradient of pH, temperature, and conductivity. Different types of acidophiles inhabit its waters, including representatives of the Acidithiobacillia class. In this study, we investigated the occurrence, distribution, and diversity of Acidithiobacillia linages along this natural gradient, to improve our understanding on their ecology. For this, we recovered genomes from culturable isolates and MAGs from metagenomes sampled along RA. De novo and reference-based binning were applied to the datasets to recover population-specific reads, using both local and global reference genomes and MAGs. This allowed us to assess population structure and diversity along the gradient. Results obtained showed that the acidithiobacilli, as a group, are highly abundant in the water column at RA, being readily detectable in the total community DNA of the sites along Upper RA, and also at the springs that source RA, yet rarely further downstream. Up to 10 lineages of the class co-exist at these sites, which is rather unusual and represents a unique opportunity to understand the ecological interactions between species of this taxon. Some of the lineages studied had differential distribution and relative abundances per site. Other lineages, showing ample and even distributions across sites, showed clear evidence of local diversification. These results indicate that some of the species of Acidithiobacillia have ecotypes which partition differentially along URA and that characteristics of the habitat play a relevant role in defining their distribution.

W198 - Antagonistic effects of rhodococci on exposure to pharmaceutical pollutants

Presenting Author - *Elena Tyumina, Perm Federal Research Center Of The Ural Branch Of The Russian Academy Of Sciences, Russian Federation*

Author/s – *Elena Tyumina, Grigory Bazhutin, Irina Ivshina*

Abstract Content

Background: Population growth, urbanization, and the emergence and spread of new diseases have inevitably led to an increase in the production and consumption of pharmaceuticals, elevating the risk of environmental exposure. Active pharmaceutical ingredients entering the environment have a negative impact on invertebrates, vertebrates, microbiota, and plants, accumulate in food chains, and disrupt the structure and functioning of ecosystems. Rhodococcus spp. are among the microorganisms that carry out the natural self-purification processes from xenobiotics and have the highest diversity of degradable contaminants and a broad range of adaptability.

Objectives: Investigation of the features of the interaction of rhodococci with pharmaceutical pollutants, the study of stress reactions of rhodococci, and triggered adaptive mechanisms in response to separate and combined exposure to pharmaceutical pollutants.

Methods. We used light, atomic force, confocal laser scanning, and transmission electron microscopies. The antioxidant enzyme activity in rhodococci was measured spectrophotometrically. The Illumina platform was used to study the rhodococci genome structures.

Results. On the example of ibuprofen, naproxen, diclofenac, ketoprofen and meloxicam—non-steroidal anti-inflammatory drugs ubiquitously detected in the environment—stress reactions of rhodococci under individual and combined exposure to the pharmaceutical pollutants were revealed. A catalog of genes encoding enzymes for the initial steps of ibuprofen, ketoprofen, and diclofenac oxidation was compiled using high-throughput next-generation sequencing in conjunction with bioinformatics analysis. The information obtained is crucial to understand the mechanisms that shield native microbiota from pharmaceutical contaminants' adverse effects and to develop efficient techniques for eliminating them from aquatic and terrestrial environments.

W199 - Impact of amoxicillin therapy on pre-weaned calves intestinal microbiota and its resistome

Presenting Author - *Agnese Lupo, French Agency For Food Environmental And Occupational Health & Safety (ANSES), France*

Author/s – *Thibault Destanque, Tony Rochegue, Joanna Fourquet, Marisa Haenni, Jean-Yves Madec, Claire Hoede*

Abstract Content

Background: Amoxicillin is largely used in human and veterinary medicine. Its impact on the microbiota composition and selection of antibiotic resistance genes (ARGs) has been investigated in humans, but similar studies in animals are lacking.

Objectives: Analyzing the impact of amoxicillin therapy on the intestinal microbiota of calves and its resistome.

Methods: Feces were collected from three calves receiving intramuscular amoxicillin (to treat non-intestinal infection) before (T0), at the end (T1), one week after (T2) the treatment and from three healthy calves, not receiving amoxicillin. After DNA extraction, shotgun sequencing was performed using NovaSeq (Illumina). MetaGWGS pipeline v.2.2 (https://forgemia.inra.fr/genotoul-bioinfo/metagwgs) was used for the analysis. ARGs (n=44) were quantified by qPCR with hydrolytic probes.

Results: At T0, Actinobacteria, Bacteroidetes and Firmicutes were the most abundant phyla in both treated and untreated pre-weaned calves. At T1, Bacteroidetes and Proteobacteria raised in treated calves along with genera *Bacteroides*, *Odoribacter*, and *Escherichia*, hosting potentially pathogenic species. Furthermore, during T0-T2, species' alpha diversity stagnated in treated calves, whereas it increased in untreated ones. ARGs (blaTEM, tetA, strA and strB) raised in treated calves at T1 and remained more abundant at T2 than T0.

Amoxicillin affects the composition of pre-weaned calves' microbiota and selects ARGs. The stagnation of alpha diversity in pre-weaned, treated calves suggests a delay in microbiota maturation with unknown consequences for the host health. Standardization of procedures/data analysis are necessary for precisely comparing the impact of different antibiotic therapies, which could help improving antibiotics usage preserving microbiota health and mitigating the resistance crisis.

W200 - The phyllosphere on the gradient of environmental to host-associated microbiome

Presenting Author - *Wenke Smets, University of Antwerp, Belgium*

Author/s – *Mason Chock, Corinne Walsh, Caihong Vanderburgh, Steven Lindow, Noah Fierer, Britt Koskella*

Abstract Content

Leaves harbor distinct microbial communities that can have an important impact on plant health and microbial ecosystems world-wide. Nevertheless, the ecological processes that shape the composition of leaf microbial communities remain unclear, with previous studies reporting contradictory results regarding the importance of bacterial dispersal versus host selection. This discrepancy could be driven in part because leaf microbiome studies typically consider the upper and lower leaf surfaces as a single entity despite these habitats possessing considerable anatomical differences. We characterized the composition of bacterial phyllosphere communities from the upper and lower leaf surfaces across 24 plant species. Leaf surface pH and stomatal density were found to shape phyllosphere community composition, and the underside of leaves had lower richness and higher abundances of core community members than upper leaf surfaces. We found fewer endemic bacteria on the upper leaf surfaces, suggesting that dispersal is more important in shaping these communities, with host selection being a more important force in microbiome assembly on lower leaf surfaces. Our study illustrates how changing the scale in which we observe microbial communities can impact our ability to resolve and predict microbial community assembly patterns on leaf surfaces.

W201 - A study on molecular characterization of resistance determinants & mobile genetic elements in MDR bacteria from natural water

Presenting Author - *Insha Sultan, Jamia Hamdard University, Department of Biochemistry, School of Chemical and Life Sciences, India*

Abstract Content

Background: Antibiotic resistance is a global concern. The expansion of resistance determinants through horizontal transfer is linked with mobile genetic elements (MGEs). Heavy metals also create consequential health hazards. Metal resistance along with antibiotic resistance genes (ARGs) and MGEs facilitates bacteria to gain resistance

Objective: The present work was carried to study ARGs CTX-M, AmpC, qnrS, MGEs like ISecp1, TN3, TN21, Int I from Wular and Dal lakes of Kashmir; India. The genetic environment of CTX-M-15, in-silico docking and mutational studies were performed. Co-occurrence of ARGs and HMRGs, PCR based replicon typing (PBRT) and conjugation assay was also done

Methods: Collected isolates were screened for ESBL production following CLSI. Molecular characterization of resistance genes and Genetic environment was performed using PCR, cassette PCR, and sequencing. Plasmid incompatibility was determined by PBRT

Results: Out of 201 isolates from 16 locations 33 were ESBLs producers. 30 ESBL isolates were positive for CTX-M gene, followed by AmpC (17), qnrS (13), ISecp1 (15), TN3 (11), TN21 (11), Int I (18), SulI (14). Genetic environment of blaCTX-M-15 was observed as (ISEcp1-blaCTX-M-15-orf477), classical promoter -10 TACAAT and -35 TTGAA was found at the 3' region. CTX-M-15-ISEcp1 (R299L) docking and mutation showed reduction in hydrogen bonds. Co-occurrence of antibiotics and HMRGs (mer, sil and ars) was found in 18, 14 and 8 isolates. PBRT showed Inc groups (B/O, F, 11, HI1, FIA, HI2, N, FIB, L/M). Molecular analysis of transconjugants showed transfer of ARGs, MGEs and HMRGs in *E. coli* J53 AZ R strain.

W202 - Importance of microbial screening for the conservation of cultural heritage

Presenting Author - *Tereza Branysova, University of Chemistry and Technology, Czech Republic*

Author/s – *Nikola Lisa, Nicole Petru, Michal Durovic, Katerina Demnerova, Hana Stiborova*

Abstract Content

Cultural heritage objects carry valuable information from our history. Unfortunately, they are commonly subject to biodeterioration, i.e. unwanted changes caused by the activities of organisms, primarily microorganisms. This process causes irreversible damage to cultural heritage objects and, thus, incalculable losses of information. To tackle this issue, our study addressed not only the contamination of audio-visual materials but also microbial contamination of the air, as it is known to have a significant impact on the biodeterioration of cultural heritage objects. To gain a comprehensive knowledge of biodeterioration, two approaches, culture-dependent and culture-independent, were used for microbial screening. To conduct the culture-dependent approach, four distinct media were used to capture microscopic fungi, and an additional four media were used to capture bacteria. The isolates were then identified using MALDI-TOF MS and, if necessary, Sanger sequencing. To conduct the culture-independent approach, DNA from polyurethane sponges (swabs) and PTFE membranes (air) were isolated. The amplicons for Illumina MiSeq sequencing were prepared using two-step PCR with specific primers for 16S gene and ITS region. The obtained sequences were assigned to microbial species, and the data were statistically evaluated. The results from both approaches were compared, and factors that may influence the presence of microorganisms on the materials, such as the composition of audio-visual materials or type of audio-visual materials, were tested. This work provides a comprehensive understanding of the microorganisms present on audio-visual materials and will help design targeted disinfection or prevention methods.

W203 - Microbial diversity as a barrier to antimicrobial resistance: A Pan-European study

Presenting Author - *Uli Klümper, Technische Universität Dresden, Germany*

Author/s – *Giulia Gionchetta, Elisa Catao, Helmut Bürgmann, Christophe Merlin, Thomas U. Berendonk*

Abstract Content

During the last century environmental microbiomes have constantly been subjected to invasion by antimicrobial resistant bacteria (ARB) and their associated resistance genes (ARGs). For an invasion event to be successful the invader has to overcome the biotic resilience of the habitat, which is becoming more difficult with increasing biodiversity. The capacity to exploit resources in a given habitat is enhanced when communities exhibit greater diversity, which reduces opportunities for invaders, leading to lower persistence.

In the context of AMR dissemination, ARB reaching a natural community may thus persist longer when biodiversity of the indigenous community is low, hence increasing the chance of ARG transfer to community members. Reciprocally, high microbial diversity could serve as a long-term barrier towards invasion by ARB and ARGs.

To test this hypothesis, a sampling campaign across seven European countries was carried out to obtain >200 environmental samples of low-impact origin. Samples were taken from contrasting environments: static, structured forest soils, or dynamic river sediments. Microbial diversity and abundance of 36 ARGs were determined.

In soils higher diversity, evenness and richness were all significantly negatively correlated with the relative abundance of the majority (>85%) of ARGs. Furthermore, the number of detected ARGs per sample was inversely correlated with diversity. However, no such effects were detected for dynamic, regularly mixed river sediments. In conclusion, we demonstrate that diversity can indeed serve as the proposed barrier towards AMR in the environment, with this effect mainly realized in static, structured environments, where long-term, diversity-based resilience against invasion can evolve.

W204 - Nitrogen recycling in microbial communities in a high altitude hydrothermal system

Presenting Author - *Coral Pardo, Universidad Católica Del Norte, Chile*

Author/s – *Pablo Paquis, Vilma Pérez, July Florez, Claudio Quevedo, Sara Cuadros, Martha Hengst*

Abstract Content

The nitrogen cycle is fundamental to sustain life as we know it, however the agricultural industry have pushed it beyond sustainability, and technological fixes will continue to be limited by our understanding of the underlaying microbiology. Moreover, high-altitude hydrothermal systems are natural laboratories occurring under a plethora of extreme physicochemical parameters, that allow to study the adaptations of microbial life to thrive in such conditions. Lirima is a poly-extremophile ecosystem comprising high-altitude hydrothermal ponds that harbor oligotrophic conditions, where other life forms are scarce, therefore microorganisms must be responsible to sustain critical geochemical cycles. Thus, we aimed to determine the metabolic potential associated to nitrogen cycling of the microbial community that inhabits this poly-extreme environment. We sampled three ponds with 42, 53 and 72 °C of water temperature, with high concentrations of metal(oids), especially in the hotter pond. Moreover, associated microbial communities have low diversity (ranging from 13 to 27 species), and all were dominated by bacteria belonging to the *Thermus* genus, with 1-16% of presence of Archaeal taxa. Analyses of the metagenomes reveals that the pathways associated with nitrogen fixation are absent in this community, therefore additional and potential novel nitrogen recycling mechanism must be in place in order to sustain basic life functions. Also, dissimilatory nitrate reduction and nitrite reduction pathways were enriched, while nitric oxide reduction was absent on the hotter pond where nitrous-oxide reduction was prevalent. These adaptations can have an important biotechnological impact given the novelty associated with these ancient, extremophile, pristine environment.

W205 - Effect of rice straw amendments in rice postharvest soil on the CH4 emissions and soil microbial community

Presenting Author - *Marc Viñas, Institute Of Agrifood Research and Technology (IRTA), Spain*

Author/s – *Mar Carreras, Belén Fernández, Miriam Guivernau, Cristy Medina, Mar Catalá, Yolanda Lucas, Joan Noguerol, Carles Alcaraz*

Abstract Content

Background: rice paddy fields are agrosystems representing an important source of anthropogenic methane emissions. Common rice straw incorporation into the soil after the harvest in a flooded fallow season, causes the highest peak of CH4 emissions.

Objectives: we hypothesize that an increase of straw loading into paddy soils, boost CH4 emissions and shifted soil microbiota, affecting the potential strategies of C utilization and methanogenesis.

Methods and Results: *in-vitro* 60d. slurry anaerobic experiments with different straw content in soil (0-0,35-2% w/w) revealed positive relation of CH4 emission with straw content, and with inorganic nutrient amendment. Interestingly, the CH4 accumulated in 0,35% straw assay were in the same range than detected at field scale during the fallow season in a previous study conducted in the Ebro Delta (Martínez-Eixarch et al., 2018). 16S-metabarcoding and 16S rRNA/mcrA assesmentn revealed an important effect of straw on soil microbiota. After 29 days of incubation, the addition of 2% straw caused a clear depletion of alpha diversity of bacteria (H: 7,2 (t0) to 6,5), and also a beta-diversity differentiation and growth of total bacteria and archaeal communities. An important relative decrease of Proteobacteria, Actinobacteria, Chloroflexi, and Armatimonadetes phyla, and *Methanosaeta* was revealed at 2% straw. Contrary, an increase of Bacteroidetes, and Oxalabacteriaceae, and methanogens such as *Methanosarcina*, and *Methanocella* were also depicted.

Conclusions: the amount of straw incorporated into the soil increases CH4 emissions and also modulates the biodiversity of bacteria and archaeal communities.

W207 - Date palm root and soil fungal communities are distinct as well as connected and impacted by irrigation water

Presenting Author - *Sunil Mundra, United Arab Emirates University, United Arab Emirates*

Author/s – *Sunil Mundra, Subha Chandran, Dinesh Sanka Loganathachetti*

Abstract Content

Date palms (*Phoneix dactylifera*) are widely cultivated in arid agroecosystems, where knowledge of irrigation water source effect on below-ground fungal communities is limited. We studied soil and root-associated fungal (RAF) communities of date palms under different irrigation regimes (freshwater vs saline groundwater) using ITS2 metabarcoding. Compared to the soil, RAF diversity was lower and communities were distinct. Co-occurrence analysis showed a relatively complex and connective (average degree, clustering coefficient and density) as well as highly co-occurring RAF community compared to soil. The RAF and soil fungal communities were also distinct between irrigation water sources; wherein water pH and electrical conductivity (EC) were the major structure factors, while soil pH and EC chemistry were additional factors in soil. Drift (stochastic) was the dominant process in both root and soil under saline groundwater irrigation and its relative importance was higher in root than soil. Saline groundwater irrigation enriched the abundance of specific saprotrophic genera in root (*Acrocalymma*, *Coprinopsis* and *Myrothecium*) and soil (*Chaetomium* and *Preussia*) compartments. In addition, the abundance of saprotrophs was higher in roots under saline groundwater irrigation, while the opposite pattern was observed in soil. Taken together, we show that the RAF communities are complex and connected; saline groundwater irrigation distinctly alters fungal communities in root and soil and select specific fungal community suitable for promoting host growth under extreme conditions of saline agroecosystems.

W208 - Microbial biotransformation of antimony in an urban environment

Presenting Author - *Claire Da Costa, Université Paris-Saclay, France*

Abstract Content

Antimony (Sb) is a toxic metalloid mostly found as a co-contaminant of arsenic in mining sites. However, its growing anthropogenic use (e.g. flame retardants, ammunition, paint pigments, brake pads) results in increased contamination of urban areas due to waste incineration, material erosion and road traffic. In addition to abiotic transformations of Sb, microorganisms are expected to actively participate in Sb speciation in receiving ecosystems, thus influencing its mobility, toxicity and bioavailability. In this context, we characterized microbial communities thriving in a highway stormwater pond system with elevated Sb concentrations relative to the geochemical background. In sediment collected across the pond, 16S rRNA amplicon sequencing revealed changes in the composition of total and metabolically active microbial communities along with shifts in the concentration and speciation of Sb. Specifically, changes in proportion of active bacterial genera potentially involved in Sb(III) oxidation (*Thiobacillus*, *Hydrogenophaga*, *Bradyrhizobium*) were observed across the samples. In addition, an enrichment in Sb-reducers (*Dechloromonas*) was reported in sediments where Sb(III) was predominant. Moreover, a shotgun metagenomic analysis indicated that microbial genes involved in Sb oxidation (e.g. aioA, arsH, arsV) were present across the ponds. In contrast, genes involved in reduction reactions (e.g. anrA, arrA) were mostly reported in sediments immersed under reducing conditions. Although poorly characterized in environmental samples, the anoA gene, encoding a Sb-specific oxidase, was also detected in the sediments. Therefore, a set of universal primers was designed to quantify this gene for which only strain-specific primers were available so far.

W209 - Microbial live interactions with textiles

Presenting Author - *Vukašin Janković, Institute Of Molecular Genetics And Genetic Engineering, University Of Belgrade, Serbia*

Author/s – *Jasmina Nikodinovic-Runic, Tatjana Ilic-Tomic, Milena Stevanovic, Marija Nenadovic*

Abstract Content

Microorganisms, especially soil-dwelling *Streptomyces*, have the potential to both degrade and colour a variety of textiles. Pigments from Streptomycetes could serve as colouring agents for different natural and synthetic fabrics. Apart from pigments, *Streptomyces* can produce a variety of enzymes. Several of these enzymes show favourable application in the depolymerization of synthetic materials such as polyamide and polyurethane.

The aim of this study was the assessment of live interactions of pigmented *Streptomyces* strains from the lab collection using polyamide (PA) and Polyamide/Elastane (PA/EA) knits as substrates.

Cultivation of pigment-producing *Streptomyces* strains was done following the standard microbiological protocols, using two different growth media with the addition of PA and PA/EA knits into flasks. Cultures were incubated at 30°C for 7 and 14 days under static and dynamic conditions. Materials were recovered and their colour coordinates, colour difference (ΔE), and fastness were determined, and their surface changes were examined by Scanning Electron Microscopy (SEM).

The incubation of knits with living bacterial cultures resulted in both live dyeing and degradation, depending on the strain used. The intensity of color yield was larger under dynamic culture conditions. Therefore, *Streptomyces* strains could be successfully applied in the development of greener dyeing and degradation bioprocesses.

W210 - Expression of PET-hydrolyzing enzymes in *Streptomyces* spp.

Presenting Author - *Milena Stevanovic, Institute Of Molecular Genetics And Genetic Engineering, University Of Belgrade, Serbia*

Author/s – *Brana Pantelic, Vukasin Jankovic, Jasmina Nikodinovic-Runic, Sandra Vojnovic*

Abstract Content

Plastic waste has become a serious global challenge that calls for sustainable solutions and requires rapid actions. Biocatalysis could present an adequate answer to this problem by providing different enzymes capable of degrading plastic polymers. *Streptomyces* strains as predominant soil inhabitants have also adapted to the presence of variety of plastic waste in natural environments, so they have been examined for the plastic degrading capabilities.

The aim of this work was to improve the biocatalytic properties of *Streptomyces* strains for their use in biodegradation of plastic polymers and develop a system for heterologous expression of polyethylene therephtalate (PET) degrading enzymes in *Streptomyces* spp.

Well studied *Streptomyces* lividans TK24 and S. albus NRRL B-1335, as well as two newly isolated Streptomycetes were used for expression of benchmark PETases and cutinases. Enzymes were cloned into pGM1202 *Escherichia coli–Streptomyces* shuttle vector and subsequently introduced into *Streptomyces* hosts either by polyethylene glycol-mediated protoplasts transformation or by electroporation. Cell-free extracts and supernatants of transformed cells were tested on different plastics using bis(2-hydroxyethyl) terephthalate (BHET), polycaprolactone (PCL) and Impranil as substrates in plate assays.

Expression of leaf-branch compost cutinase in *S. albus* and *S. lividans* resulted in an 8.5- and 2.5-times increase in esterase activities, respectively. Introduction of the enzyme into newly isolated strains that already showed some plastic degrading activity resulted in synergistic activity of the recombinant strains.

W211 - Evolution of microbial community dynamics during field retting of hemp "*Cannabis Sativa L.*"

Presenting Author - *Eliane Bou orm, IMT Mines Alès, France*

Author/s – *Eliane Bou Orm, Anne Bergeret, Suvajit Mukherjee, Sandrine Bayle, Jean-Charles Benezet, Luc Malhautier, Sébastien Grec*

Abstract Content

Hemp is a bast fibre plant from which lignocellulosic fibres with interesting mechanical properties can be used for industrial purposes (textiles, biocomposites). Field retting of hemp stems is a bioprocess in which the stem cell wall polysaccharide network is degraded by a complex microbial consortium to facilitate further extraction of fibres. So far, this process is carried out by farmers in an empirical way and depends strongly on pedoclimatic conditions, which makes difficult the control of its efficiency.

The aim of this work is then to unravel the biodiversity-ecosystem function relationship for a better control of this ecosystem. Retting is as a result examined by exploring the temporal dynamics of the microbial communities by considering four characteristic ecological indicators (density, diversity, structure, and activity). On one hand, a DNA metabarcoding approach and enzymatic activities measurements are performed during the retting process. On the other hand, the stem and extracted fibres color, the fibre biochemical composition and the stem microstructure are evaluated.

Results show that the first two weeks of retting are characterized by intense microbial colonization and significant biochemical changes in the composition of the stem cell wall (degradation of lipophilic extracts and increase in cellulose content), leading to progressive decohesion of the fibre bundles confirmed by microscopic observations. Furthermore, this study provides the first thorough description of the hemp field retting microbiome. The relationship between the microbial communities involved in retting and intrinsic characteristics of fibres are examined to provide recommendations and perspectives on possible improvements in retting management.

W212 - Bacteria chemotactic motion in a microfluidic channel: Influence of the surface

Presenting Author - *Asma Braham, University of Lyon, France*

Author/s – *Laurence Lemelle, Romain Ducasse, Eleonore Mottin, Vincent Calvez, Christophe Place*

Abstract Content

Bacteria travel long distances using a flagellar bundle. When a motile bacterium approaches a surface, hydrodynamic interactions result in a directed circular motion of its trajectory. However, the trajectory of a bacterium is marked by reorientations and stops that are the result of a change in the direction of rotation of the bundle of flagella. This change of direction occurs periodically, about every 1s, for 100ms for the bacterium *E. coli*. This random reorientation allows bacteria to increase its effective diffusion near a surface to explore their environment.

Bacterial chemotaxis, which takes advantage of a frequency bias in reorientation, allows the redistribution of a bacterial population to avoid toxic environment and go toward preferential regions. We study the effects of known chemorepellent substances such as Ni2+ cations on bacterial redistribution. For this purpose, we conduct experiments on bacteria motility in dark field video microscopy by varying the condition of chemical agents in microfluidic channels.

An asymmetric wave was generated by a chemorepulsive diffusion profile of Ni2+. Individual bacteria biasing their motion into the direction of propagation was observed. A minimal model coupling the diffusion of Ni2+ and the chemotactically-enhanced displacement of bacteria reproduces the wave's asymmetrical shape together with the average speed of the wave.

Near the surface, the behavior of the bacteria is modified by the local hydrodynamics. This change is investigated and substantiates an environmental colonization, mainly driven by the wave collective motion in the bulk, but developing its own characteristics on the surface.

W214 - Molecular Epidemiology and Antimicrobial Resistance determination by Whole-genome Sequencing of *Clostridioides difficile* from En

Presenting Author - *Khald Blau, University Of Applied Sciences Emden/leer, Germany*

Author/s – *Judith Hoellwarth, Fabian Berger, Alexander Mellmann, Claudia Gallert*

Abstract Content

Clostridioides difficile is the most common pathogen causing antimicrobial-associated diarrhea in humans and some animal species, but can be present also in various environments outside healthcare institutions. Thus, the objective was to investigate the prevalence and the molecular characteristics of toxin genes, antimicrobial resistance, mobile genetic elements (MGEs), and ribotype (RT) diversity of *C. difficile* isolated from environmental samples (e.g., sewage, sewage sludge and calf feces) which collected from Ostfriesland, Germany. *C. difficile* spores were recovered after selective enrichment and PCR-ribotyping of obtained strains was performed. Whole genome sequencing (WGS) was used to determine core genome multilocus sequence typing (cgMLST), toxin-encoding genes, antimicrobial resistance (AMR) genes, and MGEs. Antimicrobial susceptibility of vancomycin, metronidazole, moxifloxacin, tetracycline, clindamycin, ciprofloxacin, clarithromycin, and rifampicin was determined by E-test or disk diffusion method.

Out of 169 *C. difficile* isolates, 150 (88.75%) were toxigenic. The most common RTs were RT127, RT126, RT001, and RT078 in wastewater samples, faeces of calves, and digested sludge-amended soils. All isolates were susceptible to vancomycin and metronidazole, whereas a considerable number of isolates were resistant to clindamycin, fluoroquinolones, rifampicin, and macrolides. Thirty sequence types (STs) were identified, the most common being ST11, ST2, ST3, and ST109. The majority of analysed genomes belonged to MLSTs of clades 1 and 5. Many strains carried AMR genes, [aac(6')-Ie-aph(2'')]-Ia, ant(6)-Ib, aph(3')-IIIa-sat4-ant(6)-Ia cluster, tetM, gyrA and/or gyrB, or ermB, conferring resistance to aminoglycoside, tetracycline, fluoroquinolones, or macrolide-lincomycin-streptogramin B (MLSB) antibiotics. Some of AMR genes were associated with conjugative or mobilizable transposons, Tn6215, Tn916 or Tn5397.

W215 - Soil fungi and soil organic carbon stocks in Arenosol profile on Scots pine stand

Presenting Author - *Diana Sivojiene, Lithuanian Research Centre for Agriculture and Forestry, Institute of Horticulture, Lithuania*

Author/s – *Jelena Ankuda, Kęstutis Armolaitis, Audrius Jakutis, Donata Drapanauskaitė, Jūratė Aleinikovienė, Leho Tedersoo, Vladimir Mikryukov*

Abstract Content

The global warming is currently very relevant around the world. Soils, especially in the forests, are one of the most biologically diverse ecosystems and have a high potential for significant carbon sequestration. Therefore, it is important to comprehensively study soil organic carbon, soil microorganisms, and other parameters at different soil depths of forest soils. The main task of this study was to investigate microbial diversity and abundance, and soil chemical parameters at various depths of Arenosol in a 60-year-old Scots pine (*Pinus sylvestris L.*) stand (southwestern Lithuania) with a focus on main groups of soil fungi, on soil pHCaCl2, organic carbon (SOC) and total nitrogen (STN) contents. Soil samples were collected from 7 different 5 cm thick soil layers up to a depth of 200 cm. eDNA extracted from these samples was amplified. The PCR products were sequenced using PacBio third generation sequencing platform Sequel instrument. Soil pHCaCl2 showed a significant increase with soil depth, in particular the highest concentrations of SOC and STN were observed in forest floor, and these significantly decreased down in the soil profiles. The most abundant phyla in all soil depths were Ascomycota and Basidiomycota, which together accounted for more than 90% of fungi sequence reads. An increase in the abundance of saprotrophs, that dominated at all soil depths, was observed at soil depth 10 - 200 cm. The abundance of Ectomycorrhizal fungi decreased at soil depth 10-150 cm. The amount of plant pathogens in soil depth gradient 0–150 cm was increasing.

W216 - Benzalkonium Chloride Resistance in *Staphylococcus* spp. from International Space Station

Presenting Author - *Olivia Barber, Northwestern University, United States*

Author/s – *Anahid A. Moghadam, Shayan Malik, C. Mark Ott, Erica M. Hartmann*

Abstract Content

Background: The International Space Station (ISS) is a unique, isolated environment that has been subject to extensive microbial characterization. To reduce bacterial load and ensure crew member safety, cleaning has been done for decades primarily using benzalkonium chloride (BAC), a biocidal disinfectant linked to antimicrobial resistance.

Objectives: To investigate connections between long-term BAC use and antimicrobial resistance.

Methods: We classified levels of BAC resistance in 73 ISS *Staphylococcus* spp. isolates: *S. aureus* (n = 5), *S. haemolyticus* (n = 7), *S. hominis* (n = 56), and *S. lugdunensis* (n = 5). An initial estimate of the minimum inhibitory concentration (MIC) was determined by growing isolates on media containing BAC. Broth dilution testing was then performed to gain more specific MIC values for both standard strains and the ISS isolates.

Results: Differences in MIC were observed both within and between species. All S. haemolyticus ISS isolates tested had at least double the BAC MIC compared to the S. haemolyticus control strain. There were also MIC differences between the other ISS *Staphylococcus* spp. compared to their respective standards. There were no significant differences in MIC considering collection date, sample location, or sample type. That resistance is not consistently associated with time suggests that other factors, e.g., stochastic introduction of BAC resistance genes with arrival of crewmembers, are responsible. By comparing the resistance of a single bacterial genus from the ISS environment to Earth-based standards, we will gain insight into how the widespread use of BAC may be impacting the antimicrobial resistance crisis.

W217 - Unusual regulation of genes necessary for carbon dioxide fixation by marine chemolithoautotroph *Thiomicrospira pelophila*

Presenting Author - *Jana Wieschollek, University of South Florida, United States*

Author/s – *Kathleen Scott*

Abstract Content

Carboxysomes and dissolved inorganic carbon (DIC) transporters act together to form carbon dioxide concentrating mechanisms (CCMs), which facilitate DIC fixation by autotrophic bacteria when DIC is scarce. Autotroph *Thiomicrospira pelophila* has carboxysomes, and encodes six potential DIC-transporters, more than usual. We conducted experiments on how carboxysomes and multiple transporters are integrated into a functioning CCM in *T. pelophila*.

T. pelophila was grown in chemostats, under DIC-limitation or ammonia limitation, to induce its CCM. Potential DIC-transporter genes were heterologously expressed in *E. coli*, in order to determine capability for DIC transport. Random mutagenesis was used to clarify which genes are essential for CCM function. Electron microscopy was used to track carboxysome abundance, and qRT-PCR to track transcript abundance.

T. pelophila grew under low DIC conditions (>10 µM), evidence that it expresses a functional CCM. 4 out of 6 transporters were capable of DIC uptake when expressed in *E. coli*. Disruption of the carboxysome operon via random mutagenesis lead to high DIC requiring mutants, suggesting that carboxysomes are part of the CCM. A slight increase in carboxysome abundance was apparent under low DIC conditions; however, this increase is far smaller than in other autotrophs. Transcript abundances from carboxysome-related genes in *T. pelophila* do not differ under low vs. high DIC conditions, unlike other organisms with CCMs. Only one transporter was upregulated under low DIC conditions.

T. pelophila's CCM differs from those of other organisms, both with respect to carboxysome and transporter regulation, and response to environmental cues.

W218 - Assessing the antimutagenic activity of lactic acid bacteria isolated from human samples and fermented foods of Nigeria

Presenting Author - *Rachael Duche, Federal University Of Agriculture, Nigeria*

Author/s – *Tochukwu N T Nwagu, Harsh Panwar, Lewis I Ezeogu*

Abstract Content

Background: Probiotic bacteria have found wide applications in the medical sciences by recent consideration of their antimutagenic potentials.

Objective: The objective was to investigate the possible application of selected Lactobacillus strains isolated from both human and food sources in the prevention of mutagenesis and the risk of cancer.

Methodology: Isolates were identified by MALDI-TOF biotyper and 16S rDNA sequencing. Viability tests were performed based on MTT assay using HT-29 cell lines, while the antimutagenicity assay was based on Ames test.

Results: Identification results revealed 5 species *Lb. casei*, 2 *Lb. paracasei*, 3 *Lb. brevis* and 1 *Lb. pentosus*. HT-29 cells when co-cultured with *Lactobacillus* isolates displayed high viability; BK5 (99.2%), 3BM3 (96.2%), NON4 and PT1 (94.4%), 15ST2 and LGG (92.4%), 3BM1 (90.4%), 8BM6 (83.2%) and AK1 (81.9%) except 8BM9 (23.6) and GR11 (20.0%). *L. rhamnosus* GG was included as reference probiotic strain. *Salmonella* Typhimurium genotypes were confirmed according to Ames protocol. Antimutagenic activity against B[a]P with or without S9 mix among the selected strains was strong (above 40%). Strain 3BM3 exhibited the highest inhibition on TA100 without S9 mix (59.8±0.30) and with S9 mix (73.8±0.23); while KN3 exerted the most inhibitory effect against B[a]P on TA98 without S9 mix (51.4±0.00) and with S9 mix (76.1±0.83) compared to the other two *Lactobacillus* strains. No significant difference ($P>0.05$) was observed between inhibitions on TA98 and TA100. All strains displayed good antimutagenic properties and may be explored as potential probiotics after validating safety and efficacy in animal models

W219 - The effect of cadmium and copper on the net N2O production of deep-sea isolates

Presenting Author - *Leonor Pizarro, Interdisciplinary Center Of Marine And Environmental Research (ciimar), Portugal*

Author/s – *Laurine Mathé, Catarina Magalhães, Maria de Fátima Carvalho, Miguel Semedo*

Abstract Content

Deep-sea bacteria have high environmental importance due to their active role on nutrient cycling, among other activities. Some of these bacteria are responsible for maintaining low levels of nitrous oxide (N2O), a powerful greenhouse gas, by reducing it to dinitrogen gas (N2), through the activity of the N2O reductase enzyme, expressed by the nosZ gene.

The expansion of deep-sea mining operations anticipated over the coming decades could expose more marine microorganisms to dangerous levels of metals like cadmium and copper, as well as other rare elements. However, little is known about how vulnerable the N2O reduction potential is to metal exposure, particularly in deep-sea environments.

With this study, our goal is to understand the potential effects of cadmium and copper exposure on the N2O reduction metabolism of two deep-sea isolates: *Shewanella loihica* PV-4 and *Thalassospira indica* PB8B.

Cadmium and copper exposure experiments were performed in semi-closed bioreactors under aerobic conditions. When growth reached mid-exponential phase, anaerobic conditions were created for stimulating denitrifying conditions. Headspace gas samples were taken during anoxia to measure N2O accumulation over time and RNA extracted from cell suspension to measure the expression of the genes involved in nitrite (nirK) and N2O (nosZ) reduction.

Our results suggest that cadmium have an impact on net N2O production in the deep-sea bacteria studied, and that this may be due to changes in transcriptional control. Our findings will contribute to the global efforts of assessing the potential impacts of deep-sea mining on important ecosystem functions.

W221 - Impact of *Lactobacillus* exopolysaccharides on vaginal microbial biofilms

Presenting Author - *Beatrice Vitali, Dipartimento di Farmacia e Biotecnologie; Università di Bologna, Italy*

Author/s – *Barbara Giordani, Marina Naldi, Ülfet Erdoğan, Vanessa Croatti, Manuela Bartolini, Carola Parolin,*

Abstract Content

Background: Exopolysaccharides (EPS) are high molecular mass polymers produced by microbial cells and represent structural components of the extracellular matrix surrounding biofilms. EPS are involved in adhesion, auto-aggregation and prevention of pathogen growth. Little is known about EPS produced by vaginal lactobacilli, although anti-inflammatory and antitumoral effects have been proposed.

Objectives: EPS released by vaginal *Lactobacillus crispatus* and *Lactobacillus gasseri* were chemically characterized and tested for their capability to modulate biofilm formation towards beneficial vaginal lactobacilli and pathogens commonly responsible for vaginal infections.

Methods: EPS composition was analysed by LC-UV and LC-MS; EPS activity towards biofilms was evaluated in terms of residual biofilm cell viability quantified by MTT reduction assay, and biomass quantified by Cristal Violet staining.

Results: EPS released by vaginal *Lactobacillus* strains are heteropolysaccharides, D-Mannose and D-Glucose are the most abundant monomers in *L. crispatus*- and *L. gasseri*-derived EPS, respectively. EPS promoted the formation of biofilms of beneficial *L. crispatus*, *L. gasseri* and *Limosilactobacillus vaginalis* strains, in a dose-dependent manner. Notably, EPS mostly stimulated the biofilms of the same producer species rather than that of other species. Conversely, EPS significantly reduced biofilms of bacterial (*Escherichia coli*, *Staphylococcus* spp. *Enterococcus* spp., *Streptococcus agalactiae*) and fungal (*Candida* spp.) pathogens. *L. gasseri*-derived EPS showed the best anti-biofilm profile.

The present study provides insights into the functionality of EPS released by the most preponderant Lactobacillus species in the vaginal microbiota and supports their employment as a therapeutic/preventive strategy to counteract vaginal infections.

W222 - Microbiology of activated carbon filters used in the fourth stage of wastewater treatment plants

Presenting Author - *Yulduzkhon Abdullaeva, University of Münster, Germany*

Author/s – *Marie Löwe, Benedikt Kuhs, Bodo Philipp*

Abstract Content

Conventional wastewater treatment plants with their three steps of purification do not sufficiently remove micropollutants such as pesticides, pharmaceuticals including hormones and antibiotics as well as microplastics and nanoparticles. Such micropollutants can affect aquatic ecosystems and enter the food chain via bioaccumulation in aquatic organisms. To remove these trace chemicals from effluent water, improved wastewater treatment techniques are being installed. One of those techniques is adsorbing micropollutants with activated carbon (AC) filters. Previous studies have shown that microbial biodegradation can support the physicochemical purification process in AC filters but the microbiome and microbiological processes are largely unexplored. The complex environmental conditions within AC filters might facilitate the evolution of new metabolic pathways for micropollutant degradation as well as dissemination of antibiotic resistance genes via the horizontal gene transfer.

Physiological experiments with model bacteria from genera that can be expected in real AC filters have shown that aromatic-degrading *Pseudomonas* spp. can grow by desorbing adsorbed substrates from AC and that *Sphingomonas* spp. can form massive biofilms on AC. Selective enrichment of bacteria has led to the isolation of several strains with high affinity for AC particles that are currently being characterized for identifying molecular properties that facilitate growth in such filters. Furthermore, DNA extracted from operating AC filters is currently being sequenced for microbiome analysis.

Our further research seeks to understand microbial communities and their function in AC filters with the perspectives of risk assessment regarding the spreading of antibiotic resistance as well as bioaugmentation by adding micropollutant-degrading bacteria.

W223 - Identifying the presence of multidrug resistant bacteria and plasmids during unplanned water reuse for crop irrigation

Presenting Author - *Maria Blanca Sanchez, Institute IMDEA, Spain*

Author/s – *Lorena Martínez-García, Beatriz Peinado, Ana de Santiago-Martín, Raffaella Meffe, Gloria Teijón, Virtudes Martínez-Hernández,*

Abstract Content

The use of surface water highly impacted by wastewater treatment plant (WWTP) effluents for crop irrigation is defined as unplanned water reuse. This water usually contains different chemical and biological contaminants of emerging concern, including antibiotics and antibiotic resistant microorganisms and resistance genes that can propagate towards other environmental compartments. The objective of this study was to identify in the water-soil continuum the presence of microorganisms usually found in the human gut microbiome with a multidrug resistant phenotype (MDR) and plasmids able to provide resistance as a consequence of the irrigation of maize with highly impacted surface water. The presence of Enterobacteriaceae (coliforms) was analyzed in the irrigation water, in the water infiltrating through the soil, and in the soil itself throughout the maize growth period (3 months). A group of 246 coliforms isolated from water (irrigation and infiltrating) and soil were selected and their antibiotic resistance phenotype studied following the guide of Clinical and Laboratory Standards Institute (CLSI, 2018). Most of the isolated (92.7%) were susceptible or showed resistance to one or two of the eight tested antibiotics and only few showed clinical resistance. Eighteen isolates showed MDR phenotype, 6% in irrigation water, 11% in infiltrating water and 2.2 % in soil. The presence of plasmids responsible of the low susceptibility was analyzed in five MDR isolates. Data showed the presence, in all isolates, of plasmids that were capable of increasing the resistance to antibiotics when introduced into susceptible bacteria.

W224 - Microbiome sequence analysis of the UK Crop Microbiome CryoBank resource

Presenting Author - *Payton Yau, Scotland's Rural College, United Kingdom*

Author/s – *Payton Yau, Sue Jones, Nicola Holden*

Abstract Content

The UK Crop Microbiome CryoBank is a BBSRC-funded Bioinformatics and Biological Resource of cryopreserved and characterised crop microbiomes, which aims to underpin national and international crop research. Material is from six major UK crops: (barley, oats, oil seed rape, sugar beet, vining peas and wheat) grown in three different soil types from regions across the UK. The resource comprises preserved microbiomes and culturable isolates focused on crop rhizospheres; and sequence data for the microbiomes and isolate genome sequences; plus associated metadata for the crop and soils. The complete resource for the ongoing project is discoverable via an online database: AgMicrobiomeBase(https://agmicrobiomebase.org/). Crop and soil microbiomes are being sequenced for taxonomic groups of by metabarcoding for bacterial (16S) and fungal communities (ITS - selected samples) and overall community structure by shotgun metagenome sequencing. Culturable isolates are being whole genome sequenced by de novo sequencing. Sequence data analysis pipelines are being optimised for protocol standardisation and to provide the optimal state-of-the-art data outputs. Active involvement with EBI MGNify will serve to improve microbiome datasets utility. One of the key aims of the project is to provide legacy so that future analyses can be applied to the datasets using updated tools, for advanced analyses. The main sequence data analysis aims are to (i) provide an accessible dataset; (ii) comparisons of the soil and crop sample type communities; (iii) standardised protocols for use with inherently complex microbiota communities. The datasets and resources are openly accessible for academic, policy and industry interests.

W225 - Microbiology in indigenous space – a New Zealand Māori perspective of rediscovering native species

Presenting Author - *Eva Biggs, Manaaki Whenua - Landcare Research, New Zealand*

Author/s – *Alexander Fergus, Floyd Walker, Nikki Harcourt, Claudia Lange*

Abstract Content

Āwheto, also known as a vegetable caterpillar, is a natural structure formed by the fungus *Ophiocordyceps robertsii* infecting New Zealand's endemic Ghost moth caterpillars. The fungus mummifies the caterpillar when it is underground and shoots a thin stroma through its head to reach the surface and spread its spores. In Māori culture, āwheto has been important in tattooing ceremonies and for medicinal purposes. Indigenous knowledge concerning āwheto is slowly being lost, resulting in a limited understanding of āwheto ecology. We have been working with a Māori trust, used microbiological approaches to rediscover āwheto on their land and enabled reconnection of the owners with their land.

We used a culturally-informed soil sampling approach to identify candidate sites where we found āwheto. We examined the vegetation, soil microbial communities, and other soil-dwelling invertebrates and identified potential predators at sites with āwheto. Pure fungal strains were isolated from sampled āwheto and cultured.

Sites where āwheto were found shared several botanical and topographical characteristics and differed from sites without āwheto. We detected several animal visitations, but none fed on āwheto or disturbed sites. This might contribute to the prolonged persistence we observed of the āwheto structure on the forest floor.

The entomopathogenic fungus was confirmed as *O. robertsii* and the insect host was identified as Dumbletonius unimaculata. We have detected presence of *O. robertsii* on other soil-dwelling invertebrates without visible infection.

This collaboration between western science and indigenous knowledge produced fruitful and impactful outcomes with immediate adaptation.

W226 - Sustainable cultivation of *Arthrospira maxima* in organic based media with potato peel wash water

Presenting Author - *Nadine Juarez-Rendon, University Of Glasgow, United Kingdom*

Author/s – *Ian Watson*

Abstract Content

From pigment production such as chlorophyll and carotenoids to macromolecules extraction of proteins and carbohydrates, microalgae offer sustainable and high nutritional value for human consumption. Upgrading from pilot-scale to industrial production poses limitations and bottlenecks; one of these being the cost of nutrient supply. To investigate alternatives to commercial nutrients, *Arthrospira maxima* was cultivated in organic wastewater from potato peels. Potato peelings and ionized water were mixed for 1, 3, 24 and 48 h at ambient temperature with constant aeration (4 mL/min) with an air pump. The water was extracted, sterilized, and used to grow microalgal cultures at 33°C to assess the impact of washing time on the suitability of the wastewater as a nutrient replacement for microalgae growth; growth was compared to control samples grown with SAG media.

All experiments were done in triplicate over twelve days. Biomass concentration (g/L) and microscopic characteristic were analysed. Results showed that the maximum biomass was found in samples with 48, 3, and 24 h treatment, with biomass productivity of 0.456, 0.354 and 0.156 g/L, respectively. Microscopic analysis indicated that the algae filaments were shorter and straighter in organic waste media compared to control samples. It is concluded that organic waste based on potato peels is a potentially eco-friendly media replacement for the cultivation of *Arthrospira maxima* at scale. Furthermore, no heating was required for this process which reduces the energy inputs. Experiments are planned to improve the biomass concentration and identify the impact on the algae filaments after cultivation.

W227 - Unravelling the microbial ecology of slow sand filters

Presenting Author - *Valentina Attiani, Wageningen University & Research, Netherlands*

Abstract Content

Slow sand filtration is a reliable drinking water treatment technology for the removal of microorganisms, biodegradable-organic-carbon(BDOC) and particulate matter, and the production of biologically stable water, which prevents the growth of undesired pathogens. The role of physical-chemical processes in this treatment have been clarified in the past, but the (micro)biological processes still remain largely unknown.

The main objective of this work is to unravel the microbial ecology of different slow sand filters (SSFs) in the Netherlands by comparing variables such as sand depth and sampling points. A subsequent objective is to identify the key-microbes in BDOC degradation with the biorthogonal non-canonical amino acid tagging(BONCAT) technique coupled with fluorescence-activated cell sorting (FACS)[4, 5].

Full-scale SSFs from three drinking water treatment plants (DWTPs) were sampled at different points at increased distance from the influent water inlet, and from different depths of the sand bed. The DNA was isolated and then used for 16s rRNA gene amplicon sequencing.

The most abundant taxa observed in all SSFs were Nitrospiraceae, Gemmataceae, Pierllulaceae, Nitrosomonadaceae families. The depth of the SSFs and the DWTPs significantly influenced the bacterial community composition, whereas the spatial sampling points did not have a significant influence. This might be due to an even distribution of the nutrients coming from the water influent, and that the conditions were similar across the whole filter area. The most abundant taxa were similar in all SSFs. Additionally, the results obtained so far with the BONCAT-FACS until are promising for the identification of the BDOC-degrading microbes.

W228 - Impact of fertilization regimes on the soil microbiome of grassland ecosystems

Presenting Author - *Rostand Chamedjeu, Institute of Microbiology and Biotechnology, Germany*

Author/s – *Kunal Jani, Karoline Jetter, Patrick Schäfer, Lena Wilfert, Simone Sommer, Christian Riedel,*

Abstract Content

Land-use intensification has an important impact on soil microbial diversity. One of the anthropogenic factors of land-use intensity and a major driver of changes in microbial biodiversity of grassland ecosystems is fertilization, often leading to the loss of biodiversity and selection for particular taxa with adaptation capacity to changes. Although manure from animals as fertilizer provides good nutrient supply and growth conditions for plants, there is an increasing concern about its use because of its potential contribution to the selection on soil bacteria, potentially influencing their stability and function. Here, we report the effects of intensive organic fertilization on soil bacteria diversity and their most related core microbiota. Soil samples were collected from grassland ecosystems of the Swabian Alb, managed under three fertilization regimes (pig slurry, cow-manure and biogas digestate) in comparison to control areas. DNA extraction was performed on soil samples using ZymoBIOMICS™ DNA Miniprep Kit and sequenced with Illumina MiSeq, targeting the V4 region of 16S rRNA. To characterize the total bacteria community composition and compare between treatments, we calculated beta-diversity indices. We found highest bacterial diversity in microbial communities of control sites and a significant difference on soil microbial diversity with 47.8% divergence explained by treatment (fertilization regime). The most abundant and ubiquitous genera in the study areas were *Gaiella*, *Mycobacterium*, *Bacillus*, *Bradyrhizobium* and *Streptomyces*. These genera are known to be involved in soil functionality with link to nutrient cycling. This knowledge will contribute to the design of management strategies for sustainable ecosystem functioning and resilience.

W229 - Shedding light on the total and active core microbiomes in slow sand filters used to produce safe drinking water

Presenting Author - *Xi Bai, University of Amsterdam, Netherlands*

Author/s – *Inez J. T. Dinkla, Gerard Muyzer*

Abstract Content

Slow sand filters (SSF) are widely used in the production of drinking water as a last barrier in the removal of pathogens. This removal mainly depends on the 'Schmutzdecke', a biofilm-like layer on the surface of the sand bed. However, the mechanism and microbes involved in the removal of pathogens are unclear. So far, most previous studies used extracted DNA to determine the microbial community composition, missing the active community members. In our study, we determined and compared the DNA- and RNA-displayed communities in the Schmutzdecke from 10 full-scale slow sand filters and further explored the SSF core microbiome in terms of both presence (DNA) and activity (RNA). Discrepancies were observed between the total and the active community. The DNA-displayed community may be inflated, while the RNA-displayed community could reveal low abundance (or rare) but active community members. The overall results implied that both DNA and RNA data should be considered to prevent the underestimation of organisms of functional importance but lower abundance. The microbial communities of the studied Schmutzdeckes were shaped by the influent water. Nevertheless, a core microbiome was shared by the mature Schmutzdeckes from independent filters, representing a dominant and consistent microbial community in slow sand filters. The core microbial community structure was influenced by the operational parameters. To our knowledge, this is the first study revealing differences in the core microbiomes in terms of presence and activity in slow sand filters.

W230 - Trace Element-Related Phenotyping of the *Arabidopsis* Phyllosphere Community

Presenting Author - *Jan Friedrich Plewka-Mandelkow, Ruhr University Bochum, Germany*

Author/s – *Ute Krämer*

Abstract Content

In plants, we have a controlled plastic root uptake and distribution of metals through metal homeostasis. The vacuoles and apoplast of leaves are stored in toxic metals. Microbes require essential metals, such as copper and zinc, for survival. However, microbes are sensitive to excess micronutrients and chemically similar non-essential toxic trace elements such as cadmium. We hypothesized that plant metal homeostasis could influence plant-associated microbial growth and, by extension, community structure. The magnitude and variation of trace element tolerance of plant-associated bacteria are unknown. Here we screened 224 representative strains of the *Arabidopsis thaliana* phyllosphere on synthetic media containing increasing concentrations of metals. Overall, ~40 % of the variance in metal tolerance could be explained by the genus. Cadmium was the most toxic in the context of the employed medium. Of the previously identified keystone strains that control the phyllosphere microbial community structure, some strains showed high metal sensitivity, which suggests that minor changes in phyllosphere metal concentrations can affect bacterial community structure. To evaluate the relevance of metal tolerance levels, we quantified the elemental contents of *A. thaliana* apoplastic wash fluid and found that Cd is available in a concentration toxic for some bacteria. In the end, we analyzed already published KEGG data and showed that known metal resistance mechanisms could not explain tolerance profiles satisfactorily. In summary, our results agree with the possible role of plant metal homeostasis in shaping phyllosphere bacterial community structure and build the first step in answering our hypothesis.

W231 - VOC Cambers: novel devices for the assessment of volatile-mediated microbial interactions

Presenting Author - *Samuel Álvarez-García, University Of Lyon, Spain*

Author/S – *Alba Manga-Robles, Hugo Mélida, Pedro Antonio Casquero, Antonio Encina*

Abstract Content

Volatile organic compounds (VOCs) are a major element in both intra and inter-specific microbial interactions. These molecules intervene in relevant processes including recognition, communication, and competition, both between microbial strains or between microbes and other organisms.

The study of VOCs has been gaining attention during the last decades, particularly regarding biocontrol and plant growth promotion. Nevertheless, their study still faces several limitations derived from the absence of adequate lab gear.

VOC Chambers are newly developed devices for the performance of volatile assays and specifically designed to overcome some of the main problems researchers face when assessing these molecular dialogues. They can be used to evaluate microbe-microbe, plant-microbe, and invertebrate-microbe interactions, as well as the study of volatile-phase essential oils and other molecules in gaseous state. This communication strives to present the possibilities offered by VOC chambers for the insight into VOC-mediated interactions and to illustrate them through the obtained results.

Our findings demonstrate that VOC Chambers provide higher homogeneity and replicability of results, being able to reveal differences between treatments that were not detected by traditional methodologies. They also avoid cross contamination between cultures, are less time consuming, and easier to manipulate and set up. Moreover, these devices allow to adjust ventilation rate and gas exchange with the environment, which have been proven of the utmost importance for the outcome of volatile interactions. These results confirm that specific and more flexible new methodologies, such as the VOC chamber, are needed to further advance the shared knowledge regarding microbial volatile interactions.

W232 - Microbial community species composition and dynamics in the rhizospheric zone of *Stachytarpheta jamaicensis* and *Piper sarmentosu*

Presenting Author - *Pooja Sharma, National University of Singapore, Singapore*

Author/s – *Yong Wei Tiong, Yen Wah Tong*

Abstract Content

Aim of this study is the identification of microbial species composition and dynamics in the rhizospheric zone *Stachytarpheta jamaicensis* and *Piper sarmentosu*m after applying the food waste anaerobic digestate (liquid by-product) was compared to biochar (B) and organic compost (OC) on the field trial in 146 days. In *Stachytarpheta jamaicensis* and *Piper sarmentosu*m, the pH values in the rhizospheric zone were shown (7.1-7.5). The Illumina MiSeq sequencing was used to analyze the sequencing of the 16S rRNA V3-V4 hypervariable region, which revealed operational taxonomy units (OTUs) produced from the rhizospheric soil of *Stachytarpheta jamaicensis*. Results showed that the microbial analysis of the rhizospheric zone of *Stachytarpheta jamaicensis* revealed the microbiota is dominated by Bacteroides in D (PST001) and B (PST002), Gammaproteobacteria in B (PST002), D+B (PST003), and OC (PST004). Major phyla detected in the root zone of *Stachytarpheta jamaicensis* and *Piper sarmentosum* were *Pseudomonas*, Acidobacteriota, Actinobacteriota, Firmicutes, Halobacteriota, Proteobacteria, Verrucomicrobiota, respectively. There is a dominant microbial community in the rhizospheric zone of *Piper sarmentosu*m, where Prevotallaceae and Methanosaetaceae are found in D (PST001) and OC+ D (PST006), whereas Lachnospiraceae, Microscillaceae, *Nitrosomonas* and Pedosteurellaceae are dominant in B (PST002) & OC (PST004). *Stachytarpheta jamaicensis* and *Piper sarmentosu*m roots contain a microbial community that promotes plant growth. As plant growth-promoting microorganisms (PGPM), the majority of detected microbial species are involved in nutrient immobilization.

W233 - "In vitro" study of human gut microbiota related to gluten metabolism: from pure culture to community approach

Presenting Author - *Yaiza Carnicero-Mayo, University of Lyon, Spain*

Author/s – *Navasa Nicolás, Miguel Ángel Ferrero, Luis Enrique Saenz de Miera, Javier Casqueiro*

Abstract Content

Celiac Disease is an immune-mediated enteropathy with partially unknown etiology. Nowadays, the idea that gut microbiota plays an important role in Celiac Disease pathogenesis is widely accepted. Several studies have shown that gluten is metabolized by members of microbiota. This research has usually been performed in pure culture of isolated bacteria. Intestinal microbiota comprises myriads of microorganisms that co-exist and interact which each other. We had no reference that gluten metabolism by commensal microbiota had been studied using models based in microbial communities. Thus, we attempted the isolation and stable culture of microbial communities involved in gluten metabolism from human gut. For this purpose, a stool sample from a healthy volunteer was used to inoculate six different cultures. Some of those cultures contained non-digested-gluten as a main nitrogen source, while others did not. Also, half of them were performed in unbuffered media and the remaining, in buffered media. Subcultures were performed every 24 hours. Samples from cultures were taken every day and communities composition was studied via 16S rDNA sequencing. Mostly bacteria belonging to Firmicutes phylum (54 %) were found. Medium pH and non-digested-gluten presence were shown to significantly affect communities microbial composition. Furthermore, communities composition remained stable through subculturing in the same medium. Hence, this study shows the development of the first method for culture and stable maintenance of gut communities related to gluten metabolism. These communities could constitute a more realistic approach for the study of gluten metabolism by commensal microbiota.

W234 - Influence of the afforestation process on soil fungi and soil organic carbon stocks in Arenosols

Presenting Author - *Jelena Ankuda, Lithuanian Research Centre for Agriculture and Forestry, Institute of Horticulture, Lithuania*

Author/s – *Diana Sivojienė, Kęstutis Armolaitis, Audrius Jakutis, Donata Drapanauskaitė, Jūratė Aleinikovienė, Leho Tedersoo, Vladimir Mikryukov, Mycology and Microbiology Center, University of Tartu, Tartu, Estonia.*

Abstract Content

The abundance and taxonomic composition of soil fungi can affect the intensity of decomposition of soil organic matter and thereby influence climate warming. Therefore, it is very important to study not only soil chemical parameters but also soil fungi in different ecosystems. The main objectives of this work were to understand the influence of the afforestation process on soil fungi, soil organic carbon (SOC), and other chemical parameters of the soil, and which ecosystems are more conducive to stopping climate change and are less sensitive to climate warming. Soil samples were taken in the autumn of 2020 Arenosols in 5 ecosystems in South Lithuania (Arable land, Managed grassland, Natural grassland on abandoned arable land, Coppicing grassland, and Planted premature silver birch stand) and in 2 ecosystems in Central Lithuania (Young coppice of Scots pine and Natural mature silver birch stand). Environmental DNA extracted from these samples was amplified using eukaryotic primers targeting the full-length ITS region. The PCR products were sequenced using PacBio third generation sequencing platform Sequel instrument. It was found that the highest SOC and soil total nitrogen (STN) concentrations were in soil on Natural mature silver birch stand, and the lowest concentrations occurred in the soil on Arable land and Natural grassland on abandoned arable land. The highest number of OTUs classified as fungi were in Natural mature silver birch stand, but the lowest in Coppicing grassland. Obtained data showed, that the afforestation process is "climate-friendly", as it promotes the accumulation of SOC in the soil.

W235 - What can metagenome-assembled genomes tell us about carbon transformation in slow sand filters?

Presenting Author - *Tage Rosenqvist, Lund University, Sweden*

Author/s – *Johanna Hilding, Catherine J. Paul*

Abstract Content

Slow sand filters (SSFs) are commonly used for producing drinking water. Water is driven through a sand bed by gravity and undesired chemicals and microorganisms adsorb to, and are degraded in the biofilm attached to the sand. The microbial communities in SSFs are diverse and complex, and thus require cultivation-independent techniques to understand their contribution to SSF function.

The study examined the potential for microbial communities to transform organic matter. SingleM OTUs and metagenome-assembled genomes (MAGs) from water and biofilm of SSFs with and without ozonation as pre-treatment were compared. Functional, taxonomic and antibiotic resistance gene (ARG) annotations were performed. Ozonation breaks organic matter into small carbon compounds, so the gene content of MAGs associated with ozonated or non-ozonated filters were expected to be adapted towards metabolizing lower or higher molecular weight organic matter, respectively.

253 MAGs were generated. Sphingomonadaceae, Hyphomicrobiaceae and Burkholderiaceae OTUs were more abundant in the SSF receiving pre-ozonated water. The abundant MAGs from this SSF were enriched with genes conferring the ability to use 1 or 2 carbon compounds as carbon sources. In the biofilm receiving non-ozonated water, 16 families of carbohydrate active enzymes were enriched within the MAGs, including cellulases (GH5, GH9), sialidases (GH33) and α-amylases (GH13). Preliminary ARG analysis did not identify differences between filters. This study identified microorganisms oriented towards the metabolism of different fractions of organic matter in SSFs, providing targets for future modelling and community engineering efforts through modification of inflowing nutrients to biofilm.

W236 - Influence of water depth on biofilm microbiomes on decaying water lily leaves

Presenting Author - *Brianne Palmer, Rheinische Friedrich-Wilhelms-Universitat Bonn, Germany*

Author/s – *Sabina Karačić, Gabriele BierBaum, Carole Gee*

Abstract Content

Background: The process of tissue preservation is a primary focus in taphonomy. One prevailing theory is that mineralizing biofilms might be involved in the fossilization of soft tissue, such as leaves or skin. However, the composition and dynamics of microbial communities in plant biofilms are poorly understood. Diatoms might also play a role.

Objectives: We aim to identify the microbial community composition in the leaf biofilms of two water lily genera (Nymphaea and Nuphar) during the decay process, from leaves floating on the water or buried in the sediment. We predict that the buried leaf biofilms contain diverse niches and would therefore have a different microbial community composition compared to the floating leaf surface.

Methods: We used 16S rRNA (bacteria and archaea), 18S rRNA (diatoms), and ITS (fungi) amplicon sequencing to describe the prokaryotic and eukaryotic communities inhabiting the leaf surfaces in a freshwater pond. These data are supplemented with scanning electron micrographs of the biofilms.

Results: There was no statistical difference in the microbial communities between the water lily genera, but the communities varied with water depth. Rhizobium, *Pseudomonas*, and Rhodotorula were more abundant in the buried leaves while Bacillus were enriched in the floating leaf biofilms. Diatoms were more abundant in the buried biofilms, which was also documented by SEM. The buried leaf biofilms had greater richness compared to the floating biofilms, providing a more diverse environment for microbes perhaps involved with mineralization and the early stages of the fossilization process.

W237 - Biofilm colonization and succession in a full-scale partial nitritation-anammox moving bed biofilm reactor

Presenting Author - *Carolina Suarez, Lund University, Sweden*

Author/s – *Tage Rosenqvist, Ivelina Dimitrova, Christopher J. Sedlacek, Oskar Modin, Catherine J. Paul, Malte Hermansson, Frank Persson*

Abstract Content

Biofilms are frequently used to retain biomass during biological nitrogen removal processes in wastewater treatment. In the partial nitritation-anammox (PNA) process aerobic ammonia-oxidizing and anaerobic ammonia-oxidizing (anammox) bacteria together convert ammonium in nitrogen-rich wastewater to nitrogen gas. Little is known about how these biofilm communities develop, and whether knowledge about assembly in natural communities can be applied to PNA biofilms.

The start-up of a full-scale PNA moving bed biofilm reactor was followed for 175 days using shotgun metagenomics. Environmental filtering likely restricted initial biofilm colonization, resulting in low phylogenetic diversity, with the initial microbial community comprised mainly of Proteobacteria. Facilitative priority effects allowed further biofilm colonization, with initial growth of aerobic colonizers promoting the establishment of secondary anaerobic taxa like methanogens and anammox bacteria.

Although aerobic nitrifiers were early biofilm colonizers, species replacement was observed among members of the guild. Among the early colonizers were known 'oligotrophic' ammonia oxidizers including comammox *Nitrospira* and *Nitrosomonas* cluster 6a AOB. Increasing the nitrogen load in the bioreactor allowed colonization by 'copiotrophic' *Nitrosomonas* cluster 7 AOB and resulted in the exclusion of the initial ammonia- and nitrite oxidizers. During the final stages of colonization, the abundance of anammox bacteria rapidly increased and putative nitrite oxidizers in the phylum Chloroflexota were observed.

W239 - Genetic Engineering of gentisate degradation pathway in *Pseudomonas bharatica* CSV86T

Presenting Author - *Sukhjeet Kaur, Indian Institute Of Technology Bombay, India*

Author/s – *Sukhjeet Kaur, Dr. Rohit Srivastava, Dr. Petey V. Balaji, Dr. Prashant S. Phale*

Abstract Content

Pseudomonas bharatica CSV86T is an ideal host for genetic engineering for a number of reasons. Strain CSV86T has the novel property of preferential utilization of wide range aromatics (naphthalene, benzoate etc.) over glucose either via catchehol or proto-catechuate pathway. In addition to that to diverse metabolic capabilities, the strain CSV86T is plasmid free, virulence factors free and whole genome sequence is available. The present study is an endeavor to genetically engineer strain CSV86T. Introduction of gentisate pathway will diversify the metabolic capabilities of CSV86T. The genes mcbIJKL (3.2kb) encoding salicylate-5-hydroxylase and mcbOPQ (2.5kb) encoding gentisate dioxygenase, maleylpyruvate isomerase and fumarylpyruvate hydrolase from *Pseudomonas* sp.C5pp were cloned in pSEVA624 and pSEVA234 respectively. The clones were individually transformed in CSV86T. The expression of the respective clones was confirmed using enzyme activity, spectral scan or thin layer chromatography. The recombinant of pSEVA234 were grown on salicylate and p-hydroxybenzaote. The specific enzyme activity of gentisate dioxygenase obtained (260±0.02 nmoles min-1mg-1) and (310±0.03 nmoles min-1mg-1) on salicylate and p-hydroxybenzaote respectively. Further spectral scan was also performed, which showed peaks at 330nm (maleyl pyruvate) and 340 nm (fumaryl pyruvate). Similarly, salicylate pathway recombinant in pSEVA624 was subjected to spent media analysis by thin layer chromatography. The results of thin layer chromatography analysis showed gentisate spot, suggesting bio-transformation of salicylate into gentisate by salicylate-5-hydroxylase. Thus genetic engineering of strain CSV86T provide pathway for utilization of gentisate produced intracellularly as an intermediate in aromatics degradation.

W240 - Generalists, specialists: Integrated approaches to decipher fundamental functional ecology and habitat specific alterations

Presenting Author - *Shekhar Nagar, Acharya Narendra Dev College (University of Delhi), India*

Author/s – *Shekhar Nagar, Rup Lal, Ram Negi*

Abstract Content

Background: The increasing availability of metagenome-assembled genomes and environmental metagenomes provides unprecedented access to the metabolic potential and functional differences within the habitats. The hot spring microbiome with its diverse habitats and relatively well-characterized microbial inhabitants offers an opportunity to investigate core and habitat specific community structures at an ecosystem scale.

Objectives: Here, we employed tailored genome-resolved metagenomics and a novel approach that offers metagenomic overlaps to investigate the core and habitat-specific microbial diversity and multifunctionality of microbial residents of three habitats: microbial mat, sediment and water.

Methods and Results: The 16S rRNA network analysis revealed 6% of the Ecosystem core community (ECC) and 72% of the Habitat specific community (HSC) in all three habitats. Strain-level resolution of metagenome-assembled genomes defined the habitat specific genotypes (HSGs) and comparative metagenomic analysis exposed ecosystem-core genotypes (ECGs). Further, the functional attributes of ECGs revealed a complete metabolic potential of nitrate reduction, ammonia assimilation and sulfate reduction. The highest cycling entropy scores of N cycle suggested the enrichment of nitrogen fixing microbes commonly present in all three habitats. While specifically HSGs possessed the amino acid transport and metabolism functions in microbial mat (9.5%) and water (13%) and 19% of translation, ribosomal structure and biogenesis in sediment. Our findings provide insights into population structure and multifunctionality in the different habitats of hot spring and form specific hypotheses about habitat adaptation.

W241 - Trophic interactions between *Bifidobacterium* spp. and the butyrogenic gut commensal *Megasphaera elsdenii*

Presenting Author - *Sainan Zhao, Nanyang Technological University, Singapore, Singapore*

Author/s – *Raymond Lau, Ming-Hsu Chen*

Abstract Content

Trophic interactions among intestinal microorganisms are key factors that shape microbiota composition and metabolite generation. Previously, our fermentation trials revealed that hydrolyzed beechwood xylan with smaller chain lengths induced significant butyrogenic effects, which were closely correlated with the abundance of genus *Bifidobacterium* and species *Megasphaera elsdenii*. We hypothesized that *M. elsdenii* was responsible for butyrate production and that its growth relied on cross-feeding with *Bifidobacterium*. To test the hypothesis, we isolated three bacterial species (*B. longum*, *B. pseudocatenulatum*, and *M. elsdenii*) from the fecal cultures fermenting hydrolyzed xylan, conducted pairwise coculture studies of *Bifidobacterium* spp. and *M. elsdenii*, and performed integrated analyses utilizing whole genome sequencing, metabolite quantification, and metatranscriptomics. Results indicated that, despite possessing multiple copies of genes encoding carbohydrate active enzymes used for xylan degradation, *B. pseudocatenulatum* and *B. longum* exhibited preferences for xylose oligomers with specific chain length. In contrast, *M. elsdenii* hardly grew on any xylose-based substrates by itself. The growth of *M. elsdenii* was markedly enhanced by coculturing with bifidobacterial strains, while *B. longum* and *B. pseudocatenulatum* responded differently to the presence of *M. elsdenii*. Coculturing led to the disappearance of lactate and elevated levels of butyrate. Metatranscriptome analysis disclosed the upregulation of *M. elsdenii* genes involved in lactate-consuming and butyrate-producing pathways. We concluded that lactate produced by the hydrolyzed xylan consumer *Bifidobacterium* fueled the growth of *M. elsdenii* and initiated the butyrate generation. Our findings demonstrate an alternative pathway of butyrate formation through metabolic cross-feeding in the colonic environment.

W242 - Removal of antibiotic resistant bacteria and genes from wastewater treatment effluents with ultrafiltration membranes

Presenting Author - *Fátima Rojas-Serrano, The Helmholtz Centre of Environmental Research, Germany*

Author/s – *Matthias Schmidt, René Kallies, Florian Schattenberg, Susann Müller, Anja Worrich*

Abstract Content

Wastewater treatment plants (WWTP) are known hotspots for the development of antibiotic resistance. Antibiotic resistance genes (ARGs), the genetic elements conferring resistance to antibiotics, are normally found inside bacterial cells. However, during biological treatment, these elements (e.g. plasmids) can be released into the surrounding environment, where they can be acquired by other bacteria via horizontal gene transfer. WWTP effluents become vehicles for the dissemination of not only antibiotic resistant bacteria (ARB), containing intracellular ARGs, but also extracellular ARGs (eARGs) and, therefore, both should be considered when designing preventive strategies. In this study, post-treatment of secondary effluents with ultrafiltration membranes was investigated to verify the membrane efficiency for removing eARGs and ARB. The feedwater used was a synthetic effluent matrix containing either target eARGs (cell-free extracts of IncW plasmid R388, conferring resistance to sulfonamides) or ARB (*P. putida* KT2440 harboring the same plasmid). The lab-scale set-up consisted of cross-flow membrane cassettes (100KDa, polyethersulfone), each operated in batches over 4 weeks, approximately 20 hours of operation divided into 12 consecutive experiments. Quantification of eARGs and ARB was performed by quantitative polymerase chain reaction (qPCR) and flow cytometry, respectively. Membrane fouling was recorded during operation as permeate flow rate decrease whereas the membrane surface was inspected at the end of the experiments with scanning electron microscopy (SEM). The results showed high removal efficiencies for ARB (99.97% average) and eARGs (99.4% average) regardless of the degree of membrane fouling, proving that membrane technology is a promising alternative to minimize environmental spread of antibiotic resistance.

W243 - Assessing impact of combination of AMF and mycorrhiza-associated bacteria on growth and development of Zea mays

Presenting Author - *Varsha Varsha, The Energy and Resources Institute (TERI), India*

Author/s – *Varsha Varsha, Mandira Kochar, David Cahill, Lambert Brau, Leena Johny*

Abstract Content

Microbial communities play a significant role in the physical and chemical makeup of soil, influencing the nutrient balance in the ecosystem and plant growth. Most terrestrial plant roots are naturally associated with an arbuscular mycorrhizal fungus (AMF) and its associated bacteria. Recent studies have isolated AMF-associated bacteria (AAB), however, the effects of the combination of AMF and these AAB towards plant growth and development remain unexplored. In this study, the effects of co-inoculation of AMF and AAB to promote the growth and development of Zea mays under in vivo conditions were investigated. We investigated *Rhizophagus irregularis* (AMF) and *Priestia filamentosa* (AAB). Tassel and silk emergence in co-inoculated plants were two weeks prior to single inoculations of AMF and AAB and 4 weeks prior to uninoculated plants. Co-inoculation enhanced plant height (2-fold), plant biomass (3-fold) and stem diameter (2-fold) in comparison to uninoculated plants. AAB also positively influenced AMF spore density in co-inoculated plants. Results thus demonstrated a functional tripartite association between AMF, AAB, and host plant which beneficially impacted host plant development. Understanding these interactions between AMF and AAB in relation to plant growth will provide vital information for the development of next generation biofertilizers.

W244 - Impact of fertilization regimes on the diversity of root and flower microbiomes of *Trifolium pratense* in grassland ecosystems

Presenting Author - *Karoline Jetter, Institute of Microbiology and Biotechnology, Germany*

Author/s – *Kunal Jani, Rostand Chamedjeu, Simone Sommer, Lena Wilfert, Patrick Schäfer, Christian Riedel*

Abstract Content

Just as animals or humans, plants rely on communities of microbes to sustain their health. An imbalance in the microbiome caused by an external factor can provoke severe problems for the plant. Fertilization is an important component of agricultural land use but has a high disturbance potential on ecosystems and their services. By targeting grassland sites managed with different fertilization regimes, we investigate the change in the functional diversity of root and flower microbiomes of the legume *Trifolium pratense* acting as key stone species in the trophic chain. Plant samples were collected on grassland sites that are treated by four different fertilization regimes and samples were collected before and after fertilization. Subsequent sequencing of the microbiome revealed a significant impact of fertilization on microbial diversity in different plant compartments. The most intensive fertilization with pig slurry significantly reduced the alpha diversity in root associated microbiomes of *Trifolium pratense* compared to unfertilized plots and plots fertilized with cow manure or biogas digestate. This effect is even more pronounced in the endosphere microbiome given that comparing samples before and after fertilization with pig slurry resulted in a significant decrease in alpha diversity after fertilization. In contrast, the microbial community in flowers showed an increased alpha diversity in intensively fertilized sites compared to unfertilized control sites. In all compartments pig slurry fertilization further caused a change in beta diversity. Further studies will aim at understanding to what extent these changes affect plant health and ecosystem functioning.

W245 - Characterization of ammonia-degrading microbial community from an activated sludge for landfill leachate treatment

Presenting Author - *Rossana Petrilli, University Of Camerino, Italy*

Author/s – *Attilio Fabbretti, Kathleen Pucci, Graziella Pagliaretta, Valerio Napolioni, Maurizio Falconi*

Abstract Content

Background: The growing amount of municipal solid waste and its management in landfills caused an increasing production of leachate, a liquid formed by percolation of rainwater through the waste. Leachate contains high ammonia levels that can damage the bacterial population present into the activated sludge of municipal wastewater treatment plants (MWTPs) with subsequent reduction of the biological removal of nitrogen.

Objectives: The aim of the present work was to study the microbial community of activated sludges and eventually to produce an optimized microorganism's mixture consisting of selected bacteria highly resistant to ammonia stress and very active in nitrogen removing.

Methods: To find out a minimal bacterial population capable of breaking down high concentration of ammonium, activated sludge enrichment experiments based on Repetitive Re-Inocula in leachate medium (NH_4^+-N = 350 mg/L) were performed. Selected bacterial species were identified by metagenomic approach based on Next Generation Sequencing (NGS). Therefore, bacteria were isolated on minimal leachate solid medium at high ammonia concentration (300 mg/L), and identified by 16 rDNA amplification and sequencing.

Results: NGS data demonstrated that seven bacterial families (Alcaligenaceae, Nitrosomonadaceae, Caulobacteraceae, Xanthomonadaceae, Rhodanobacteraceae, Comamonadaceae and Chitinophagaceae) were predominant in the enriched sludge and strongly contributed to ammonia oxidation. In addition, we found that three isolated species (*Klebsiella* sp., *Castellaniella* sp., and *Acinetobacter* sp.) performed heterotrophic nitrification coupled with aerobic denitrification where NH_3 is converted to gaseous nitrogen products (N_2O or N_2).

Our results are promising to increase the efficiency of the biological ammonia removal in MWTPs reducing time and energy costs.

W246 - Inhibition of *Aspergillus brasiliensis* growth by non-thermal plasma

Presenting Author - *Jana Jirešová, University Of Chemistry And Technology Prague, Czech Republic*

Author/s – *Jana Jirešová, Eliška Lokajová, Kamila Zdeňková, Lucie Dorazilová, Zuzana Rácová, Josef Khun, Jan Hrudka, Vladimír Scholtz*

Abstract Content

Aspergillus brasiliensis, a member of the genus *Aspergillus*, is a common contaminant in cosmetics, food, or building materials. It is therefore desirable to develop new methods of inhibiting its growth. Recently, despite other options for use of the non-thermal plasma (NTP) in biology and medicine, new possibilities has been described as a tool for inactivation of fungi especially micromycetes. NTP is the partially ionized gas that is not in thermodynamic equilibrium.

This contribution follows our previous study of inactivation of dermatophyte micromycetes and the healing of onychomycosis and is focused on a method of treating several considerable building materials to inhibit the growth of microscopic filamentous fungi based on NTP using a direct bipolar corona discharge generated in a point-to-ring electrode system at atmospheric pressure and ambient temperature.

Aspergillus brasiliensis CCM8222 (ATCC 16404) was used for our experiments. A suspension of concentration of approx. 200 CFU/1 mL was inoculated on the surface of malt extract agar in the form of three 0.1 mL droplets. The inoculated plates were incubated at 23C for 7 days, during which they were consecutively exposed to NTP. The intervals between exposures were 24 hours, the duration of each individual exposure was 10 minutes. The results show that a single treatment causes a slowing down of mould growth, while repeated treatments may cause complete inhibition. Analogous results are anticipated for the growth on building materials, details will be presented on stage.

W247 - The quest for the ideal dew retting promoting micro-organism

Presenting Author - *Hanne Pappaert, Research group of Food Microbiology and Food Preservation, Belgium*

Author/s – *Veronique Troch, Leen De Gelder*

Abstract Content

Dew retting of hemp stems lying on the field is a spontaneous microbiological process in which pectin and hemicellulose are degraded in order to release the cellulose bast fibers from its stem. The process is weather dependent and strongly affects the desired fiber quality needed for textile applications. Adding specific bacteria or consortia on the stems during retting might achieve a more controlled process. We set out to isolate, characterize and identify the ideal retting micro-organism, which will degrade hemicellulose and pectin without degrading cellulose, of which the hemp fiber is made.

In 2021 samples of both hemp stems and underlying soil were taken from field trials in Bottelare, Belgium. Bacteria were isolated using the dilution to extinction method and identified through 16s rRNA gene sequencing. After identification, the bacteria were screened for their ability to degrade pectin, hemicellulose and cellulose. In total 57 bacteria were successfully isolated and identified. These bacteria belonged to 4 main phyla; 29% Actinobacteria, 27% Bacteroidota, 12% Firmicutes and 32% Proteobacteria. After screening for degradation of the plant biopolymers, six isolates with the right characteristics were found, namely the potential of degrading hemicellulose and pectin without degrading cellulose, belonging to the genera *Clavibacter, Microbacterium, Rhodococcus, Flavobacterium, Pedobacter and Luteimonas* sp. These isolates are being screened further through in-vitro mini-rettingexperiments to assess the extent to which they are able to release the fibres without damaging them.

W248 - Similar mechanisms are involved in *E. coli* prolonged survival in autoaggregates on Earth and under space flight conditions

Presenting Author - *Pavel Domnin, N. F. Gamaleya Scientific Research Institute Of Epidemiology And Microbiology, Russian Federation*

Author/s – *Svetlana Ermolaeva, Yusef Khesuani, Vladislav Parfenov, Alexey Kononikhin, Maryam Abdulkadieva*

Abstract Content

Background: For bacteria growing in suspension, the spaceflight provides low shear conditions without convective flows and microgravity that prevents sedimentation and was shown to result in bacterial autoaggregation. The growth in autoaggregates within the liquid bulk on Earth is well studied in main features but it is less known about late cultivation stages because of aggregate sedimentation. Using magnetic levitation conditions when gravity is counterbalanced by the magnetic force allows prolonged cultivation of autoaggregates without sedimentation on Earth.

Objectives: To compare proteome patterns of *E. coli* grown under spaceflight and magnetic levitation conditions upon prolonged cultivation.

Methods: The probiotic strain M17 was grown aboard of ISS and under magnetic levitation conditions on Earth for 6 days before samples were taken for proteome analysis. Control bacteria were grown under standard cultivation conditions without shaking.

Results: Up-regulation of autotransporter Ag43, Vitamin B12 transporter BtuB, and enzymes of the glycosylate shunt was observed under spaceflight and magnetic levitation conditions. While Ag43 is known to require for *E. coli* autoaggregation, both the glyoxylate shunt and BtuB have multiple functions including utilization of metabolites released by destroyed bacteria. Utilization of metabolites supplied by lysed cells has to be effective in autoaggregates where dead and alive bacteria are in close proximity while it is useless for a planktonic culture under gravity conditions when dead bacteria sank to bottom.

W249 - Application of non-thermal plasma on building material's fungi

Presenting Author - *Eliška Lokajová, University Of Chemistry And Technology Prague, Czech Republic*

Author/s – *Eliška Lokajová, Kamila Zděňková, Jana Jirešová, Zuzana Rácová, Petra Tichá, Mária Domonkos, Pavel Demo, Vladimír Scholtz*

Abstract Content

Fungi are a part of everyday life and their products are in many cases used in the food industry, but they do not always have a positive effect on their environment. Among others they are a major problem in the construction industry, where they infect a significant part of the materials used. In addition to unsightly colour maps, they can cause material degradation and health complications. The treatment of building materials against mould has been a discussed topic for several decades. The development of the non-thermal plasma (NTP) applications could contribute to solving this problem.

Our research was focused on evaluating the impact of NTP application to fungi growth on building materials such as OSB board, fibreboard, plasterboard etc. The samples of building materials (5x5 cm) were inoculated by fungal inoculum containing approx. 40 - 2500 spores; *Aspergillus brasiliensis, A. versicolor, Uloclacium* sp*., Cladosporium* sp*., Verticillium* sp*., Acremonium murorum or Fusarium* sp*.* isolated mostly from building materials were tested. Selected samples were exposed to NTP, in different exposure schemes. Subsequently, they were incubated and the growth and sporulation was evaluated by image analysis and evaluated as relative surface coverage. We found such conditions of NTP application that were able to reduce or completely inhibit the growth of micromycetes. The NTP application can be useful for example in temporarily wet buildings, or during water accidents, where it is necessary to slow down the growth of fungi before the subject dries out again.

W250 - Comparative and functional metagenomics analysis of rhizosphere plant communities from the Atacama Desert and Antarctica.

Presenting Author - *Karla Leal, Universidad de la Frontera, Chile*

Author/s – *Karla Leal, María José Contreras, Kattia Nuñez, Olman Goméz, Pedro Zamorano, Leticia Barrientos, Pablo Bruna, Andrés Santos, León Bravo, Bernardita Valenzuela, Francisco Solís, Giovanni Gahonna*

Abstract Content

The rhizosphere plays an essential role in the subsistence of plants, especially those under high environmental stress, which has led to greater interest in determining how rhizosphere bacteria contribute to plant survival in these environments. This work aimed to determine the microbial structure and functional predictions among five plant species inhabiting different extreme environments (desert and Antarctic) including *Croton chilensis, Eulychnia iquiquensis*, and *Nicotiana solanifolia* from Paposo, Antofagasta, Chile; and *Deschampsia antarctica* and *Colobanthus quitensis* from King George Island, Antarctica. DNA extraction and sequencing were performed by 16S rDNA analysis using the 16S SQK-RAB204 kit from Oxford Nanopore Technologies. Taxonomic determination were performed with Silva 138 SSU Software and classification using Centrifuge Software. The results revealed 3019 16S rRNA sequences, of which 781 are shared among all plants. The percentage distribution of abundant bacteria, within the common among all samples, were *Haliangium*, *Bryobacter*, unclassified (closest phylum: *Chitinophageceae*) and MNDI. Alpha diversity analyses showed differences in Shannon's and Simpson's indices (ANOVA p-value>0.05), with a higher value for desert samples. In addition, nitrification, chlorate reduction and chitinolysis were the main functions which could be related to stress resistance common for all the studied plants. Therefore, this study provides new information about rhizosphere microorganisms on plants exposed to polyextreme environments, which might be used for future agricultural applications regarding stress resistance.

W251 - FArhodopsins, a widespread paralog of proteorhodopsin in aquatic bacteria with streamlined genomes

Presenting Author - *Jose Manuel Haro-Moreno, Miguel Hernandez University, Spain*

Author/s – *Jose Manuel Haro-Moreno, Mario López-Pérez, Francisco Rodriguez-Valera, Ramunas Stepanauskas, Valentin Gordeliy, Alexey Alekseev, Elizaveta Podoliak, Kirill Kovalev*

Abstract Content

Microbial rhodopsins have been shown to play a key role in the ecology of aquatic microbes. More than one rhodopsins are often found in a single genome (paralogs) which usually have different functions. However, most of their diversity is found in the vast reservoir of uncultivated microbes. Therefore, we screened a large dataset of open ocean single-amplified genomes (SAGs) for co-occurrences of multiple rhodopsin genes. Such cases were found among *Pelagibacterales* (SAR11), HIMB59 and the *Gammaproteobacteria Pseudothioglobus* SAGs. These genomes always had a bona fide proteorhodopsin and a separate cluster of genes (genomic island) containing a quite divergent second rhodopsin associated with a predicted flotillin coding gene and have thus been named flotillin-associated rhodopsins (FArhodopsins). Metagenomic recruitment indicated that FArhodopsins are mainly associated with the lower layers of the epipelagic zone. In addition, metagenomic screening in aquatic environments revealed that these FArhodopsins are also present in freshwater microbes although in these they lack the retinal binding lysine. Their characteristic genomic context found in both marine and freshwater environments indicates a novel potential involvement in membrane microstructure that could be important for the function of the co-existing proteorhodopsin proton pumps. The conservation of FArhodopsins in diverse and globally abundant microorganisms suggests that they may be important in the adaptation to the twilight zone of aquatic environments.

W252 - Molecular bases of the interactions between clover plants and *Novosphingobium* sp. HR1a during rhizoremediation.

Presenting Author - *Ana Segura, Estación Experimental del Zaidín-CSIC, Spain*

Author/s – *Lázaro Molina*

Abstract Content

Rhizoremediation is based on the ability of microorganisms to metabolize nutrients from plant root exudates and, thereby, to co-metabolize or even mineralize toxic environmental contaminants. *Novosphingobium* sp. HR1a is a good biodegrader of polycyclic aromatic hydrocarbons (PAHs) and other aromatic compounds, and also a good colonizer of rhizospheric environments. The main objective of our research is to investigate the molecular interactions between clover plants and the bacteria, *Novosphingobium* sp. HR1a, during rhizoremediation. For this, we used a combination of physiological, transcriptomic and chemical methodologies. We have demonstrated that this microbe is able to co-metabolize PAHs and nutrients exudated by clover roots, being this ability mediated by the action of the PahT regulator. Furthermore, the bacterial genes responsible for PAH degradation are induced in the rhizosphere, and we have identified the individual carbon sources, among those exuded by the clover plants, that induced these genes.

Clover root morphology is altered in the presence of *Novosphingobium* sp. HR1a. However, in the presence of a mutant in the LuxR-solo regulator, LuxR874, root morphology is recovered. We have found a good correlation between some plant hormone levels, the root architecture and the presence/absence of the wild-type or mutant bacteria. Our data suggest that LuxR874 is a repressor of QS responses in *Novosphingobium* sp. HR1a. We are now studying the implications that this phenotype might have during rhizoremediation of PAHs.

W253 - Bacterial community composition associated with contrasting vegetation cover of Antarctic plants

Presenting Author - *Olman Gómez-Espinoza, Universidad de la Frontera, Chile*

Author/s – *León A. Bravo, Leticia Barrientos Diaz, María José Contreras Rivas, Karla Leal Villegas, Kattia Núñez-Montero*

Abstract Content

The Antarctic Peninsula has suffered from a strong warming between the 1950s and 2016, accelerating the expansion of the two Antarctic vascular plant species inhabiting this continent: *Deschampsia antarctica* and *Colobanthus quitensis*. However, during the expansion of these plants, the soil and rhizosphere bacteria have gone unnoticed, even though the plant-bacteria relationship is critical to plant survival. For example, the increase in temperature could positively or negatively modulate the bacterial community, favoring specific bacterial genera, which in turn may contribute to Antarctic plants' response to climate change. However, only some studies have focused on the analysis of the rhizosphere microbiota of these two plants. Therefore, using a 16S ribosomal RNA metagenomic approach, we aimed to characterize the diversity and bacterial community composition from the rhizosphere and of the two Antarctic plants at three sites in the Antarctic Peninsula with contrasting soil conditions: high vegetation cover, low vegetation cover and moraine (close to glacier with almost no-vegetation). To complete the objective, 16S rDNA amplicon sequencing, bioinformatic analyses, and soil physicochemical properties measurements were performed using three samples with three replicates per sample collected in the Antarctic Peninsula. Our results showed a negative correlation of soil water content, P, N, K, and organic matter with vegetation coverage, where moraine showed significantly lower parameters. On the other hand, the contents of Ca were significantly higher on the moraine. Microbial community analysis showed a typical profile of phylum regardless of the sample and plant species, which include Proteobacteria, Bacteroidota Myxococcota, Acidobacteriota, and Actinobacteriota.

W254 - Contribution of rhizobiomes from extreme Chilean Altiplano and the Atacama Desert native plants to endure a warming planet

Presenting Author - *Juan Castro-Severyn, Universidad Católica Del Norte, Chile*

Author/s – *Jonathan Fortt, Alessandra Choque, Coral Pardo-Esté, João Saraiva, Ulisses Nunes da Rocha, Francisco Remonsellez, Claudia P. Saavedra, Eduardo Castro-Nallar*

Abstract Content

In the climate change scenario, food demand is estimated to affect more than 1 billion people in over 100 countries. In particular, Chile with naturally low precipitation patterns, is facing a water crisis. Therefore, it is necessary to optimize soil and water usage, where stress-resistant crops are key. Rhizospheric microbes (rhizobiome) improve plant physiology, adaptation and resistance to abiotic stress (salinity and drought). Hence, we set up to characterize the rhizobiomes from plants thriving in the Salar de Huasco (SH) (Chilean Altiplano) and Yungay HyperArid Core (YHAC) (Atacama Desert), to elucidate microbes/plants interaction, suggesting functional associations enabling them to thrive under harsh conditions. For this, we took 200 rhizospheric soil samples from SH plants (*Deyeuxia curvula* and *Werneria incisa*) and 30 samples from YHAC plants (*Suaeda foliosa* and *Distichlis spicata*) for 16S rRNA amplicon sequencing and physicochemical/elements analysis. Our results in SH show that the geographical location and plant species vary significantly in diversity and electric conductivity, humidity and some elements (As, Li, B) seem to be influencing communities segregation. Differences in taxa abundance and presence are evident between plants, where *Pseudomonas* genus and Actinobacteriota members are dominant. In YHAC it is possible to observe the “fertile island effect” as plants under extreme drought conditions can acidify the soil and decrease EC. Also, *Suaeda foliosa* have higher TC, TOC and C/N ratio, which can favor heterotrophic microorganisms. The knowledge about microbial communities inhabiting extreme ecosystems holds the potential to improve the growth efficiency and abiotic stress resistance in crops.

W255 - Different transcriptional response of two closely related photoheterotrophic *Gemmatimonas* strains to changing light regimes

Presenting Author - *Karel Kopejtka, Institute of Microbiology of the Czech Academy of Sciences, Czech Republic*

Author/s – *Sahana Shivaranu, Karel Kopejtka, Jürgen Tomasch, Mohit Kumar Saini, Michal Koblížek*

Abstract Content

Background: The bacterial phylum *Gemmatimonadota* contains two members capable of performing aerobic anoxygenic photosynthesis to date. Microaerophilic *Gemmatimonas* phototrophica AP64T and strictly aerobic *G. groenlandica* TET16T were both isolated from freshwater environments and are closely related. Their genomes contain identically organized photosynthesis gene clusters (PGC) with sequentially higly-similar genes, suggesting that they share a common ancestor. However, compared to *G. phototrophica* AP64T, *G. groenlandica* TET16T lacks genes coding for anaerobic versions of some photosynthesis-related enzymes, while possessing a gene repository for coping with oxidative stress.

Objective: Here, we compared genomes, transcriptomes, and physiology of both strains to study evolution of phototrophy in the Gemmatimonadota phylum.

Methods: Using RT-qPCR and RNA sequencing we studied transcriptional response of both strains to changing light regimes.

Results: In *G. phototrophica* AP64T, the change from heterotrophic growth in the dark to photoheterotrophic growth in light was accompanied by a weak and transient downregulation of the PGC and upregulation of the ferrochelatase gene, suggesting a re-routing of the porphyrin anabolism away from bacteriochlorophyll synthesis. In *G. groenlandica* TET16T the transcriptional response of PGC was also transient but stronger. In contrast to *G. phototrophica* AP64T, a singlet oxygen defense was activated in *G. groenlandica* TET16T.

W256 - Phosphate-related environmental genomic islands as drivers of adaptation to different oceanic regions in the streamlined HIMB59

Presenting Author - *Carmen Molina-Pardines, Miguel Hernandez University, Spain*

Author/s – *Carmen Molina-Pardines, Jose M. Haro-Moreno, Mario López-Pérez*

Abstract Content

Background: The marine alphaproteobacterial HIMB59, the closest order to the SAR11 clade, is one of the most abundant groups inhabiting the oligotrophic surface waters of the global ocean. However, despite its abundance, the small number of cultured representatives (only one to date) as well as the scarce representation in metagenome-assembled genomes (MAG) has hampered the study of HIMB59 ecogenomics.

Objectives: In this study, we used all publicly available HIMB59 genomes (mostly single-amplify genomes [SAGs]) to provide a genomic overview of their phylogeny, metabolism, biogeography and microdiversity.

Results: Metagenomic recruitment showed distribution patterns that allowed the description of different ecogenomic units within the two families defined by our phylogenomic analyses. One of them (GCA002718135-1.A), for which no pure cultures have been isolated, showed an abundant global distribution in surface waters. Comparison with other representative marine microbial groups with multiple ecological strategies that share the same marine habitat such as SAR11 or *Prochlorococcus* revealed an enrichment in glycosyl hydrolases, especially of α-N-acetylgalactosaminidase and sugar transporters in this family. Microdiversity analysis showed the presence of metagenomic islands related to phosphate (P) acquisition and metabolism throughout the entire group. Different environmental versions of the high-affinity phosphate transporter (PstSCAB) and auxiliary proteins were colocalized in the different versions of the metagenomic islands, which showed a differential environmental distribution correlated with P bioavailability. These results suggest the presence of environmental isofunctional (eco-paralogs) variants in streamlined microbes to allow their adaptation to multiple ecological niches in response to different nutritional requirements.

W258 - Improvement of Hygiene Environment in Poultry House by 365 nm UVA-LED Irradiation

Presenting Author – *Mutsumi Aihara, Tokushima University Graduate School, Japan*

Author/s – *Mutsumi Aihara, Takaaki Shimohata, Shiori Kitayama, Shiho Fukushima, Kai Ishida, Masaaki Shimizu, Eiichi Marutani, Ryohei Yamamoto, Akiko Tomihisa, Takahiro Wada, Masayuki Okamoto, Hiroshi Ito, Takashi Tanizaki, Chikamitsu Oshima, Hiroshi Suzuki, Takashi Yasuno, Takahiro Emoto, Masatake Akutagawa*

Abstract Content

Background: Poultry meat consumption and production are increasing on globally, and demand is expected to continue to rise. As poultry farming system scale become larger, measures to prevent infectious diseases and further improve productivity are required. Contact infection is the main route of infection for *Escherichia coli, Salmonella, Campylobacter* and other bacteria that cause infections in poultry meat. In terms of infection prevention, it's important to construct a system that aims to improve the hygiene environment in poultry houses for the stable production. We attempted to control microorganisms using a diode that can emit ultraviolet light at 365 nm (UVA-LED) which has high bactericidal effect and is resistant to physical damage, into the poultry house.

Objectives：UVA-LED lighting equipment was installed on the ceiling of the poultry house to evaluate the number of bacteria and microbiota of poultry bedding by comparing the UVA-irradiated and non-irradiated areas, then examine the possibility of improving the hygiene environment.

Methods: A small-scale poultry house containing 100 broiler chickens, as ceiling light UVA-irradiated and non-irradiated areas were set and compared. The number of bacteria and microbiota of the poultry bedding were analyzed. Similar experiments were conducted in a large commercial poultry house containing 7710 broiler chickens.

Results: UVA-LED irradiation did not significantly reduce total bacterial counts. However, we found that γ-proteobacteria including *E. coli* and Salmonella, tended to decrease after UVA-LED irradiation, indicating that UVA-LED irradiation could control pathogenic bacteria for the hygiene environment in poultry houses.

W259 - In-depth analysis of a pristine oil microbiome reveals global distribution of plasmids

Presenting Author - *Julia Plewka, Environmental Metagenomics, Research Center One Health Ruhr of the University Alliance Ruhr, Faculty of Chemistry, Germany*

Author/s – *Armando Alibrandi, Till L. V. Bornemann, Cristina Moraru, Rolando Di Primio, Jens Kallmeyer, Alexander J. Probst, Sarah P. Esser, Tom L. Stach, Katharina Sures*

Abstract Content

Oil reservoirs are society's primary resource of hydrocarbons. However, microbial and metagenomic investigations are mainly conducted years to decades after industrial use, allowing water contamination and community exchanges. Here, we present the metagenomic analysis of the pristine Filicudi oil reservoir and its comparison to ten other hydrocarbon-associated sites. We identified 28 different microorganisms belonging to 22 genera in the Filicudi site. Thirteen metagenomic assembled genomes (MAGs) were reconstructed, allowing a detailed analysis of the metabolic capacity of organisms existent in the oil field. Beyond the diversity of microorganisms, we identified 51 plasmids and 95 viruses; the majority of both were presented as novel when compared to elements in public databases. We clustered the microbial single-copy gene rps3, plasmids, and viruses from all oil-associated sites and the Filicudi oil, revealing an overlap of elements across geologically distant locations. While rpS3 genes from Filicudi oil were not found at any other site, three out of the 95 viruses from the Filicudi samples were also detected in a heavily water-flooded oil reservoir in Shandong province in China. We, therefore, conclude that our samples are most likely uncontaminated and represent a pristine microbiome from an oil reservoir. Importantly, we did find an overlap between plasmids from the Filicudi oil and globally distributed sites, indicating that plasmids have fewer barriers when traveling between and settling in ecosystems compared to viruses and microorganisms. Our results shed new light on the genomic capacity and fluidity of oil microbiomes in their natural state.

W260 - Assessing microbial hydrogen oxidation in seafloor habitats

Presenting Author - *Nicole Adam-Beyer, Geomar Helmholtz Centre for Ocean Research Kiel, Germany*

Author/s – *Mirjam Perner*

Abstract Content

Molecular hydrogen (H2) can serve as an energy source for microorganisms in marine seafloor habitats. Microbial hydrogen oxidation plays a pivotal role in primary biomass production but also fosters fermentation by maintaining low hydrogen concentrations in different seafloor habitats, e.g. in deep-sea hydrothermal vent systems and anoxic sediments. The interconversion of H2 to protons and electrons is catalyzed by hydrogenase enzymes, which so far have all been described as metalloenzymes requiring a complex maturation apparatus.

Here we will present data from recent hydrogen amended sediment incubation experiments where we monitored hydrogen consumption alongside with shifts in the respective microbial communities based on RNA analyses. Hydrogen consumption was observed in all incubations, yet differences occurred in the specific rates and in the communities apparently responsible for the utilization of the hydrogen. Since the majority of the microbes are currently not culturable, we recently established a function-based screen to recover hydrogenases from metagenomic fosmid libraries. We will introduce some novel hydrogenases recovered by applying this screen to fosmid libraries of seafloor habitats.

W261 - Comparative genomics and phenotypic characterisation of antibiotic resistance in *Escherichia coli* isolated from marine sediments

Presenting Author - *Isabel Erb, Lund University, Sweden*

Author/s – *Carolina Suarez, Ellinor Frank, Johan Bengtsson-Palme, Elisabet Lindberg, Catherine J. Paul*

Abstract Content

High counts of *E. coli* have been frequently reported during the summer in marine bathing waters. Sediments were investigated as potential reservoirs and live *E. coli* were isolated from sediments. While this indicated pollution of the sediments, it was unclear if there were potential health risks associated with these bacteria, or if they possessed genotypic or phenotypic traits that would clarify the source and/or explain their ability to survive in brackish sediments.

In this study, we demonstrate that isolated *E. coli* from marine sediments show signs of adaptions that may facilitate their survival through wastewater treatment and that sediments are a reservoir for pathogenic *E. coli*. Thirty-seven *E. coli* isolates were sampled and sequenced from marine sediments of one region. Whole Genome Sequences were determined, assigned to phylogroups and sequence types (STs) and genes associated with virulence and antibiotic resistance were identified. Sources that had been associated with assigned STs of the isolates were analysed in the database EnteroBase. In addition to genetic comparisons, cultures of isolates were examined for halotolerance, biofilm formation and resistance to five different classes of antibiotics.

This study suggests that *E. coli* in marine sediments may survive due to their ability to form biofilms and grow in the presence of salt may be pathogenic. In addition, as antibiotic resistance genes (ARGs) of one isolate found in a plasmid conferred a resistance phenotype in the laboratory, there is a risk for ARG transmission in marine sediments.

W262 - Metaproteomic investigation of marine sediment to evaluate the metabolic potential for hydrocarbon degradation

Presenting Author - *Anne Ostrzinski, University Greifswald, Germany*

Author/s – *Anne Ostrzinski, Benoît Kunath, André R. Soares, Alexander J. Probst, Anke Trautwein-Schult, Dörte Becher, Cedric C. Laczny, Jens Kallmeyer, Rolando di Primio*

Abstract Content

The EU-funded project PROSPECTOMICS aims to develop a fundamentally new approach for oil and gas exploration in marine environments based on molecular biological techniques. Besides using geological and geochemical parameters, the project partners study the microbial community in marine near-surface sediments by applying several meta-omics approaches to differentiate between sediment sites above hydrocarbon reservoirs and control sites.

For the proteomics analysis, several protein extraction protocols for marine sediment were tested and compared for extraction efficiency. We found continuous-elution electrophoresis to be most efficient to extract proteins from sediment samples. Eluted proteins were digested using trypsin and resulting peptides were separated by high-performance liquid chromatography and analyzed via mass spectrometry. Subsequently, proteins were identified using a sample-specific metagenome-based protein database.

In first experiments, only a small number of less than 20 proteins could be identified in these challenging environmental samples. Using electrophoresis for protein extraction, significantly more proteins could be recovered from the sediment samples compared to other previously published extraction methods. In the first analyzed sediment core from a hydrocarbon-positive site more than 800 proteins were identified. Most of the proteins could be assigned to Methanomicrobia and were involved in methanogenesis or one-carbon metabolic processes.

The investigation of the proteome is of special interest because in contrast to genomic data, which provide information on microorganisms present in environmental biological samples, proteomic data reflect the actual level of metabolic activities in a microbial community. The combination of the meta-omics approaches can thereby provide comprehensive knowledge about metabolic processes in environmental samples.

W263 - Investigating the effect of cover crops on soil biodiversity in apple orchards

Presenting Author - *Rita Noto, Free University of Bozen-Bolzano, Italy*

Author/s – *Raphael Tiziani, Mauro Maver, Stefano Cesco, Luigimaria Borruso, Tanja Mimmo*

Abstract Content

Cover crops are a valuable source for feeding microorganism and micro-meso fauna and impact thus fundamental biogeochemical cycles in agricultural ecosystems. This study aims to evaluate the effect of different mixtures of cover crops on the dynamics on the functional and taxonomic soil biodiversity. The study is conducted in six apple orchards in South Tyrol with different agronomic practices in collaboration with local farmers. Soil samples are collected at two depths (0-30, 30-60 cm) two times a year. Soil taxonomic biodiversity will be investigated by targeting the bacterial 16S rRNA gene, fungal ITS2 region and micro- and mesofauna CO1 gene. Soil microbial respiration and functionality will be evaluated determining selected enzyme activities related to the carbon cycle. The data will be analysed by multivariate statistical methods to elucidate possible links among soil biodiversity, soil respiration and functionality and their contribution to ecosystem services of the apple orchards.

W264 - Determinants of active bacterial taxa in soil microbial communities and their contribution to soil ecosystem functions

Presenting Author - *Johannes Sikorski, Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Germany*

Author/s – *Selma Vieira, Ellen Kandeler, Marion Schrumpf, Markus Fischer, Norbert Hölzel, Jörg Overmann, Yu Shang, Katharina J. Huber*

Abstract Content

The identification of active bacteria and their ecological functions in complex habitats such as soil is a major challenge. Here we apply an improved approach using 16S rRNA/rDNA ratios obtained from amplicon sequencing in which a novel statistical procedure reliably (false-positive rate 0.3%) distinguishes false-positives caused by stochastic sequencing noise. Contrary to the current perception, rare but also dominant taxa were identified as active and constitute up to 1% of all bacteria. Using 60 soil samples from German grasslands (https://www.biodiversity-exploratories.de/en/), we investigated a wide set of physicochemical soil properties, microbial biomass, land use intensity values and traits of the accompanying plants and soil animal communities to identify drivers of the activity status (high rRNA/rDNA ratios) of individual bacterial sequence variants. We show that counts of active taxa had the strongest statistical explanatory power for soil respiration and enzymatic activities related to the carbon cycle (glucosidase, xylosidase and chitinase) but not to the nitrogen or phosphorus cycle. Using qPCR for the analysis of laboratory growth cultures of active and non-active representative taxa from the soil samples, we determined that high rRNA/rDNA ratios typically occur at the peak of growth rates, suggesting cellular replication in soil. In the era of easily accessible high-throughput sequencing, our approach provides a feasible way to detect active sequence variants and offers a higher potential to understand particular soil functions than custom alpha- or beta-diversity estimates.

W265 - The impact of *Bacteroides thetaiotaomicron* on *Bilophila wadsworthia*, a sulphidogenic member of the human gut microbiota

Presenting Author - *Jade Davies, Quadram Institute, United Kingdom*

Author/s – *Lizbeth Sayavedra, Melinda Mayer, Arjan Narbad*

Abstract Content

Bilophila wadsworthia is a sulphite-reducing bacteria producing hydrogen sulphide (H2S), a highly toxic gas. *B. wadsworthia* is enriched in disease states including inflammatory bowel disease and colorectal cancer, but is commonly present in the healthy commensal human gut microbiota. Understanding how abundance and metabolism of *B. wadsworthia* can be influenced has important implications for human health. *Bacteroides thetaiotaomicron* (Bt), one of the most prominent gut bacteria, can release sulphate for use by sulphate-reducing bacteria. However, until now, the interactions with *B. wadsworthia* remained unexplored. Here, we investigated the differential impact of two Bt strains on *B. wadsworthia*'s growth and H2S production.

B. wadsworthia was cultured independently with or without two Bt strains. We quantified Bt and B. wadsworthia using species-specific qPCR and measured the sulfidogenic activity of *B. wadsworthia* via the H2S concentration using an optimised colourimetric assay. Surprisingly, the effect on the growth of B. wadsworthia was strain specific. With one Bt strain (Bt1), a slight increase in *B. wadsworthia* biomass was observed at 8 hours; however, the H2S concentration in the co-culture was significantly increased, indicating that the presence of Bt1 increased the H2S concentration per *B. wadsworthia* cell. In contrast, co-culture with a second Bt strain (Bt2) showed that the H2S concentration and B. wadsworthia abundance were significantly decreased. Our study highlights how strain-level variation of one species can produce strikingly opposite effects on the growth of a potential pathobiont, and suggests that *B. wadsworthia* H2S production in the gut may be affected by other microbiome members.

W266 – Effects of the conservation tillage practices on the soil microbial community

Presenting Author - *Ana ibañez sanchez, University of León, Spain*

Author/s – *Aurora Sombrero, Arturo Santiago Pajón, Yolanda Santiago Calvo, M. Carmen Asensio-S.-Manzanera*

Abstract Content

Backgrounds: Growing human population leads to increasing demand for food amidst limited land resource, with a continuous search for higher yields in lesser times. It brings on an agronomy strongly dependent on machinery, which deteriorates soil structure, and decrease biodiversity and microbial activity, organic material and soil organic C content.

Global challenges are calling for a change. This is how conservation agriculture was born, including “no till” (NT) and different kinds of “minimal tillage” (MT). However, although it is clear that agronomic practices define the bacterial community structure, little is known about how different long-term conservation tillage practices may affect the microbial diversity.

Objectives: The objective was to examine the effects of two conservation tillage practices (MT and NT) on soil microbial community after 19 years of regime.

Methods: Soil samples were collected at 15 cm depth: four replicates, consisting of three mixed cores, from each treatment (two tillages, three crops). Soil microbial community was analysed by 16S rRNA gene sequencing by Biome Makers (Sacramento, CA). Demultiplexed sequencing data were analyzed with QIIME 2 software and statistical analyses were performed using RStudio-4.1.2 software.

Conclusions: Tillage regime, crop, and, specially, growing season, emerged as the most important factors driving microbial diversity. A total of 34 phyla were identified, being *Actinobacteria, Proteobacteria and Acidobacteria* the most abundant. Overall, our findings evidenced that soils under MT host a richer and less even bacterial community than under NT for all growing seasons, being *Chloroflexi* and *Verrucomicrobia* the most affected phyla.

W267 - Phylogenomic analysis and comparative genomics of insect-endosymbiont '*Candidatus Erwinia dacicola*' and free-living *Erwinia* species

Presenting Author - *Ilaria Checchia, University Of Verona, Italy*

Author/s – *Gioele Lazzari, Elisa Salvetti, Luigi Casillo, Giuseppe Firrao, Luca Mazzon, Nicola Mori, Nicola Vitulo, Giovanna E. Felis*

Abstract Content

'*Candidatus Erwinia dacicola*' is a co-evolved symbiotic bacterium associated with olive fly *Bactrocera oleae* (*Diptera: Tephritidae*), the major pest of olive tree. It is vertically transmitted and plays a key role in insect fitness. The adaptation to its host and the related consistent genome reduction (2.69 Mb) prevents its cultivability, which determines the difficulty of describing this species in sufficient detail. Nevertheless, 'Ca. E. dacicola' has a high 16S rRNA gene similarity (> 98.36 %) with the free-living and cultivated plant pathogens *Erwinia aphidicola, Erwinia persicina*, and *Erwinia rhapontici.*

Thanks to the availability of 41 genome sequences of '*Ca E.dacicola*' (metagenome assembled genomes-MAGs) and strains of the related species, the present study aimed at i) defining in detail the phylogenomic relationship among the endosymbiont and its closely related species, ii) unraveling their variable genes and iii) analyzing intraspecific genomes diversity of '*Ca. E. dacicola'*.

Phylogenomic analysis on 27 selected genomes (2-Ca. E. dacicola, 3-E. aphidicola, 15-E. persicina, 7-E. rhapontici) confirmed that E. aphidicola was the closest relative free-living species and chosen for detailed comparative genomics. A significant diversity was observed for genes associated to metabolism and environmental information processing, as could be expected considering the different lifestyles.

Finally, all MAGs genomes of '*Ca. E. dacicola*' were used to shed light on intraspecific variability in order to possibly describe novel haplotypes.

The results provide, for the first time, a detailed overview of the genetic determinants involved in genome reduction related to host adaptation as well as of genome-level intraspecific evolution.

W268 - Prediction of a growth medium for the endosymbiont '*Candidatus Erwinia dacicola*' through genome-scale metabolic modeling

Presenting Author - *Gioele Lazzari, University Of Verona, Italy*

Author/s – *Ilaria Checchia, Elisa Salvetti, Nicola Mori, Giovanna E. Felis, Nicola Vitulo*

Abstract Content

'*Candidatus Erwinia dacicola*' is an obligate endosymbiont of the olive fly Bactrocera oleae (*Diptera: Tephritidae*), the most economically relevant pest of the olive tree Olea europaea, where larvae develop on the olive mesocarp.

'*Ca. E. dacicola*' is strictly necessary for the larval development in this environment, making it an ideal target for pest control systems.

However, the development of effective natural symbiocides would benefit from pure laboratory cultures of the symbiont.

Here we use a genome-scale metabolic model (GSMM) to infer the composition of a growth medium for '*Ca. E. dacicola*', based on tryptone soy medium, used for other phylogenetically related Erwinia species.

The GSMM was derived from a '*Ca. E. dacicola*' quality-filtered core-genome, and its analysis pointed out no auxotrophies for amino acids but gaps in the fatty acid elongation and menaquinol biosynthesis pathways, suggesting the addition of peculiar fatty acids and vitamin precursors to the base medium.

From a biological role point of view, GSMM analysis suggested that the symbiont supplies amino acids to the larvae, in line with recent literature; further, we speculate that the host provides fatty acids through the mesocarp digestion, thus completing the mutualistic relationship with '*Ca. E. dacicola*'.

Future work will be focused on in vitro cultivation for the experimental validation of this approach. GSMMs could be an important tool to support cultivation effort of uncultured microorganisms, at least those only recently adapted to their host, that could have maintained sufficient genes for an in vitro growth but could require a supplemented medium.

W269 - Assessing the physicochemical and microbiological impacts of surface water intrusion on shallow groundwater wells

Presenting Author - *Kevin Lyons, University of Oulu, Finland*

Author/s – *Vadim Yapiyev, Pekka Rossi, Katharina Kujala*

Abstract Content

Background: Intruding surface water can negatively impact the physicochemical and microbiological quality of groundwater. Understanding this impact is important because groundwater provides about 25–50% of the world's potable water supply, and poor groundwater quality is a public health risk.

Objectives: This study aims to investigate the physicochemistry and microbiology of surface water–groundwater interactions at shallow wells in Finland, and how these interactions vary through the seasons. We hypothesise that wells which are closer and more hydrologically connected to nearby surface water bodies will exhibit greater overall physicochemical and microbiological similarity to those surface water bodies than wells that are more distant and less connected.

Methods: We monitored six shallow groundwater wells and three surface water bodies in the North Ostrobothnia region of Finland twice-monthly for 12 months (October 2021–October 2022) via (i) on-site and off-site measurements of physicochemical water quality parameters, (ii) determination of stable water isotope compositions, and (iii) analysis of microbial communities (via amplicon sequencing of the V3–V4 16S rRNA gene sub-regions). Weather observations, geospatial data, and continuous water level measurements (from the groundwater wells and surface water bodies) were also considered.

Results: Initial observations suggest that at least one of the studied wells is strongly influenced by surface water intrusion – as indicated by stable water isotope compositions and water temperature measurements. Multivariate analysis of the physicochemical data is ongoing. 16S rRNA gene amplicons have been sequenced and will be analysed via the QIIME 2 bioinformatic pipeline to determine taxonomic classifications and diversity metrics.

W270 - The effect of land management and a changing climate on arbuscular mycorrhizal fungal communities in New Zealand

Presenting Author - *Fionnuala Bulman, Lincoln University, New Zealand*

Author/s – *Eirian Jones, Amanda Black, Leo Condron, Steve Wakelin*

Abstract Content

Increasing the resilience of soil ecosystems is key to sustainable agricultural production in the face of our changing climate1. Arbuscular mycorrhizal fungi (AMF) are symbionts that form an intricate and intimate relationship with plant roots, making them a key component of soil ecosystems2. AMF are crucial to both aboveground and belowground processes, and play an essential role in ecosystem resilience, with communities able to adapt to changing environmental conditions3.

Exploring the drivers of AMF community composition, and how the AMF community relates to plant outcomes in the face of stress are both important knowledge gaps. This project hopes to further the knowledge base of how land management shapes AMF communities and how those communities affect the resilience of plants in both agricultural and native ecosystems in New Zealand.

Soil and plant roots from across a mosaic landscape, containing both agricultural and native forest ecosystems, have been repeatedly sampled for one year. The agricultural systems are perennial kiwifruit orchards and annual maize cropping. The spatiotemporal diversity of the AMF community from each site is currently being characterised by phospholipid fatty acid analysis and next generation sequencing. Preliminary results indicate ecosystem-driven differences in AMF diversity and community structure. Once characterised these communities will be used to examine the role of AMF in the resilience of plant-soil ecosystems to a changing climate (future proposed work).

W271 - Antibiotic Susceptibility Profile and Complete Genome Analysis of Multidrug-Resistant *Enterococcus faecalis* EN010

Presenting Author - *Soo-Jeong Lee, Seoul National University, Korea, Republic of*

Author/s – *Soo-Jeong Lee, Joon-Gi Kwon, Jihye Yang, Sung-Woo Choi, Li-Ha Kim, Ju-Hoon Lee*

Abstract Content

Antibiotic resistance (AMR) is a major health problem rapidly spreading worldwide. Its serious health impacts increase the interest in antibiotic resistance bacteria including enterococci. *Enterococcus* spp., gram-positive bacteria that inhabit the intestines of warm-blooded animals and environments such as plants, water, and soil, are intrinsically resistant to a broad range of antibiotics. In this study, 27 *Enterococcus* spp. were isolated from different environmental sources including four crops, three agricultural water, and two hospital wastewaters. Among isolated 27 *Enterococcus* spp., 11 strains with multidrug resistance to clindamycin, erythromycin, minocycline, and tetracycline were selected. Based on the minimum inhibitory concentrations (MIC) results, *E. faecalis* EN010 with the highest MIC values (Clindamycin: 48ul/mL, Erythromycin: >=256 ul/mL, Minocycline: 24 ul/mL, Tetracycline: 48 ul/mL) was finally selected. Complete genome analysis of *E. faecalis* EN010 was conducted using MINion based on Nanopore technique. The complete genome sequence of *E. faecalis* EN010 consists of a 2,916,542 bp circular double-stranded DNA containing erythromycin, clindamycin, and aminoglycoside resistance genes, ermB (EN010_1772), lsa(E) (EN010_1757), ANT(6)-la (EN010_01749), and AAC(6`)-le-APH(2``)-la (EN010_01769), between the IS6 family (EN010_1734, EN010_1776). In addition, the tetracycline resistance gene, tet(M), was carried within an entire copy of the transposon Tn916 (EN010_00977~01001). Consequently, this study demonstrates that multidrug-resistance resistance in *E. faecalis* EN010 can be attributed to the transmission of potential mobile genetic elements (MGEs) by horizontal gene transfer (HGT).

W275 - Blind spots of bacterial diversity: bacterial communities of endemic plants in Afromontane and Páramo ecosystems

Presenting Author - *Selma Vieira, Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Germany*

Author/s – *Selma Vieira, Johannes Sikorski, Dereje Hailu, Ellen Kandeler, Maria Mercedes Zambrano, Jörg Overmann*

Abstract Content

Tropical montane grassland and shrubland ecosystems are consistently poorly represented in biodiversity studies, despite being important reservoirs of carbon stocks and species diversity. In this study we assessed bacterial communities in the bulk soil and rhizosphere of different plant species endemic to protected areas of the North Andean Páramo (Colombia, South America) and the Ethiopian Highlands (Ethiopia, Africa), at altitudes between 2353 and 4169 m, by employing high-throughput sequencing, targeting the V3 region of the 16S rRNA gene. Bacterial richness was much higher for Ethiopian soils when compared to Colombian soils, which likely is due to the more acidic conditions found in the latter. Correspondingly, pH was also found as the largest driver of bacterial community composition on both continents. Although the plant species covered by our study exhibited very distinct traits, their rhizosphere effect was only limited, whereas soil properties exerted a much larger influence on rhizosphere bacterial community composition than plant species. Interestingly, most of the sequencing inventories were shared between both countries, despite representing only a small fraction of all sequence types found for both Colombia (28.1%) and Ethiopia (7.3%). These sequence types can also be identified in other sequencing inventories, providing evidence for the cosmopolitan nature of many soil and plant associated bacteria.

W276 - Formulations based on essential oils for the environmental microbial control

Presenting Author - *Giulia Lombardini, Catholic University of The Sacred Heart, Italy*

Author/s – *Maura Di Vito, Stefania Garzoli, Teresa Spanu, Mattia Di Mercurio, Maurizio Sanguinetti, Francesca Bugli*

Abstract Content

Background: Resistant pathogenic microorganisms in the air are major threats to human health. Antimicrobial effectiveness of essential oils (OEs) and their vapours are important for the fight against drug resistance and environmental microbial control.

Objectives: The objective of the study was to evaluate the effectiveness of single or mixed EOs (M-EOs) to control the growth of bacterial and fungal environmental/hospital strains.

Methods: 15 OEs were tested against bacterials resistant strains (*Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella pneumoniae*) and fungal strains (*Aspergillus fumigatus, Aureobasidium pullans melanigenum, Cladosporium cladosporioides, Alternaria alternata, Chaetomium globosum, Candida auris*). Broth micro-dilution tests according to EUCAST guidelines were used to obtain MIC and MBC values. The most active EOs were formulated in mixtures and the antimicrobial efficacy of their volatile compounds was evaluated using both micro-atmosphere tests and confined nebulization models. Starting from a suitable bacterial or fungal suspension, the charge variation was evaluated by making 2 nebulisations/minute for 10 or 20 minutes in an environment of 0.8 m3. The air quality was assessed by sampling on sorbent tubes followed by thermal-desorption and GC-MS analysis.

Results: After a single nebulization, data show a statistically significant reduction (between 50%-80%) depending on the bacterial species, and a time-dependent inhibition of fungal growth (between 77%-95%). Furthermore, the quality analysis does not identify concentrations of volatile compounds harmful to the human health. The air treatment by M-EOs nebulization is a possible, green, and safe treatment against hospital strains and antibiotic resistance in line with the one health approach.

W277 - Exploration of bacteriophage mediated hydrocarbon degradation gene's distribution in nature

Presenting Author - *Jinlong Ru, Christian Griebler University of Vienna, Germany*

Author/s – *Jinling Xue, Linda Cova, Li Deng*

Abstract Content

Microbial bioremediation is a widely accepted approach for hydrocarbon degradation, but the role of bacteriophages (phages) in mobilizing hydrocarbon-degradation genes (HYDEGs) within microbial communities has not been extensively studied. Increasing evidence suggests that phages can influence host communities and metabolism, contribute to better adaptation, and increase competition in the community. However, little is known about the role of phages in hydrocarbon degradation. In this study, we analyzed viral proteins in public databases and found that phages encode auxiliary metabolic genes (AMGs) that may be involved in various types of hydrocarbon degradation. Both aerobic and anaerobic hydroxylases that initiate the degradation process (CYP153, AlkB, AlmA, LadA, PmoC) were observed in viral genomes. We identified 77 viral genomes that encode high-quality vHYDEGs, suggesting that viral AMGs could contribute to alkane and aromatic hydrocarbon degradation, which could be beneficial for both biodegradation and phage propagation. We also constructed a comprehensive database to make it easier to use for future screening and validating processes of these viral-encoded HYDEGs (vHYDEGs). This study provides the first insight into the existence of various HYDEGs in viral genomes, their evolutionary origins, functions, and pathways. It will facilitate further searches and screenings of vHYDEGs with preferred features, and open new perspectives on the role of phages in hydrocarbon degradation and global carbon cycling.

W278 - Bacterial community from two freshwater aquaculture systems in Northern Germany

Presenting Author - *Júlia Clols Fuentes, University Of Rostock, Germany*

Author/s – *Julien A. Nguinkal, Patrick Unger, Bernd Kreikemeyer, Harry W. Palm*

Abstract Content

During the last centuries, the demand for fisheries products has increased considerably, leading to the rapid development of the aquaculture sector (FAO, 2020). Freshwater aquaculture in raceway systems is an inland common practice, which consists on the continuous flow of water through the tanks where fish are raised. The water is usually obtained from a natural source, filtered and discharged. Fish metabolism and other management practices, such as disinfection treatments for fish disease prevention, alter physicochemical water parameters (Keramat, 2008) and the microbial community (Lucas and Southgate, 2012; Jegatheesan et al., 2011). Therefore, knowledge of the aquatic microbial communities is required to improve management strategies and reduce the environmental impacts associated with the discharge of aquaculture wastewater. In this study, we assessed the effect of two fish farms on the water microbiome composition by analyzing the water before entering the system, named inflow water, and the wastewater, named outflow water. We additionally identified bacterial pathogens in the rearing environment associated with common fish farming practices. Molecular targeting the V3-V4 16S rRNA hypervariable region were used to infer Amplicon Sequence Variants (ASV), identify microbes, and assess their abundance and diversity. Results showed microbiome modulations after circulating through the fish rearing installations at both farms. *Flavobacterium* sp. and *Pseudomonas* sp. were the predominant potentially pathogens observed at both farms. The implications of aquaculture practices on microbial communities from two freshwater sites are being discussed.

W279 - Microbial interactions descriptions for humans and machines alike

Presenting Author - *Charlie Pauvert, RWTH Aachen University Hospital, Germany*

Author/s – *Alan R. Pacheco*

Abstract Content

Background: Microorganisms from multiple taxonomic domains engage in intermicrobial interactions to form communities in all biomes. Even with identical community members, these complex interactions can vary in time, space and due to changes in abiotic factors. The range of in silico, in vitro or in vivo methods used to study these interactions is equally wide, and all of them produce a large amount of data. However, there is no standard to report intermicrobial interactions and a dire need for an open access platform to datasets that support this evidence.

Objectives: Improve findability and integration of microbial interactions data by humans and machines by enhancing their description via a set of standardized metadata.

Methods: Microbial interaction metadata are specified using the LinkML modeling language to leverage validation capacity and interoperability with published ontologies or existing metadata. Durable hosting is enabled via interconnection to metadata in the knowledge base Wikidata embedded within a user-friendly interface and editing features for both humans and machines.

Results: Metadata concerning the interactions themselves, associated community members, their environmental context and evidence methods were formalized by types, expected values, and ontology values where appropriate, to enable comparison between studies. We illustrate this metadata scheme by describing examples of microbial interactions within hosts. Once more microbial interactions are described accordingly, original hypotheses could be drawn and tested by bioinformaticians, network scientists and microbiologists.

W280 - Understanding drinking water biofilm development using a pilot-scale distribution network operating at different temperatures.

Presenting Author - *Fien Waegenaar, Research group of Food Microbiology and Food Preservation, Belgium*

Author/s – *Thomas Pluym, Cristina Garcìa-Timermans, Bart De Gusseme, Nico Boon*

Abstract Content

Drinking water providers can ensure safe and qualitative drinking water when it leaves the drinking water treatment plants. However, during storage and distribution it is more difficult to control the drinking water quality. For example, higher temperatures in summer enhance microbial (re)growth and induce changes in the microbial community. In addition, biofilms present in distribution pipes can be a potential source of bacterial contamination or affect taste and odor.

In this study, a drinking water pilot consisting of 300m piping was used to investigate the effect of increasing temperatures (16 - 24C) in the drinking water network on biofilm development. With a unique coupon system to sample the biofilm, we were able to characterize the biofilm microbial community. Furthermore, with the use of online flow cytometry, the bulk bacterial community was analyzed, allowing to examine underlying bulk-biofilm interactions as well as interactions with physico-chemical parameters such as flow rate, conductivity and the possible formation of odor compounds.

Biofilm growth and bulk characteristics were followed during four months in terms of total cell densities, phenotypic fingerprinting, ATP activity, next genome sequencing and confocal laser scanning microscopy. Preliminary results show that temperature does not have a significant influence on biofilm formation rates, however more bulk bacteria or biofilm detachment is observed at higher temperatures (24C).

Overall, this study will provide a novel insight into the effects of drinking water biofilms and the impact of higher temperatures on drinking water quality on an unprecedented scale.

W282 - Effect of agricultural management and climate conditions on soil microbiomes and consequences for plant growth characteristics

Presenting Author - *Hamed Azarbad, Philipps-University Marburg, Germany*

Author/s – *Muriel Ornik, Renata Salinas, Martin Schädler*

Abstract Content

Global climate changes and agricultural management practices can both have major impacts on microbes. However, the effects of climate change-related stressors and management practices on microbes have often been studied separately, and it remains to be answered how the combination of such factors impacts soil and plant-associated microbiome and, subsequently, plant growth. The Global Change Experimental Facility (GCEF) in Bad Lauchstädt, Saxony-Anhalt, Germany is one of the first attempts to assess the parallel effects of predicted future climate scenarios (reduced precipitation and warming) and land-use types (including conventional and organic management) on various ecosystem processes. In this study, we extracted microbes from soil collected from the field which resulted in four unique soil suspensions (2 farming history: organic and conventional x 2 climate history: future and ambient). By inoculating seeds of wheat (*Triticum aestivum* L. NORDKAP) in sterile microboxes, we observed that soil microbial extract from organic farming with experience of future climate significantly enhanced seedlings' above-ground biomass compared to soil microbes from conventional farming and control treatments. This result provided baseline knowledge on the parallel impact of farming and climate histories on soil microbiomes and its consequent effect on seedlings' biomass. The next step is to answer open questions regarding the extent to which soil microbial extracts shape the seedling's microbiome.

W284 - Identification and characterization of bacteria majorly responsible for metabolizing aromatic amino acids in the human gut

Presenting Author - *Jae Kyeong Lee, Chung-Ang University, Korea, Republic of*

Author/s – *Kyung Hyun Kim, Ju Hye Baek, Yoonsoo Hahn, Che Ok Jeon*

Abstract Content

The metabolism of aromatic amino acids including tryptophan, phenylalanine, and tyrosine in human gut is one of important microbial metabolisms affecting on the human health because it has been known that their metabolites may be associated with diverse human health and diseases. Some bacteria and genes metabolizing aromatic amino acids have been discovered, but bacteria and genes majorly responsible for metabolizing aromatic amino acids in the human gut were not elucidated. Therefore, in this study we identified bacteria majorly responsible for metabolizing aromatic amino acids in the human gut. A total of 335 non-redundant bacterial genomes were retrieved through blastX analysis using the experimentally verified fldH, fldB, fldC, and acdA genes against genome databases and redundancy removal based on 95% average nucleotide identity. Phylogenetic analysis based on genome sequences showed that bacteria metabolizing aromatic amino acids are very taxonomically very diverse in the human gut. Metabolic gene diversity was also analyzed through sequence similarity network analysis based on fldH genes, and the abundance of each cluster was analyzed by mapping gut metagenomes into fldH genes in each cluster. Six fldH gene clusters with high abundances were selected and their representative proteins were expressed. We assayed the substrate specificities of six expressed fldH proteins for phenylalanine, tyrosine, and tryptophan to investigate key bacteria metabolizing each aromatic amino acid. Identification and characterization of key bacteria responsible for metabolizing aromatic amino acids in the human gut will provide a better understanding of human health and diseases related to aromatic amino acid metabolism.

W285 - Bacterial microbiota profiling along the life cycle of farmed gilthead sea bream (*Sparus aurata*)

Presenting Author - *Konstantinos Kormas, University of Thessaly, Greece*

Author/s – *Eleni Nikouli, Pimlapas Leekitcharoenphon, Elena Mente*

Abstract Content

Fish microbiota change along the different developmental stages and are impacted by the rearing conditions. In this study we profiled the Sparus aurata associated microbiota from the early embryonic stages until 230 days post hutch in a commercial fish farm. We followed the life cycle of the same batch of fish, in order to characterize the microbial assemblages through the investigated stages and to identify keystone bacterial operational taxonomic untis (OTUs). We sampled eight sampling points corresponding to developmental and/or dietary stages (D0, D4, D6, D14, D45, D73, D111, D230). Bacterial diversity was assessed by 16S rRNA gene sequencing of bulk DNA isolated from fertilized eggs, larvae, skin, gill and gut samples. A total of 1955 OTUs were obtained which were classified into 26 bacterial phyla and 195 families. *Proteobacteria* and *Bacteroidota* phyla dominated at the embryonic/larval stages (D0 – D73) with 91.6% of the total reads, related to *Tenacibaculum* and *Polaribacter*. At later stages (D111 & D230) *Proteobacteria* (with relative abundance 51.6%) together with Firmicutes (with relative abundance ranging from 17.7-34.1%) dominated the gut samples. In these stages *Comamomonas* was the most abundant OTU. Skin sample at D230 and gill samples (D2111, D230) were also dominated by *Polaribacter*. Fertilized eggs had the higher OTUs richness (282 ? 52.6) and the midgut at D230 the lowest (53 ? 3.2). Skin and gills shared more bacterial OTUs (33.8% at D2111 and 21.5% at D2111) compared to gut samples. Nine OTUs were shared among all the stages (*Bacteroidia, γ-Proteobacteria, Bacili*).

W286 - Impact of synthetic silver nanoparticles on biofilm microbial communities and antibiotic resistome in hybrid wastewater treatment

Presenting Author - *Jaak TRUU, University Of Tartu, Estonia*

Author/s – *Jaak Truu, Hiie Nõlvak, Kertu Tiirik, Angela Peeb, Arun Devarajan, Marika Truu, Kaja Kasemets*

Abstract Content

Background: Silver nanoparticles (AgNP) are among the most widely used engineered nanomaterials that reach the wastewater during their production, usage and disposal phase. Knowledge of the impact of AgNPs on the microbial community structure, abundance and removal efficiency of antibiotic resistance genes (ARGs) in wastewater treatment facilities, including constructed wetlands, is essential in the context of public health.

Objective: This study evaluated the impact of increased concentrations of AgNPs and Ag+ ions on the microbial community composition, system treatment efficiency and removal of ARGs in a hybrid wastewater treatment system.

Methods: Microbial community structure and antibiotic resistome diversity was assessed using shotgun metagenomics. Quantitative PCR was used to determine the abundance of ARGs.

Results: The results showed that the increased amounts of AgNPs and Ag+ ions had a modest effect on the prokaryotic community composition in the filter material biofilms and did not significantly impact the purification efficiency of the system. However, higher Ag concentrations did affect the abundance and removal efficiency of ARGs in the wastewater, resulting in an elevated ARG discharge into the environment. The study recorded enhanced relative abundance values for tetracycline, sulfonamide, and aminoglycoside resistance genes, and elevated levels of plasmid and integron-integrase genes in the biofilms of AgNP treated system. The findings indicate that further investigation is needed to understand the impact of AgNPs on the nature and characteristics of prominent resistance genes in wastewater treatment systems.

W287 - The global fungal biodiversity, biogeography and ecology viewed through the high-throughput sequencing results

Presenting Author - *Petr Baldrian, Institute of Microbiology of the Czech Academy of Sciences, Czech Republic*

Author/s – *Petr Baldrian, Petr Kohout, Tomáš Větrovský*

Abstract Content

Background: Several areas of biodiversity research including mycology have been changed dramatically with the advance of high-throughput sequencing. This opens a potential for the use of metabarcoding data in the study of global fungal biogeography and ecology.

Objectives: To determine the patterns of fungal diversity at a global scale and to identify the most important drivers of fungal biogeography and diversity.

Methods: Until 2022, the papers utilizing high-throughput sequencing approaches to study natural habitats in terrestrial ecosystems worldwide, in total >500 studies, have yielded over 1102 million sequences of the primary mycological molecular marker, the ribosomal internal transcribed spacer contained in the GlobalFungi database https://globalfungi.com (Větrovský et al., 2020).

Results: With a conservative threshold for fungal species delimitation at 97% ITS sequence similarity, the total estimated fungal richness is 6.3 million taxa, mostly *Ascomycota* (57%) and *Basidiomycota* (37%). The highest alpha diversity of fungi is associated with soil and litter habitats followed by air, plant shoots, plant roots and deadwood (Baldrian et al., 2022). The meta-study of fungal communities in soil identified climate as the most important driver of different aspects of fungal biogeography. In contrast to plants and most other taxa, tropical diversity of soil fungi was found low, and most of their diversity is concentrated at high latitudes (Větrovský et al., 2019). Climate change may significantly affect ecosystem functioning because the climatic tolerances of mycorrhizal fungi, important for plant nutrition, appear to be significantly more constrained by climatic variables than those of plant pathogenic fungi.

W288 - Space flight on ISS selects for different bacterial species/strains in the mouse gut

Presenting Author - *Lee Kerkhof, Rutgers, The State University of New Jersey, United States*

Author/s – *Lee Kerkhof*

Abstract Content

Illuminating the environmental factors which select for different bacterial species/strains will help in discovering the mechanisms promoting microbial diversity. In this report, mouse fecal samples from US-NASA mission RR9 which flew on the International Space Station were compared with ground based and vivarium controls. The gut microbiota was characterized by rRNA operon profiling using the rRNA Operon Database (rOPDB) and relative abundance testing with DeSeq2. Results indicated 26 different taxa were enriched during space flight, while 113 taxa present in ground controls were depleted. Specifically, rRNA operon reads most closely associated with 1 strain of Akkermansia muciniphila (EB-AMDK-7) were enriched in ISS mouse fecal samples, out of 33 different A. muciniphila strains detected in all RR9 mission samples. Likewise, flight rRNA operon reads most closely associated with 1 assembled genome from the Lachnospiraceae (IMSAGC015) were also enriched in ISS samples out of 30 similar Lachnospiraceae rRNA operons detected overall. Additionally, 2 strains of *Hungateiclostridum clariflavum* (DSM 19732, and 4-2a), *Rombustia ilealis* (CRIB), *Gottshalkia acidurici* (9a), and *Clostridium butyricum* (NBRC 13949) were enriched in ISS flight samples among other bacteria. Since the rOPDB is based on genomes in NCBI RefSeq, a direct comparison of strain genetic potential becomes feasible. For example, *A. muciniphila* (EB-AMDK-07) harbors unique genes involved in glycosyl- and acyl- transferase while Romboutsia ilealis (CRIB) contains unique genes for N-acetylmannosamine utilization. These results demonstrate rRNA profiling can discern strain selection and may provide an avenue for a microbial therapeutic intervention to improve astronaut health during spaceflight.

W289 - Investigating the impact of drought on the rhizosphere microbiome: comparing old and modern varieties of maize

Presenting Author - *Tillmann Lueders, University of Bayreuth, Germany*

Author/s – *Nicolas Tyborski, Benedikt Bartel, Andreas Wild, Tina Köhler, Franziska Steiner, Shu-Yin Tung, Sebastian Wolfrum, Johanna Pausch*

Abstract Content

Background: Drought tolerance is a key property of crops under climate change. The multifaceted mutualistic interactions between host plants and rhizosphere microbiota can foster plant stress tolerance in many ways. In comparison to todays' modern, yield-optimized varieties of maize, older landraces have been hypothesized to sustain a higher plasticity of traits conveying stress tolerance, also involving a more supportive microbiome.

Objectives: We aim to reveal compositional and functional distinctions in root-associated microbiomes for different varieties of Zea mays. We ask how host influence manifests across bacterial and mycorrhizal constituents of root microbiomes.

Methods: More than a dozen different varieties each of old and modern varieties of maize were grown in a greenhouse screening facility and at two different field sites in Bavaria. Using SSU rRNA gene amplicon sequencing, qPCR, microscopy and enzymatic activity tests, we compared bacterial and mycorrhizal rhizosphere microbiota under optimal water availability and under drought.

Results: Not surprisingly, rhizosphere microbiota were most clearly distinguishable between soils and sites. However, especially on the rhizoplane, differences in community assembly between plant varieties were detectable. Mycorrhization rates differed between maize varieties, with modern hybrids showing higher overall mycorrhization. An increased mycorrhization under water-reduced conditions was observed only in the field. Within the bacteria, a selection of specific *Glycomycetalales* and other *Streptomycetes* was observed under drought, related to strains recently shown to mitigate drought stress. Further understanding of these mutualistic interactions within the plant holobiont seems of promise to stabilize crop performance under drought in the future.

W290 - Evaluation of crAssphage as an indicator of the presence of antimicrobial resistance genes of human fecal origin

Presenting Author - *Cristina Garcia Aljaro, University of Barcelona, Spain*

Author/s – *Sara Morales-Cortes, Clara Gómez-Gómez, Laura Sala-Comorera, Lorena Rodríguez-Rubio, Maria Dolores Ramos-Barbero, Maite Muniesa, Cristina García-Aljaro*

Abstract Content

Background: Studies suggest the potential of bacteriophages as a vehicle for the transmission of antibiotic resistance genes (ARGs). Many ARGs have anthropogenic origins and urban wastewater is one of the main vehicles for their dissemination in the environment. For this reason, finding an indicator of the presence of ARGs could facilitate their monitoring.

Objectives: The aim of this research is to evaluate crAssphage, as indicator of the presence of ARGs of human origin in fecal polluted waters.

Methods: The presence and concentration of crAssphage and genes conferring resistance to different antimicrobials, were evaluated by qPCR in 50 wastewater samples from two different wastewater treatment plants. Detection of ARGs was done in total DNA and phage DNA fractions.

Results: No statistically significant differences were observed in the abundance of the different markers between both wastewater treatment plants (Kruskal Wallis p>0.05). The mean concentration of crAssphage in the total DNA fraction was 5.44 log10 GC/mL and 4.35 log10 GC/mL in the phage DNA fraction. The most persistent and abundant ARGs in both plants were tetW, sul1, blaTEM y qnrS, with mean concentrations higher than 6.3 log10 GC/mL in the total DNA fraction, while 2.5 log10 GC/mL were detected in the phage DNA fraction. CrAssphage presented significant correlations (p<0.05) with blaTEM, qnrS, blaVIM y blaOXA-48 (Spearman, ρ=0.381-0.787) in the total fraction and in the phage fraction with sul1 and qnrS (Spearman, ρ=0.357 and 0.610). The results support the possibility of using crAssphage as an indicator of the presence of some ARGs of clinical importance transported through bacteriophages.

W291 - High diversity of the emerging pathogen *Acinetobacter baumannii* in livestock and human wastewaters

Presenting Author - *Stefanie P. Glaeser, Justus-Liebig University Giessen, Germany*

Author/s – *Dipen Pulami, Peter Kämpfer*

Abstract Content

Carbapenem-resistant *Acinetobacter baumannii* are causing tremendous treatment problems in hospitals. There is still a knowledge gap on the abundance and stability of acquired resistances and the diversity of resistant Acinetobacter in the environment. In this study the diversity and antimicrobial resistances of *Acinetobacter* spp. released from livestock and human wastewater into the environment was studied. Fifty-two *A. baumannii* isolates were cultured from raw and digestated manure of different biogas plants and most stages of the rural wastewater treatment plants (WWTP) (no hospital wastewater receiving) and the two studied urban WWTPs receiving veterinarian and human hospital wastewater. Multi-locus sequence typing (MLST) identified 23 novel and 12 known sequence types (STs) of *A. baumannii*. Most novel ST were cultured from livestock samples and the rural WWTP. *A. baumannii* isolates from livestock and the rural WWTP were susceptible to carbapenems, colistin, ciprofloxacin, ceftazidime, and piperacillin. In contrast, *A. baumannii* isolates from the two urban WWTP showed a clinical linkage with respect to MLST and were multi-drug resistant (MDR). The presence of viable *A. baumannii* in digested manure and sewage sludge confirmed the survival of the strict aerobic bacteria during anoxic conditions. The study showed the spread of diverse Acinetobacter strains into the environment with a strong association of clinically MDR *A. baumannii* strains from the inflow of hospital wastewater to WWTPs. A more frequent detection of Acinetobacter in sewage sludge than effluent waters indicated that particle-attachment of Acinetobacter cells must be considered by the risk assessment of those bacteria.

W292 - Circulation of *Campylobacter* spp. between poultry and cattle farms

Presenting Author - *Muriel Guyard, French Agency For Food Environmental And Occupational Health & Safety (ANSES), France*

Abstract Content

Campylobacter is the main cause of zoonosis in Europe with more than 127 000 campylobacteriosis cases reported in 2021. Poultry is generally recognized as the major source of human contamination but source attribution studies report that cattle is a significant source as well. The aim of this study was to collect data about Campylobacter contamination in “mixed” farms with both poultry and cattle productions and determine if circulation of isolates between different livestock productions is possible.

Sixteen French mixed farms with free-range poultry and cattle were sampled (animals, building, pasture, etc.), in direct environment (manure pits, traffic lane), on shared livestock equipment (tractors, straw spreader…) and in pets. Feces, bootsocks and environmental swabs were analyzed for detection and enumeration of thermotolerant *Campylobacter* spp.

Campylobacter was detected in all farms. *Campylobacter* was isolated from 97% of the poultry samples (feces, houses, open-air range) and from 55% of the cattle samples (feces, houses and pasture). Campylobacter was also isolated from common area (manure pits, traffic lane), common equipment and pets. Campylobacter jejuni was isolated from the sixteen farms, additionally Campylobacter coli was isolated from six farms. Whole genome sequencing was performed on Campylobacter isolates from three farms and core genome MLST revealed potential epidemiological links between isolates from poultry and cattle or from cattle and shared equipment in one farm. These results demonstrate that Campylobacter can circulate between poultry and cattle on a farm and emphasize equipment, direct environment and pets as a potential risk of transmission between livestock productions.

W293 - Developing bacteriophage biocontrol for blackleg disease of potato.

Presenting Author - *Ashleigh Holmes, The James Hutton Institute, United Kingdom*

Author/s – *Prisca Hill, Michael Porter, Sonia Humphris, Kiri Mack, Ian Toth, Alison Blackwell*

Abstract Content

Background: Potato is third most consumed crop in the world but are at risk to substantial losses through disease and damage during both plant growth and tuber storage. There is no available treatment to control blackleg, and associated tuber soft rot, caused by Pectobacterium species. Here, we present the application of a biocontrol based on bacteriophages; naturally-occurring viral pathogens of bacteria that provide an effective, low risk solution to bacterial pathogens of crops, increasing productivity and decreasing waste.

Objectives: Our project aims to trace bacteriophage movements within plants to inform mode of action and explain efficacy results for a mix of bacteriophages targeting blackleg pathogens.

Methods: To determine whether bacteriophage treatment affected bacterial colonisation of the stem or roots of potato plants, we tested phage treatment regimens under glasshouse and hydroponic conditions. Confocal fluorescence microscopy was used to investigate the localisation of phage and bacteria expressing fluorescent protein (FP) on potato roots.

Conclusions: Phage treatment can significantly reduce the numbers of bacteria recovered from the roots of potatoes grown hydroponically. The impact of bacteriophage treatment on the development of blackleg disease on potato is more complex than phage induced lysis of susceptible bacteria.

W294 - Periodic investigation of the presence of the pathogenic bacterium *Erwinia amylovora* in apple tree stumps remaining after fire blight disease in Rosaceae plants

Presenting Author - *Seong Hwan Kim, Dankook University, Korea, Republic of*

Author/s – *Jun Woo Cho, Hyeung Jin Noh*

Abstract Content

Erwinia amylovora causes fire-blight disease in Rosaceae plants such as apples and pears. Since the disease rapidly spreads and causes huge damage on host plants, the bacterial species has been regulated as a high-risk plant quarantine pathogen in many countries. In the early stage of the first outbreak in Korea, all apple trees in the orchard where the disease occurred must be cut down, pulled out and buried. In recent years, diseased apple trees have been cut down, buried, or burned as control methods have changed. As a result, stumps remain after fire blight control. Concerns remain about the possible presence of *E. amylovora* in stumps. Thus, this study was performed to monitor and investigate the possible presence of *E. amylovora* in stumps. For this aim, the control treatment status of infected trees and surrounding treated trees that were controlled from 2019 to 2022 in 50 orchards in six regions was checked. No *E. amylovora* was detected in the plants around the stumps. Tissue samples from the stump were collected and the survival of *E. amylovora* pathogen was investigated. As a result, the fire blight pathogen was not present. It is expected that these result data can be used as useful scientific data for verifying the safety and effectiveness of the changed control method of fire blight.

W295 - Characterization and validation of environmental epoxy-degrading microorganism

Presenting Author - *Carlos Barreiro, University of León, Spain*

Author/s – *Sonia Garrido-Chamorro, Roberto Martínez-Santos, Elías R. Olivera, Alejando Chamizo-Ampudia, Luis Getino, Carlos Barreiro*

Abstract Content

Background: Thermoset polymeric composites are widely used in several industrial sector (construction, aeronautic, automotive) where both lightness and great mechanical performance are required. These epoxy reinforced materials present two main components: i) epoxy resins and ii) fibres (synthetic or vegetal derivates). However, their crosslinked microstructures hamper the recycled. Thus, the principal final fate, up to date, is the composite burn as energy source or their accumulation in landfills.

Objectives: Nowadays, the "Rs" concept, which fuels the Circular Economy idea, includes up to six ways (Rationalize, Reduce, Redesign, Reuse, Repair, Recycle) of materials processing aimed to avoid their traditional destruction. Thus, the European Union project, ESTELLA (Project no.: 101058371), funded through the Horizon Europe Framework Programme, is focussed in three of these manners (Redesign, Reuse, Recycle), where the biotechnological approach is playing a relevant role. Recently, the isolation of the poly(ethylene terephthalate) (PET)-degrading bacterium, the description of the wax moth caterpillars as polyethylene degraders and the description of a few bacterial species of the Rhodococcus and *Pseudomonas* genus able to use epoxy resins as carbon source have boosted the research on the biotechnological degradation of plastic and recalcitrant materials.

Methods and Results: Aimed to increase the array of epoxy degrading microorganisms, the optimization of the isolation conditions has been sequentially tackled by: i) culture conditions (media, aeration…); ii) environmental sampling protocols; iii) scaling processes (scale-up / down); iv) MALDI-biotyper identification and v) lab validation. As a result, the working conditions have been optimized and the first results were obtained.

W296 - The impact of plant-parasitic nematodes on the bacterial and fungal microbiome of the potato-growing soils

Presenting Author - *Diogo Proença, University of Coimbra, Portugal*

Author/s – *Paula V. Morais, Isabel Conceição, Marta Acin-Albiac, Beatriz Garcia-Jimenez, Alberto Acedo*

Abstract Content

Background: Potato (*Solanum tuberosum*) is an important staple crop worldwide and thousands of ha of potatoes are grown every year with great social and economic importance in Europe. Portugal is facing an increasingly lower potato yield average comparatively to European Union and one of the limiting factors may be attributed to plant-parasitic nematodes(PPN).

Objectives: This work aims to assess the microbiome of potato fields of healthy soils (without PPN) and diseased soils (with PPN) to contribute to the understanding the effect of disease on the soil microbial diversity.

Methods: Forty-five fields from North to South Portugal from the representing areas producing potatoes were evaluated and 450 soil samples were collected. The soil was physicochemically characterized. The evaluation of microbiomes and the identification of nematodes were performed before(T0) and after(T1) potato cultivations. Through the bioinformatic pipeline, the taxonomy was assigned from ASVs.

Results: T1 showed significantly higher a-diversity indexes and sampling sites could explain most of this variation. Presence of *Heterodera* spp. or *Globodera* spp. significantly increased diversity indices for bacterial or fungal communities, respectively. We observed that organically managed fields had higher bacterial richness when compared to conventionally managed fields. Fungal and bacterial microbiome compositions were highly correlated with pH, organic matter content and calory necessity. All soils shared only Shingomonas as common bacterial genus (analysis with abundances >1%). It was not possible to define a specific core microbiome for all disease or healthy soils. However, several healthy soils share a specific microbiome that is not present in diseased soils.

W297 - Antibiotic resistance genes among *Escherichia coli* isolated from fecal samples of healthy farm animals

Presenting Author - *Marjanca Starcic Erjavec, University of Ljubljana, Slovenia*

Author/s – *Veronika S. Mihailovskaya, Marina V. Kuznetsova*

Abstract Content

Background: Antibiotic resistance among bacterial strains is increasing. Determining the prevalence of antibiotic resistance genes (ARG) in different bacterial populations and the means of their spread can help design measures to curb further increases in antibiotic resistance.

Objectives: The aim of our study was to determine the prevalence of several ARGs among *Escherichia coli* (*E. coli*) strains isolated from fecal samples of healthy animals from industrial and private Russian farms. In addition, to assess the possibility of horizontal ARG transfer associated with the F-conjugative plasmid, the prevalence of the conjugative F plasmid traJ gene was determined.

Methods: Seventy-two non-clonal *E. coli* isolates (according to ERIC-PCR) from feces of healthy farm animals were analyzed by PCR for the presence of several ARGs and the F-plasmid traJ gene.

Results: 77.8% of the strains contained at least one ARG. The genes blaTEM, blaSHV, blaCTX-M were found in 79.2%, 23.6% and 13.9% of isolates, respectively. The blaCMY-2 gene has not been detected. The tetA and tetB genes were detected in 34.7% and 8.3% of isolates, respectively, while the tetM gene was found in only 2.8% of isolates. The cmlA and catA1 genes were detected in 16.7% and 8.3%, respectively. 56.9% of *E. coli* contained the gene traJ. In the *E. coli* traJ+ group, the genes blaCTX-M ($p=0.036$), blaTEM ($p=0.046$), cmlA ($p=0.009$) were significantly more common. It is important to note that *E. coli* containing 5 or more ARGs were found with a frequency of 19.4%, and exclusively among *E. coli* traJ+.

W298 - CRISPR Cas systems' detection in metagenomic sequences

Presenting Author - *Lina Aitmanaitė, Vilnius University Life Sciences Centre, Lithuania*

Author/s – *Lina Aitmanaitė, Kornelija Gelūnaitė, Giancarlo Russo*

Abstract Content

Viruses and prokaryotes are in a constant evolutionary struggle, leading bacteria to evolve anti-viral mechanisms such as the CRISPR-Cas systems. The CRISPR arrays consist of repeating sequences with unique spacer sequences that act as a memory of previous bacteriophage infections, while Cas proteins provide a defence against foreign nucleic acids such as viruses or plasmids. The analysis of metagenomic sequences from various sources could have a huge potential in finding new systems or revealing complex interactions between microbial community members. Nevertheless, identifying complete CRISPR arrays from metagenomic sequences is challenging, but crucial for understanding the coevolutionary processes between phages and prokaryotes in complex microbial communities. In this study, we utilized Oxford Nanopore Technologies' long-read sequencing technologies to identify full-length CRISPR Cas systems. First, we evaluated and selected the best nucleic acid extraction protocols to obtain high-quality, high-molecular-weight DNA samples from different sources. Then, we developed an approach to enrich specific target sequences, which was a crucial step in order to create an efficient CRISPR-Cas identification protocol and avoid excessive sequencing. Finally, we identified full-length CRISPR-Cas systems from various environmental samples. The results of this study shed light on the evolution and function of CRISPR-Cas systems in complex microbial communities, and also have the potential to improve the classification of CRISPR types and even expand the toolbox of CRISPR-based genome editing.

W302 - Rock and soil niches select for highly differentiated fungal communities in hyper-arid deserts of Antarctica

Presenting Author - *Fabiana Canini, University Of Tuscia, Italy*

Author/s – *Luigimaria Borruso, Federica D'Alò, Laura Zucconi*

Abstract Content

In the extremely oligotrophic and dry deserts of Victoria Land, in Antarctica, microorganisms experience cumulative environmental stressors, reducing the trophic complexity of the communities and allowing easier identification of the drivers of communities assembly. Although DNA-based molecular techniques have drastically increased our knowledge of the prokaryotic diversity in soils from this environment, still few scattered studies have investigated their fungal composition. Many characterisations are available for the endolithic niche, where communities can thrive under buffered conditions and where highly adapted and often endemic fungal species have been described. In Antarctica, local hotspots, rather than worldwide dispersion, have been accounted as sources of microbial propagules in the soils of the most extreme areas. Despite this, previous research has yet to be conducted to investigate the possible contribution of the endolithic niche to the surrounding soil diversity. To address this knowledge gap, samples were collected in three different localities at increasing distances from a colonized sandstone outcrop, which was sampled as well. The ITS1 metabarcoding analysis revealed an extremely low diversity, with very few fungal OTUs shared between rock and soil samples. Additionally, the 100-meters soil gradient considered did not show significant colonisation patterns. The two niches selected for highly differentiated communities were also highly differentiated in the three localities studied. The main OTUs indicators of the endolithic environment are lichen-forming fungi (*Lecanoraceae, Acarosporaceae*, and *Caliciaceae*). In contrast, yeast-growing taxa (e.g. *Naganishia, Malassezia*, and *Meyerozyma*) are the main indicators of soils.

W304 - Copper corrosion and microbial communities shifts in compacted bentonite: in view of a safe DGR of radioactive waste

Presenting Author - *Marcos F. Martínez-Moreno, University Of Granada, Spain*

Author/s – *Cristina Povedano-Priego, Mar Morales-Hidalgo, Adam Mumford, Jesus J. Ojeda, Fadwa Jroundi, Mohamed L. Merroun*

Abstract Content

Deep Geological Repository (DGR) is the preferred option for the final disposal of high-level radioactive waste. DGR is based on a multibarrier system where natural and engineering barriers are involved. Bentonite clay from southern Spain has been selected as filling and sealing material (engineering barrier) for future Spanish DGR. One of the most important safety requirements for repositories is the long-term confinement of radionuclides. Therefore, the mineralogical stability of bentonite, and the corrosion of metal canisters (e.g., copper), both influenced by the presence of microorganisms, need to be addressed.

This study focuses on investigating the behaviour of microbial communities under different physicochemical conditions, and the microbially induced corrosion (MIC) produced on the copper surface. For this propose, bentonite with different treatments (tyndallization heat-shock and acetate:lactate:sulfate amendment) was compacted at 1.7 g cm-3, containing a copper disc in the core, and incubated anaerobically at 30C. After one year, a multidisciplinary approach characterization was conducted.

X-Ray Diffraction was used to assess the mineralogical and chemical stability of the bentonite. 16S rRNA NGS showed the effect of the treatments on the microbial communities. Moreover, the survival of sulfate-reducing bacteria (main source of copper MIC) was demonstrated by the most probable number method. Finally, the MIC of copper was characterized by microscopic (VP-FESEM), and spectroscopic techniques (XPS and micro-FTIR).

The outputs of this study would help to understand the potential role of microorganisms from bentonite on the long-term stability and safety of the future DGR.

W306 - gNOMO2: a bioinformatic pipeline for integrated multi-omics analyses of microbial communities

Presenting Author - *Muzaffer Arikan, Istanbul Medipol University, Turkey*

Author/s – *Muzaffer Arikan, Thilo Muth*

Abstract Content

Background: In recent years, the emerging omics technologies have provided unprecedented opportunity to better understand the structural and functional properties of microbial communities. Consequently, there is a growing need for bioinformatic workflows that integrate multi-omics data and allow for comprehensive characterization of microbiomes. Previously, we introduced gNOMO, a bioinformatic pipeline specifically designed to process and analyze multi-omics data in an integrative manner.

Objectives: Here, we present gNOMO2 pipeline to facilitate reproducible and modular analysis of up to four omics levels -16s rRNA gene amplicon sequencing, metagenomics, metatranscriptomics and metaproteomics- of microbiome data in an integrative manner.

Methods: The gNOMO2 has been developed using the workflow management framework Snakemake in order to obtain an automated and reproducible pipeline. New analysis modules have been developed and integrated to the existing gNOMO pipeline.

Results: gNOMO2 pipeline includes three new modules that allow analysis of 16S rRNA sequencing data, generation of a custom protein database for metaproteomic analyses and integrated visualization of omics analysis results. Thus, gNOMO2 provides a modular and reproducible tool for extensive taxonomic and functional analyses of microbial communities in both model and non-model organisms and paves the way for new insights in microbiome investigations.

W307 - Biosynthetic potential of rare microbiota: The case of *Leeuwenhoekiella parthenopeia*

Presenting Author - *Paulina Corral, University of Naples Federico II, Italy*

Author/s – *Paulina Corral, Antonia Feola, Giuliano Gattoni*

Abstract Content

Uncultured microbiota represents a promising source to synthesize molecules of pharmaceutical interest. Here, to increase the probability of cultivation of rare marine bacteria, seasonal and dynamic sampling approaches for cold months and natural events such as Dial Vertical Migration (DVM). This approach gave rise to the isolation of *Leeuwenhoekiella parthenopeia*, a rare bacterium which cell lipidic extract is able to arrest the proliferation of prostatic tumor cells (DU-145) and glioblastoma (U-87 MG) human tumor cell lines. Comparative genome analysis of the Biosynthetic Gene Profile (BGP) of the strain L. parthenopeia Mr9 with all members of the genus *Leeuwenhoekiella* was approached to determine if the cytotoxic activity exhibited in vitro against human tumor cells is intrinsically correlated with the BGP of or if it is a common feature of all members of this genus. The study showed that all members of the genus *Leeuwenhoekiella* share a similar and streamlined content of Biosynthetic gene clusters (BGC) with low homologies with already known BGCs involved in the synthesis of compounds experimental verified. However, only *L. parthenopeia* possesses an unknown BGC of type NRPS, which suggests that this cluster of genes could be responsible of the bioactivity, making it a promising strain among other species of this genus.

W308 - Characterization of ciliate diversity and growth rates in a coupled aquaponic system using microscopy

Presenting Author - *Stefanos Moschos, University Of Ioannina, Greece*

Author/s – *Konstantinos Kormas, Sokratis Papaspyrou, Hera Karayanni*

Abstract Content

Background: Aquaponic systems combine the rearing of fish with hydroponic cultivation of plants, utilizing prokaryote-mediated nitrification. Currently, nothing is known about the protist communities that prey on the system's prokaryotes.

Objectives: The aim of this study was to elucidate whether different communities develop across compartments and understand microbial dynamics in such systems. For this we described for the first time the planktonic ciliate diversity in different compartments of a coupled experimental aquaponic system, and calculated ciliate and heterotrophic nanoflagellate (HNF) abundances and potential growth rates.

Methods: System water was filtered through 64μm and 10μm mesh filters for ciliates and HNF respectively, into polycarbonate bottles which were incubated in situ for 48 hours in fish tanks, sump tank (after nitrification) and drain tanks (after tomato plants). Sampling took place at 24-hour intervals (0, 24, 48 h). Ciliate samples were fixed with acid Lugol's solution, then inspected and counted with inverted microscopy after sedimentation. HNF samples were fixed with glutaraldehyde, stained with DAPI (4',6-diamidino-2-phenylindole) and counted with epifluorescence microscopy. The experiment was repeated at day 17 and day 127 of operation.

Results: Ciliate morphotypes were associated with the genera *Cyclidium, Halteria, Litonotus, Euplotes, Paramecium, Vorticella, and Chilodonella*. Total ciliate growth rates were higher in fish tanks (<0.05), and HNF growth rates were lower in drain tanks (p < 0.05). Shannon and Simpson diversity indices were higher at day 17 (<0.01). Cluster analysis showed that drain tank communities had the most distinct composition.

W309 - Responses of the bentonite microbial community to conditions relevant to nuclear waste repository: radiation and high compaction

Presenting Author - *Mar Morales-Hidalgo, University Of Granada, Spain*

Author/s – *Cristina Povedano-Priego, Marcos F. Martinez-Moreno, Ursula Alonso, Ana M. Fernandez, Fadwa Jroundi, Mohamed L. Merroun*

Abstract Content

Deep geological repository (DGR) is the internationally accepted option for the storage of highly radioactive waste in the near future. In this multi-barrier system, bentonite clay is considered as the best backfilling and sealing material. However, autochthonous microorganisms inhabiting this clay may compromise the safety of the system. Actually, bacterial groups like sulfate reducing bacteria (SRB) could corrode the metal canister. Moreover, DGR physicochemical relevant conditions such as irradiation, compacted bentonite density, and groundwater filtrations would induce shifts in these microbial communities influencing the storage safety.

This study aimed to investigate the effect of gamma radiation on the survival of microbial populations in compacted bentonites. For this propose, FEBEX bentonite was compacted at 1.6 g/cm3 and 100% saturated with bentonite pore water. One set of samples were treated with gamma irradiation at 14 kGy of total accumulated dose. Additionally, some samples were spiked with an SRB consortium to stimulate the bacterial activity.

After 6-month anaerobic incubation, DNA extractions and 16S rRNA gene Next Generation Sequencing were performed to study the microbial diversity of the different treatments. Most Probable Number of SRB was also estimated using anoxic Postgate's Medium. Furthermore, heterotrophic aerobic colony-forming units were calculated to assess the bacterial viability.

This work offers new perspectives into the microbiology of DGR as it provides insights into the effect of radiation on the bentonite microbial community and simulates a more realistic scenario of what might happen during storage lifespan.

W310 - Genome-resolved metagenomics of oil-degrading microbial community in the Gulf of Finland

Presenting Author - *Angela Peeb, University of Tartu, Estonia*

Author/s – *Marika Truu, Hiie Nõlvak, Lijuan Yan, Signe Viggor, Martin Romantchuk, Jaak Truu, Arslan Gafarov, Sergei L. Sokolov*

Abstract Content

Background: The Gulf of Finland (GoF), due to its location and characteristics (e.g., enclosed, shallow, nutrient-rich), is vulnerable to anthropogenic pollution. Concerns about the health of GoF are rising due to immense shipping and therefore increasing the risk of severe oil spill accidents. In case of an oil spill, clean-up methods such as booms and skimmers have been used extensively. However, bioremediation also has a high potential for removing oil pollution from the marine environment since species from more than 350 prokaryotic genera have been associated with the degradation of crude oil compounds.

Objectives: The aim of this study was to evaluate microbial community structure and its genetic potential to degrade oil compounds using multi-omics accompanied by recovery of metagenome-assembled genomes.

Methods: Surface water and sediment samples from different geographical locations of GoF were collected in winter and summer. Whole community DNA was extracted and sequenced on Illumina sequencing platform. Afterward, various bioinformatical tools were applied to analyze microbial community composition, construct metagenome-assembled genomes (MAG), and extract metabolic genes related to the biodegradation of crude oil compounds.

Results: The results indicate that several prokaryotic genera can degrade oil compounds. However, their abundance in the marine environment is highly dependent on seasonality. Moreover, the recovery of genes from bacterial metagenome-assembled genomes suggested that a number of microbes possess a variety of genes belonging to different biodegradation pathways. This property provides such microbes with the potential to degrade multiple oil-related contaminants.

W311 - New insights into cultivable bacteria from extreme aquatic environments of Roraima Tepuy Sur Cave, Guayana Highlands

Presenting Author - *Paula Suárez, Universidad Simon Bolivar, Venezuela*

Author/s – *Paula Suárez, Milagro Fernández-Delgado, Mary Luz Puche, Juan Giarrizzo, Hazel Barton*

Abstract Content

Background: The majority (>99%) of environmental microorganisms resist culture in the laboratory. Attempts to improve their recovery from the environment by traditional culture methods have had limited success and continue to be a major challenge in microbiology research.

Objectives: in this study, diffusion chambers were used to cultivate hidden bacterial communities from aquatic environments of the Roraima Sur Cave (RSC) at Tepuy Roraima, Guayana Highlands, the largest quartzite cave in the world.

Methods: Inocula from spring samples were mixed with semisolid agar, placed in membrane-sealed diffusion chambers, and incubated in situ for 72 h. The chamber-derived material was plated into oligotrophic media. Bacterial isolates were identified using 16S rRNA sequencing and phylogeny.

Results: chamber cultivation resulted in representative isolates of *Terrabacteria*, Beta and *Gammaproteobacteria*. The most common genera in RSC sequences were *Serratia* (22%), *Janthinobacterium* (14%), *Aquitalea* (11%) and *Ralstonia* (8%). Phylogenetic analyses also revealed their affiliations with *Cupriavidus, Burkholderia, Rhodanobacter, Nocardiodes* and *Rubrobacter*. These findings contribute to the exploration of uncultivable and novel microbes from extreme environments with the hidden potential to produce secondary metabolites, as well as to the conservation of pristine and highly diverse environments. Future work is needed to study the microbial diversity of these freshwater ecosystems with new sequencing techniques.

W313 - Evaluation of polystyrene biodegradation and assimilation capacity of a tropical fungus

Presenting Author - *Stephan Rohrbach, Leibniz University Hannover, Germany*

Author/s – *Gerasimos Gkoutselis, Anika Mauel, Adrian Ho, Jürgen Senker, Gerhard Rambold, Marcus A. Horn*

Abstract Content

Plastic pollution is considered to exceed planetary boundaries as it threatens wildlife, soils, and biogeochemical cycles. Environmental deterioration of plastic leads to the formation of microplastics (<5 mm). Due to its strong chemical inertness, it remains in the environment. While searching for solutions, more and more reports indicate that fungi inhere great capacities for plastic biodegradation. Natural occurring hydrophobic waxes of leaves resemble biochemical equivalents for synthetic polymers. Thus, we hypothesize that tropic phyllospheres are ideal reservoirs for fungi that evolved metabolic features to efficiently degrade polymeric compounds including plastics. To test our hypothesis, we utilized a tropical fungal isolate and examined its growth on semi-solid agar plates supplemented with various types of microplastics as sole carbon source. Microplastic stimulated colony growth. Then, 13C-labelled polystyrene was synthesized, added to fungal liquid cultures for 110 days, and 12CO2 / 13CO2 ratios as well as quantities were measured online via cavity ring-down spectrometry. Data showed 13C-polystyrene mineralisation at slow but constant rates. Lastly, fungal phospholipid fatty acids were extracted and analyzed via gas chromatography-isotopic ratio mass spectrometry to evaluate potential assimilation of polystyrene-13C-atoms, revealing a tentative trend towards polystyrene-derived carbon-assimilation. Ultimately, our approach will provide future researchers with a generic workflow for direct identification and evaluation of plastic biodegraders without the biases of conventional plastic biodegradation studies.

W315 - Microbiome of mine residues and its role in heterotrophic bioleaching and recovery of critical metals

Presenting Author - *Paula Morais, University of Coimbra, Portugal*

Author/s – *Paula Morais, Joana Caldeira, Ana Paula Chung, Romeu Francisco, Beatriz Rito, Rita Branco*

Abstract Content

Introduction: Global consumption of raw materials is expected to double in the next 40 years, while annual industrial residues generation is projected to increase by 70%, by 2050. Traditionally, the exploitation of raw materials is focused on high-grade ore deposits, extracted, and processed by conventional techniques. The waste generated (mine tailings) may become a valuable resource if new technologies are available that enable their use as secondary raw material sources. In Nature, materials are circularized by microorganisms. Therefore, to produce productive circular systems and a nature-based solution we need to transform microorganisms into essential tools and new engines of technology.

Objective: To assess the microbiome of basin tailings from a tungsten mine and determine the effect of the activation of the heterotrophic community on the mobilization of metals from the residues.

Methods: Four boreholes have been dug in the basins of the mine of Panasqueira, Portugal. The sediments collected were analysed by DNA sequencing and XRF analyses. Bioleaching measured by ICP-MS was performed by dynamic intermittent up-flow process, ensuring water saturation, and by using continuous flow, using R2A diluted medium.

Results: The four boreholes dug in the tailings basin showed differences between the 2 basins in the microbial composition of the residues. In both processes, the pH dropped rapidly to 3 remaining constant. A temporal succession of the metals leached was observed.

Conclusion: Selectively removing the Interference compounds by bioleaching increases the relative concentration of critical metals in the raw materials and increases their accessibility.

W316 - Niche formation of *Cryptomycota* in wastewater treatmentplant model systems

Presenting Author - *Katrin Stüer-Patowsky, Technical University Munic, Germany*

Author/s – *Eva Gega, Christian Wurzbacher*

Abstract Content

In this study down-flow hanging sponge (DHS) reactors (Hatamoto et al. 2018) were used as model systems to get insight into the fungal community of wastewater treatment systems. In previous studies, the application of DHS systems enabled the enrichment of the mostly neglected organisms, especially *Cryptomycota* (Miyaoka et al. 2017). Over six weeks, five reactors were run using a mixture of communal wastewater as feed to enable the growth of the microbial community. Chemical parameters were monitored and DNA and RNA were extracted to enable a quantitative (qPCR) and qualitative (Illumina sequencing) analysis of the organisms in the systems, get insight into the microbiological reactor performance and the association of *Cryptomycota* to other microorganisms. The reactors showed a good performance concerning ammonium reduction. After 20 days it was close to 100%. The BOD and total COD reduction appeared to fluctuate while the soluble COD reduction increased by around 249% from day 3 to day 41. The quantitative analysis of *Cryptomycota* sequences was normalized over 16S rDNA, based on the sampling time and the height of the reactors on day 41. It showed an increase of the average gene copy number of 1149% from day 3 to day 41. Also, we observed a 30% higher number of gene copies of active organisms in the middle of the reactors than on the top and a decrease of 7676% on the bottom. This showed them favouring the part of the system with still a high concentration of carbon sources.

W317 - Isolation and characterization of *Escherichia coli* from recreational waters

Presenting Author - *Ana Machado, ICBAS-Instituto de Ciências Biomédicas Abel Salazar, University of Porto, Portugal*

Author/s – *Lúcia Gomes, Adriano A. Bordalo, Ana Machado*

Abstract Content

Background: *Escherichia coli* is a commensal bacterium of warm-blood animals, and is considered a major microbiological indicator of fecal contamination in the aquatic environment. Several *E. coli* strains can cause both extra-intestinal and intestinal diseases in humans, being of public health concern.

Objectives: The purpose of this study was to investigate the origin and pathogenic profile of fecal indicator bacteria (FIB) in temperate recreational waters, and explore the potential implications for public health.

Methods: Surface water samples were collected from estuarine and coastal recreational waters. The identification and isolation of *E. coli* were performed using selective and differential culture media. Presumptive isolates were confirmed by PCR. Phylogenetic group and pathotype determination, with screening of the virulence markers, were performed by multiplex PCR. Avian pathogenic *E. coli*-associated genes were also explored. The susceptibility of the isolates to 22 antibiotics was screened using the Kirby-Bauer disk diffusion method.

Results: Thirty-three percent of the isolates were diarrheagenic *E. coli*. Among the pathotypes, enterotoxigenic *E. coli* (ETEC) was the most common. Phylogenetic group B1 and D, which are predominant in animals, accounted for the majority of the isolates studied. All isolates revealed resistance to at least one antibiotic. Moreover, 70% showed resistance to at least one antibiotic from 3 different classes (i.e. multidrug-resistant bacteria). All isolates showed susceptibility to imipenem and nitrofurantoin. The results suggested that non-human mammals and birds played a key role in fecal contamination, highlighting the importance of FIB characterization to improve public health risk management to ensure users safety.

W318 - Exploration and verification of hair bacteria that regulate the expression of genes related to proliferation and hair growth in

Presenting Author - *Azusa Yamada, Kyushu University, Japan*

Author/s – *Mugito Oshiro, Yoshinori Katakura, Kenji Sakai, Yukihiro Tashiro*

Abstract Content

Background: Maintaining healthy hair is one of the important factors for improving the quality of life. Hair bacteria actively proliferate on hair roots due to nutrient supplementation from host cells [1-3]. However, the effect of hair bacteria on the activities of host cells is still unknown. In this study, we investigated the effect of hair bacteria on the modulation of gene expression related to keratinocyte proliferation and the progression of hair growth.

Methods: HaCaT was used as a model cell for keratinocytes at the hair follicle, and predominant bacterial strains belonging to four genera on hair were killed by heat treatment. HaCaT cells were co-cultured with dead bacterial samples, and proliferation was evaluated by quantitative staining using tetrazolium salt and Hoechst. In this study, the expression levels of SIRT1 (anti-cellular senescence) and TERT (hair growth) genes, which are related to the maintenance of healthy hair follicle cells, were quantified by RT-qPCR. In addition, the expression levels of two genes were also quantified in cell wall and cytoplasm fractions of bacterial cells.

Results: Cell activity and NADH levels in HaCaT cells increased with co-culture with predominant hair bacteria such as Cutibacterium, *Pseudomonas* and Staphylococcus. These bacteria also promoted the expression of SIRT1 and TERT in HaCaT. In addition, the fraction of bacterial cell walls upregulated SIRT1 expression three times more than the fraction of bacterial cytoplasm. These results suggested that hair bacterial strains, especially bacterial cell walls, would play a role in regulating several genes in keratinocytes at hair follicle.

W319 - Effect of biopolymer stabilization in mine residue previously biotreated

Presenting Author - *Joana B. Caldeira, University of Coimbra, Portugal*

Author/s – *António A. Correia, Rita Branco, Paula V. Morais*

Abstract Content

Background: Bioleaching is a metal recovery method using biological processes. Promoting mine residues' bioleaching and biotransformation of leached waste into technosols is a strategy that achieves objectives for sustainability. Soil's microbiome plays a role in soil processes and functions, usually not considered when developing technosols.

Objectives: This work aims to recover residues' metals and, with bioleached residues, develop technosol with relevant mechanical characteristics. Bioleaching was promoted by biostimulation of residues' microbial community or bioaugmentation with strain D.polyhydroxybutyrativorans B2A2W2, followed by stabilization with biopolymers carboxymethyl cellulose (CMC) or xanthan gum (XG).

Methods: Mine residue was biotreated by biostimulation or bioaugmentation with B2A2W2 using laboratory drainage-flow systems. Then the residue was stabilized with 1% w/w of biopolymers CMC and XG, and Portland cement. Stabilized biotreated residues were submitted to second leaching. Leachates were analyzed for heavy metals concentration, biological activity, pH, and residues characterized by mechanical characteristics.

Results: XG promoted metal leaching for biotreated residues, whereas CMC showed a similar leaching profile to cement. Stabilization of bioaugmented residue with CMC led to a reduction in the leachate concentration of Zn (1.9 times), Si (1.2 times) and Fe (44.6 times), and an increase of viable microorganisms (2log) compared to non-stabilized residue. Fe-bioleaching is time-dependent and increased with XG addition, achieving 530mg·l−1 Fe in leachate. Bioaugmented residue stabilized with XG had best results regarding unconfined compression strength.

The findings from this study showed that biotreated residues' stabilization with biopolymers produces a mine residue with improved soil mechanical characteristics, increase biological activity and pH, and decreased metal leaching.

W320 - Direct-geneFISH to link antibiotic resistance gene presence and phylogeny in model strains and microbial populations

Presenting Author - *Gangan Wang, The Helmholtz Centre of Environmental Research, Germany*

Author/s – *Cristina Moraru, Sarah Haenelt, Florin Musat, Niculina Musat*

Abstract Content

Linking antibiotic resistance genes (ARGs) with cell phylogeny can provide direct proof on the antibiotic resistance potential of a specific bacterial group and can be used to trace the abundance and spread of such genes in microbial populations. Here we applied a modified direct-geneFISH approach, which combines rRNA-targeted catalyzed amplification reporter deposition - fluorescence in situ hybridization (CARD-FISH) and in situ gene detection, for visualization, identification and quantification of ARG containing cells. Three sul1-targeting polynucleotide probes and a non-sense probe (Nonsul) were designed to specifically target sul1.The proof of principle experiment was conducted on model organisms: *Citrobacter* EC35 and *Acinetobacter defluvii* strains that contain the sul1 gene, and *Pseudomonas Putida* KT2440 and *Desulfosarcina* BuS5 strains that do not contain this gene. The method was applied on naturally formed river biofilms and on a mixture of biofilm samples and sul1-containing *Acinetobacter defluvii*. The results showed the sul1 gene presence only in the sul1-containing strains, and no false positive signals were detected. Direct-geneFISH on biofilm sample showed an overall low abundance of Acinetobacter like phylotypes and the absence of sul1 gene signal in these populations. However, the sul1-containing *Acinetobacter defluvii* could be traced back in the artificially constructed mixture. In summary, our preliminary results showed that direct-geneFISH method can be used to detect and trace back the ARG-containing bacteria in model systems and environmental samples.

W321 - The phytobiome of the medicinal plant *Origanum vulgare*: linking the bacterial endophytic communities to the essential oil

Presenting Author - *Giulia Semenzato, University of Florence, Italy*

Author/s – *Sara Del Duca, Alberto Vassallo, Roberta Ascrizzi, Marinella De Leo, Luisa Pistelli, Giovanni Emiliani, Renato Fani*

Abstract Content

Background: Antimicrobial resistance is a global concerning issue associated with high morbidity and mortality. Multidrug-resistance bacteria (MDRB) may be untreatable with conventional antibiotics; hence, it's important to prioritize the development of alternative therapies. Medicinal and aromatic plants represent a natural source of bioactive molecules. In particular, antibacterial, antifungal and antiviral activities have been reported for *Origanum vulgare* subspecies and their essential oils (EOs).

Objectives: The aim of this work is to characterize the bacterial endophytic community associated with *O. vulgare* subspecies, to select a collection of bacteria able to synthesize antimicrobial molecules and to understand if the aroma profile of plant EOs might be influenced by the presence of the endophytes and/or if some EO compounds might be synthesized by the endophytes themselves.

Methods: To this purpose, endophytic communities were isolated from different plant compartments and the EO was extracted from the same plants. The bacteria were characterized through 16S rDNA sequencing and RAPD analysis; antibiotic resistance and antagonistic interactions were also evaluated. In addition, the genome of some of the isolates was sequenced.

Results: The analysis of RAPD profiles and the taxonomic affiliation of the endophytic community revealed a high degree of biodiversity and a low degree of strain sharing between plants and compartments of the same plant. Moreover, some endophytes inhibit the growth of MDRB through the synthesis of volatile organic compounds, some of which also found in *O. vulgare* EO chemical composition. The analysis of the genomes might shed light on the metabolic pathways involved.

W322 - Effect of growth conditions on Ochrobactrum tritici 5bvl1 strain Rare Earth Elements immobilization capacity

Presenting Author - *Beatriz Rito, Coimbra University, Portugal*

Author/s – *Romeu Francisco, Carina Coimbra, Rita Branco, Paula V. Morais*

Abstract Content

Rare Earth Elements (REE) are considered critical raw materials (CRM) with a wide application in modern technologies. Yet current efforts to recycle them are seemingly relatively ineffective, highlighting the need for the development of specific recovering methodologies with high selectivity and purity.

The objective was to evaluate the ability of Ochrobactrum tritici 5bvl1 strain to immobilize REE from a multimetal synthetic leachate, studying the effect of supplementation with KH2PO4 during growth, as well as the effect of the inoculum cell density on the immobilization process.

Two types of 5bvl1 strain inoculum were performed: a low starting OD600nm of 0.06 and a dense cell suspension with 0.5 final OD600nm, in the presence or absence of phosphate supplementation; followed by inoculation on synthetic leachate (8.2 ppm Y; 10 ppm Sc; 8.5 ppm La; 30.7 ppm Ce; 1.9 ppm Pr, 8.5 ppm Nd) during 3 days incubation.

The ICP-MS results showed that 1) higher cell density yielded better REE bioaccumulation, 2) phosphate was required to stabilize the metals in the cells and to increment the immobilization, and 3) the metal accumulation per biomass was higher at 24h. The REE found in higher quantities in the cells grown under these conditions were Nd (17 µg/mg protein) followed by Y (16 µg/mg protein) and La (15 µg/mg protein).

SEM-EDS demonstrated that the cell morphology was not affected by REE accumulation. SEM-EDS and ICP-MS results evidenced an improved immobilization of all tested REE but particularly Nd when cells were supplemented with phosphate during growth.

W323 - The influence of external redox potential and pH on *Thermus scotoductus* K1 during aerobic utilization of glucose with different concentrations.

Presenting Author - *Hripsime Petrosyan, Department of Biochemistry, Microbiology, and Biotechnology, Yerevan State University, Armenia*

Author/s – *Hovik Panosyan, Karen Trchounian*

Abstract Content – This study represents the investigations of *Thermus scotoductus* K1 growth parameters in presence of different concentrations of glucose. Bacteria were grown in culture medium containing peptone 1g/l, yeast 1g/l, salt solution [1], glucose as a carbon source was added 1, 2 and 4g/l. Final pH was adjusted with NaOH to 8.5, 7 and 6.

Bacterial night culture was inoculated in liquid media in flasks (with 3:2 ratio with air to provide oxygen), and incubated under 65C and 150 rpm. Sampling was done during 0-144h of growth, and measured redox potential (ORP, mV), external pH values and optical density (OD).

When bacteria grown in pH 8.5 showed ORP drop to ~ -15.3mV in 72h of growth in presence of 1g/l glucose, and ~15mV in 24h in presence of 2 g/l glucose. Meanwhile ORP values during 4g/l glucose utilization was more positive in 48-72 h of growth. At the same hours of growth pH values were the most alkaline observed above 8.2 to 9. Biomass production was optimal in presence of 2g/l glucose.

Bacteria showed strict differences in values until 24h of growth pH 6. However, after 24h they grew showing 1.2-1.6 OD, parallelly basicization of pH occurred up to bacterial optimal values. Changes of mV to the lowest values was observed in 144 h of growth -27 mV in presence of 1g/l glucose,

Data shown highlighted the essential of further research and discovery to be done to describe utilization pathways of carbon sources and bioenergetic states environment of bacterial growth.

W324 - Bacterial biopolymers sorbents for metal removal

Presenting Author - *Ana Paula Chung, University of Coimbra, Portugal*

Author/s – *Romeu Francisco, Ana Paula Piedade, Paula V. Morais*

Abstract Content

Background: Currently, increasing research interest has been directed to bio-based materials as alternatives to remove metal contaminants from wastewater. Biopolymers are excellent examples of such materials, showing excellent metal-binding properties with varying degrees of specificity and affinity due to the presence of functional groups, such as amino, carboxyl, hydroxyl, and phosphate.

Objective: In this work metal biosorption ability of cellulose-like polymers produced by two bacterial strains isolated from different environments was analyzed.

Methods: The identification of genes involved in biopolymer production and their genetic organization was performed in the RAST Server (https://rast.nmpdr.org/rast.cgi). The metal biosorption ability of both biopolymers was determined in 50 ml of saline solution (0.85% NaCl) with an initial metal concentration of 1 mM (Cu2+, Zn2+, Ga3+, Te3+, and In3+) and 20 mg or 40 mg of biopolymer.

Results: The biopolymers produced by these bacteria seem to be cellulose-like polymers. Both genomes contain genes encoding for BcsA and BcsB subunits, the minimum complex required for cellulose synthesis. The biosorption capacity of both biopolymers was similar for Ga in the two doses of biopolymer tested, ranging between 56.65 and 95.4 mg/g. In the case of copper biosorption, the biopolymer produced by strain Faro439 showed a higher biosorption capacity (93.72 mg/g) when compared to the one produced by strain 20M4 (73.13 mg/g) at the dose of 20 mg. In presence of Zn, strain 20M4 biopolymer`s showed a biosorption capacity 4 times higher (80.2 mg/g) compared to Faro 439 biopolymer. Both biopolymers were not able to biosorp Te and In.

W325 - A system for surveillance of FluA, FluB, SARS-CoV-2 and RSV using wastewater-based epidemiology

Presenting Author - *Saul Dr., Promega Corporation, United States*

Author/s – *Dongping Ma, Nathan Feirer, Brigitta Saul, Kevin Hsiao, James Cali, Subhanjan Mondal*

Abstract Content

Background: The COVID-19 pandemic has spurred investigation on the use of wastewater-based epidemiology (WBE) as a tool for monitoring the appearance and spread of COVID-19 in communities. This has raised interest in surveillance of additional respiratory viruses using WBE.

Objectives: To develop a multiplex RT-qPCR assay of detecting Influenza A, Influenza B, SARS-CoV-2, and Human Respiratory Syncytial Virus (RSV) and use it in combination with a direct capture method for concentration and purification of wastewater samples.

Methods: We optimized a RT-qPCR detection with FluA, FluB, SARS-CoV-2, RSV in FAM, Yakima Yellow, Texas Red and Cy5 channels. We also developed quantitation standards for quantitation of viral genome loads and Using Pepper Mild Mottle Virus (PMMoV) in Cy5.5 channel as an internal positive control allowed us to normalize of the viral genome loads. The PCR mastermix was formulated to tolerate PCR inhibitors in the sample.

Results: The limit of detection for FluA, FluB, SARS-CoV-2 and RSV were 10, 10, 5 and 20 copies per reaction, indicative of high sensitivity. Using this workflow, we monitored wastewater samples from two WWTP in Dane County, Wisconsin, serving a combined population of over 300,000 people, from November 2022. We have analyzed samples for over three months now and will continue monitoring it. We were able to detect all viral targets from wastewater samples during the period of testing so far.

W326 - High genomic diversity and poor geographical stratification of *Legionella pneumophila* in a local area

Presenting Author - *Andrea Colautti, University Of Udine, Italy*

Author/s – *Emanuele De Paoli, Marcello Civilini, Giuseppe Comi, Lucilla Iacumin*

Abstract Content

Over a 12-year period, 200 strains of *Legionella pneumophila* were collected from a specific geographical region from residences of patients hospitalized with legionellosis. The objective was to determine if there were any correlations between the place and time of isolation and to assess genetic stratification based on geographical location. After DNA extraction using the MagAttract HMW DNA kit, the genomes of the strains were sequenced using the Illumina MiSeq platform and assembled using the WGA-LP Pipeline (1). Phylogenetic clusters were obtained using Roary (2), pyMLST (3), Phylonium (4), and PhyloPhlAn (5) and further analyzed to identify any recurring features, such as CRISPR/Cas arrays (6), prophages (7), insertion sequences (8), and virulence factors (9). The results showed consistent phylogenetic patterns from all the clustering methods, identifying eight main clusters. However, no correlations were found between the period or area of isolation. Instead, a strong correlation was observed between the clusters and the type of patient's residence (private home, hospital, or retirement home). The clusters also differentiated based on specific genetic features, such as CRISPR/Cas and virulence factors. Comparisons with the *L. pneumophila* strains from the Genbank repository revealed that their genotypes were not specific to the overall investigated region but were part of larger clusters composed of isolates from across Europe.

W327 - Differential Production of Pigments by *Rhodanobacter* strains Under Metal Exposure

Presenting Author - *Rita Branco, Coimbra University, Portugal*

Author/s – *Paula V. Morais, Ana Paula Chung*

Abstract Content

Background: Bacterial pigments are natural pigments used in the textile, food and pharmaceutical industry that can have many biological properties.

Objectives: In this work, the production of a red pigment as a strategy to cope with metals was investigated in a group of yellow-pigmented strains from the genus Rhodanobacter.

Methods: The effect of several metals (cations and oxyanions) on growth and pigment production was assayed in several Rhodanobacter strains isolated from mine residues. Pigments were extracted using chloroform/methanol solutions and visualized on thin layer chromatography (TLC). The maximum absorption spectrum of the extracted pigment was observed by means of UV- A visible spectrophotometer within the range of 300-800 nm.

Results: *Rhodanobacter* strains highly resistant to several metals produced a reddish pigment in response to metals such as indium, aluminum, yttrium, lanthanum, vanadium and tungsten. No pigment formation was observed when *Rhodanobacter* strains were exposed to additional stress such as salt, temperature or pH. The genome analysis showed the presence of a pig-like gene cluster that encodes for xanthomonadin-like pigments, usually present in the *Xanthomonas* strains. The pairwise homologies between the proteins encoded by the pig clusters by these two group of microorganisms ranges between 32 to 73%. TLC analysis of the extracted pigments showed the presence of 2-3 yellow bands and an additional red band only in extracts of *Rhodanobacter* strains grown in presence of the critical metals. The antioxidant and antimicrobial activity of these pigments is being evaluated to assess the potential of these metabolites in biomedical applications.

W329 - Bioassays for packaging material

Presenting Author - *Markus Windisch, Medical University Of Graz, Austria*

Abstract Content

Background: The use of Bioassays for the detection of genotoxic substances in packaging material has increased in recent years. The Ames test is one of the most frequently used bioassays for this purpose. It is a model based on bacterial reversion of gene function.

Objectives: In this study, we tested 7 different packaging materials with the Ames Microplate Format (MPF) test for potential genotoxicity.

Material and Methods: 10 grams of packaging material was cut into pieces of 1 to 2 cm2 and extracted in 200 ml 99% ethanol for 6 hours at 60 C°. Final volume of extracts was 5 ml after reduction with Biotage. Genotoxicity was assessed using the Ames Microplate Format (MPF) test with the *Salmonella typhimurium* (S. typhimurium) strains TA98 and TA100 with and without addition of rat liver S9 for metabolic activation.

Results: None of the tested products revealed genotoxicity in the Ames MPF test. However, not all strains-extract combinations were perfectly compatible and additional models like eukaryotic systems have to be tested.

W330 - "MITES" as a tool to associate microbial host to virus

Presenting Author - *Francisco Nadal Molero, University of Alicante, Spain*

Author/s – *Alicia Campos-Lopez, Riccardo Rosselli, Ana-Belen Martin-Cuadrado*

Abstract Content

Miniature Inverted-repeat Transposable Elements (MITEs) are transposable elements described in genomes of all domains of life that lack their own transposase, an enzyme essential for movement through the genome. The aim of this study is to analyse if MITES can be used as trusted system to assign virus to host.

Using the software MITETracker1, MITEs were detected from 8222 Archaea and 255466 Bacteria genomes together with GenBank's and JGI's (IMG/VR v42) viral contigs. Those MITE sequences containing transposases (partial or complete) were filtered. Due to the high mutational bias of the viral genomes, a second search of MITEs was performed. Using the previous bonafide MITES, new "PseudoMITEs" were obtained by BLASTN comparisons against the genome databases. Clustering tools at 95%id in the total length allowed to identify the putative pairs "host-virus".

Thousands of bonafide-MITES were found in each group: 18091 in Archaea, 1406057 in Bacteria and 1726 in virus. By clustering tools performed in each group individually, it was probed that MITE´s specificity was maintained at genus level (only 0.03% of clusters were found beyond these taxon limits). The clustering of all the detected MITEs/PseudoMITEs at once, allowed to determine 1245 clusters (involving 935447 MITEs) where a Bacteria or an Archaea share the same MITE than virus, suggesting a transfer during the infection cycle or during a latent stay. From them, 46 clusters presented previously known host-virus associations (involving 4974 MITEs), therefore concluding that MITEs (and PseudoMITES) may be a useful association tool when present in both systems.

W331 - Treatment of urban effluent for agricultural reuse: occurrence and removal of antibiotic resistance and pathobiome

Presenting Author - *Manuela Macrì, University of Torino, Italy*

Author/s – *Manuela Macrì, Sara Bonetta, Andrea Di Cesare, Raffaella Sabatino, Marta Catozzo, Cristina Pignata, Valentina Schiavo*

Abstract Content

Treated wastewater reuse gained increasing interest due to global water shortage emergency. Although the reuse practice brings unquestionable benefits, it could lead to different microbiological risks due to its intrinsic characteristics.

The aim of this study was to evaluate the occurrence of antibiotic resistant bacteria (ARB), antibiotic resistance genes (ARGs) and potential pathogens along wastewater treatment.

In different steps of a wastewater treatment plant (WWTP) developed for reuse were investigated: i) the presence of ARB and ARGs with cultural (R2Agar supplemented/not-supplemented with ampicillin, tetracycline, sulfamethoxazole) and molecular methods (ddPCR targeting blaTEM, tetA, sulII); ii) the concentration of antibiotics and heavy metals; and iii) the pathobiome (identified by 16S rRNA amplicon sequencing).

The results obtained highlight the presence of ARB and ARGs in all WWTP steps, with decreasing trend along wastewater treatment, as also observed for some antibiotics and heavy metals. Generally, no significant difference in the ARB rates was observed in the different WWTP steps, but a correlation between the presence of some heavy metals and ARB rates was revealed. sulII and tetA were widespread with a significant reduction in the reuse samples compared to the inlet. The pathobiome characterization showed the presence of potential pathogens in inlet and final waters. However, a significant decrease in the abundance of these bacteria across the WWTP treatments was observed.

Overall these results, although showing a general efficiency of wastewater treatment in lowering all the tested contaminants, highlight the potential risk for human health in case of reuse of treated wastewaters in agriculture.

W332 - Time-kill kinetics reveal heterogeneous tolerance to disinfectants

Presenting Author - *Lydia-Yasmin Sobisch, Bundesanstalt für Materialforschung und -prüfung (BAM), Germany*

Author/s – *Niclas Nordholt, Frank Schreiber, Dominique Lewerenz*

Abstract Content

Background: Disinfection is an important mitigation strategy to control and prevent the spread of infections. Incomplete or incorrect usage of disinfection may promote evolution of resistance against disinfectants and antibiotics. Ideally, disinfection reduces the number of surviving bacteria and the chance for resistance evolution. Resistance describes the ability to grow in previously inhibitory concentrations of an antimicrobial, whereas tolerance is associated with enhanced survival of lethal doses. Individual bacteria from the same population can display considerable heterogeneity in their ability to survive treatment (i.e. tolerance) with antimicrobials, which can result in unexpected treatment failure.

Objective: In this study, we investigated six active substances of disinfectants, preservatives, and antiseptics against a population of *E. coli* to identify the presence of a tolerant subpopulation.

Methods: We performed time-kill experiments and analyzed the data with a mathematical model to statistically infer whether the data is best explained by the presence of a tolerant subpopulation.

Results: The analysis identified bimodal kill kinetics for benzalkonium chloride, didecyldimethylammonium chloride, and isopropanol. In contrast, kill kinetics by chlorhexidine, glutaraldehyde, and hydrogen peroxide were best explained by unimodal kill kinetics. These findings have implications for the risk of disinfection failure. In addition, we are currently performing adaptive laboratory evolution (ALE) experiments with the different disinfectants to investigate the potential consequences of tolerant sub-populations for the evolution of antimicrobial resistance and tolerance.

W333 - Colistin resistant *Klebsiella pneumoniae* in intensive rabbit farms after colistin ban

Presenting Author - *Marisa Ribeiro-Almeida, Icbas - School of Medicine and Biomedical Sciences, University of Porto, Porto, Portugal*

Author/s – *André Pinto de Carvalho, Paulo Martins da Costa, Ângela Novais, Luísa Peixe, Patrícia Antunes*

Abstract Content

Background: Expansion of colistin-resistant bacteria is a well-recognized public health problem linked to food-animal production, which prompted recommendations for colistin ban globally. However, their impact on intensive rabbit production and if food-chain is a reservoir of colistin resistant *Klebsiella pneumoniae*-ColR-Kp remains unknown.

Objectives: To establish the occurrence and diversity of ColR-Kp in intensive rabbit farms, after colistin ban.

Methods: Fecal samples were collected from 18 groups of reproductive-females(M) and their offspring in 2-stages [30-39 days weaning-rabbits(R1)/58-80 days pre-slaughter rabbits(R2)], housed in 8 rabbit farms (3 farms:2-years of colistin-ban;5 farms:1-year of colistin-ban). Environmental samples (feed-n=14/nest-n=8/water-n=24) were also analyzed. Samples with/without enrichment (37C/18h) were plated in SCAI+Col. Colonies identified by MALDI-TOF MS/PCR were screened for mcr(1-9). Antibiotic (disk-diffusion/microdilution-colistin) and clonality (FTIR-spectroscopy/wzi sequencing/MLST) were studied.

Results: ColR-Kp were detected in 88% (7/8) of the farms, independently of the year of colistin-ban. Ten groups-M(6farms), 11 groups-R1(7farms) and 7 groups-R2(5farms) present ColR-Kp, with persistence of ColR-Kp in M+R1+R2 in 3farms. One feed and one water sample present ColR-Kp. All ColR-Kp (n=71; MIC=4->16mg/L; no-mcr) were multidrug-resistant, including to tetracycline-93%, ciprofloxacin-89%, trimethoprim-79%, sulfonamides-76% and cefoxitin-18%. Six K-types were detected (KL19/KL64/KL113/KL10/KL13/KL38). The most dispersed K-types were KL113 (n=23, 4farms, M+R1+R2) and KL19 (n=55, 6farms, M+R1+R2+feed), with KL19-CG15 dispersed in 3 farms.

Despite the colistin ban, rabbit farms are reservoirs of MDR ColR-Kp, including the well-established KL19-CG15 lineage. The absence of mcr genes suggests other colistin resistance mechanisms and the effect of other factors contributing to ColR persistence.

W335 - Disentangling the life-history effects of long-term tillage and no-tillage on native bacterial communities across three climatic

Presenting Author - *Piera Quattrocelli, Sant'Anna School of Advanced Studies, Italy*

Author/s – *Elisa Pellegrino, Helena Gómez-Macpherson, Gernot Bodner, Bouba Traore, Laura Ercoli*

Abstract Content

Long-term conventional tillage (CT) systems affect soil physico-chemical characteristics, impacting the soil bacterial diversity and functionality. Hence, soil structure and the abundance of beneficial bacteria can be enforced by conservation tillage, like no-tillage (NT), to improve soil fertility, crop yield, and stress tolerance. Along soil practices, their interactions with climatic parameters on shaping the bacterial communities needs to be better understood to predict the outcomes on productivity and climate change.

This study aimed at testing the hypothesis that soil bacterial composition and diversity are driven by the life-history effects of long-term CT and NT systems and by climatic parameters and that this interaction affects agroecosystem productivity. Therefore, CT and NT soils were sampled from three different climatic zones classified according to Köppen climate classification: Csa (Mediterranean area, Cordoba, Spain), Cfb (Continental area, Hollabrunn, Austria), and BWh (Arid area, Sadore, Niger).

DNA was extracted and bacterial communities were characterized by Illumina sequencing using V3 and V4 region of the16S rRNA. In CT and NT systems, bacterial composition and diversity was similar among soil systems, although differences were recorded among climatic zones. Moreover, bacterial community abundance and structure was affected by the interaction of soil system and climatic zones with consequences on productivity.

Cfb and Csa zones revealed a high abundance of Nitrososphaerales and Rhizobiales involved in nitrogen cycle, while BWh climate displayed an abundance of Burkholderiales associated with biocontrol activity and plant stress tolerance. Our results provide building blocks for a synthetic agroecological framework for predicting the outcomes of agroecosystem productivity.

W336 - Heavy metal resistant microbes as a means for bioremediation of polluted environment

Presenting Author - *Armine Margaryan, Department of Biochemistry, Microbiology, and Biotechnology, Yerevan State University, Armenia*

Author/s – *Hovik Panosyan, Diana Ghevondyan, Sona Nikolyan, Nils-Kåre Birkeland*

Abstract Content

Background: Heavy metal pollution is a major global environmental concern because of its toxicity and threat to human life and the environment, however highly adapted bacteria have evolved metal resistance systems, which make them prospective for application in the bioremediation of contaminated environments.

Objectives: The main goals of the presented work were the determination of the heavy metal-resistant microbes in the Armenian mines and tailings, the selection of the highly heavy metal-resistant strains, and the study of their ability to detoxify and accumulate toxic metal ions.

Methods and Results: A total of 50 mesophilic, acidophilic, and alkaliphilic metal-tolerant bacteria, belonging to *Acidocella, Arthrobacter, Algoriphagus, Bacillus, Brevibacillus, Comamonas, Geobacillus, Micrococcus, Methylobacterium, Pseudarthrobacter, Pseudomonas, Rheinheimera, Rhodococcus, Sinomonas* and *Stenotrophomonas* genera were isolated from the mine and tailings of Armenia. Tolerance towards Cu(II), Cd(II), Zn(II), Ni(II), Co(II), Mo(II), Pb(II), and Cr(VI) was studied, and it was found that strains are highly resistant to the studied metal ions. Polyphasic approach has been used to characterize highly resistant isolates, which were selected, also, to study their ability to accumulate and detoxify heavy metals. Thus, *Acidocella* sp. K2-4 and *Rhodococcus* sp. KT1-2 strains accumulated Zn up to 50%, while KT1-2 strain also accumulated Cu up to 40% after 24 hours of growth. Hexavalent chromium detoxification ability of the *Pseudarthrobacter* sp. KJ.4-1 strain revealed after 24 hours of the growth in the present of 1.5 mM Cu and without aeration.

The results indicate that newly isolated metal-tolerant strains could have potential in bioremediation.

W337 - Genome characterization of acidophile autotrophic Arsenite oxidizer *Acidiphilium acidophilum* CJR1.

Presenting Author - *Cristian Felipe Jorquera Roman, TU Bergakademie Freiberg, Germany*

Author/s – *Michael Schlömann*

Abstract Content

Background: Metals becoming scarce have raised the interest to process also arsenic-containing minerals. The leaching of those minerals implies the release of arsenic into the environment. Arsenic (As), in its most oxidized state, arsenate (As(V)), is less toxic and mobile than one of its reduced forms, arsenite (As(III)). Hence, the oxidation of As(III) is highly important to immobilize and treat As waste.

Objective: The aim of the study was the isolation and genome analysis of a novel acidophilic chemolithotrophic As(III)-oxidizing bacteria to develop a new As-immobilization process.

Methods: For that objective, a new As(III) oxidizing strain was isolated from the ex-mining site Reiche Zeche (Freiberg, Germany). Samples were collected and cultured in 0K medium supplemented with As(III) and analyzed for As(III) oxidation activity by ion chromatography. Cell growth was observed with a Neubauer chamber under a phase-contrast microscope.

Results: One of the isolated strains, CJR1, was identified as an *Acidiphilium acidophilum*. Its genome was sequenced using the Illumina and PacBio platforms to generate a hybrid assembly. The genome analysis revealed more arsenic resistance operons, a bigger genome size than in Ac. acidophilum type strain DSM700, and the presence of genes related to its autotrophic As(III)-oxidizing lifestyle, such as carbon fixation pathways, and As(III)-oxidase operons. Also, it was possible to identify the presence of presumably new mobile genetic elements. This work opens new doors to understanding the As(III)-oxidizing autotrophic lifestyle of Ac. acidophilum CJR1.

W338 - Metagenome-Assembled Genomes from maize rhizobiome contribute to unravel potential plant growth promoting bacterial community

Presenting Author - *Jessica Ferrarezi, University Of Sao Paulo, Brazil*

Author/s – *Jessica Aparecida Ferrarezi, Maria Carolina Quecine*

Abstract Content

The rhizosphere microbiome (rhizobiome) comprises a reservoir of ecological traits that contributes to plant nutrition, health and defense, provided by plant growth-promoting bacteria (PGPB) through nutrient cycling, modulation of phytohormones and production of secondary metabolites. Metagenome-assembled genomes (MAGs) are important tools to dissect the potential of particular understudied microbes often present in the rhizobiome. Metagenomics analyses were used to investigate ecological interactions between the commercial inoculant *Azospirillum brasilense* strain Ab-V5 and the maize rhizobiome sourced from bulk soils with different levels of microbial diversity. Our objective was to describe putative metabolic contributions of particular microbes using in silico approaches on high-quality MAGs (HQMs) obtained from these metagenomes to unravel potential PGPB. We hypothesized that Ab-V5 interacted with soil native beneficial bacteria and MAGs could harbor important genes related with plant growth promotion traits. HQMs were obtained through contig binning and analyzed through quality control with MaxBin2, annotation with Prokka, Distilled and Refined Annotation of Metabolism (DRAM), and antibiotics and secondary metabolite analysis shell (antiSMASH). A total of 12 HQM were obtained, and the majority was taxonomically assigned to PGPB taxa, as follows: *Frateuria*, *Paracnuella*, *Pseudarthrobacter* (n=2), *Exiguobacterium*, *Microbacterium*, *Fictibacillus*, *Paracoccus*, *Sphingobacteriaceae* and *Opitutus*. One HQM was identified as *Ralstonia* and one as *Nitrososphaerales* (Archaea). These HQM contain genes related to nitrogen and phosphorus cycling, and biosynthetic gene clusters related to ROS stress alleviating, biofilm formation and defense, indicating their potential as beneficial microbes. This study provides new insights into PGPB candidates previously overlooked or unknown from the maize rhizobiome.

W339 - Detection and monitoring of VHS infection in Rainbow trout through environmental sampling

Presenting Author - *Giulia Zarantonello, Technical University of Denmark, Denmark*

Author/s – *Dagoberto Sepulveda Araneda, Niels Lorenzen, Argelia Cuenca Navarro*

Abstract Content

Viral hemorrhagic septicemia (VHS) is a deadly viral disease affecting salmonids which represents a major concern for European aquaculture. Current diagnostics procedures involve lethal sampling and pooling from individual fish to perform targeted detection. However, once the virus is identified in farmed animals, it is of great interest to track the viral particles in the environment when attempting to limit the spread of the disease. Here, we tested and compared non-invasive environmental samples from a controlled VHS infection trial in Rainbow trout, aiming to develop a novel strategy to monitor the pathogen presence, spread and persistence. Water samples were longitudinally collected over the experimental trial at different stages of the disease. As a reference, we assessed infectious viral units of environmental samples; environmental RNA (eRNA) quantification results were then compared across sampling and concentration methods. Notably, non-invasive sampling allows direct detection of the viral genome even after infection peak. All molecular quantification methods were more sensitive than viral replication on cell culture and, among concentration methods, water filters are the most promising for field application, with highest amount of detected VHSV genes. Additionally, since dysbiosis often occurs in association to disease, we investigated if shifts in water microbiome composition could predict disease status. Using Nanopore sequencing, we performed 16S rRNA gene metabarcoding to detect eventual water communities' dysbiosis associated to disease progression in the fish. Remarkably, environmental microbial communities were impacted by VHS infection in the host, opening new possibilities for indirect monitoring of the farmed animals' health conditions.

W340 - Potential bloom-forming phytoplankton from South West coast of Maharashtra, India and North West coast of Goa, India

Presenting Author – *Shalini Sarkar, CSIR - National Institute Of Oceanography, India*

Author/s – *Shalini Sarkar, Samir Damare*

Abstract Content

The monsoon patterns play a significant role in making the Indian Ocean a unique system and thus distinct from the other global oceans. One of the most promising features is the Northeast monsoon or Retreating monsoon during the winter months, viz November to February. Winter blooms in the northeastern Arabian sea is mainly due to convection processes driven by sea surface cooling when nutrients are injected up into the surface waters. It has been reported that dinoflagellate blooms dominate the west coast of India, while diatom blooms are dominant in the east coast . Harmful algal blooms are gaining the limelight all across the globe due to their havoc-creating capabilities. India has also seen a rise in bloom events in the last five decades, although the frequency is less than in Pacific or Atlantic waters.

To have an idea about the factors causing blooms and the species involved, a study was conducted where surface water was collected from beaches along Maharashtra and Goa coast. The unfixed samples were used for culturing the plankton. Major potential toxin producers like *Procentrum* sp., and *Gymnodinium* sp. were found in the samples. Other dinoflagellate species, like *Ceratium* sp. were also present. In order to check the ability to produce toxins by these plankton species are being grown in the laboratory. The toxin production will be confirmed by LCMS-MS analysis of the cell extracts of purified cultures.

W341 - Variations in bacterial taxonomic composition of river water treated with skimmed milk flocculation and vacuum filtration

Presenting Author - *Deyan Donchev, National Center Of Infectious And Parasitic Diseases, Bulgaria*

Author/s – *Ivan Ivanov, Ivan Stoikov, Elina Dobreva, Rumyana Hristova*

Abstract Content

Background: Skimmed milk flocculation (SMF) has been implemented in streamlined clinical sequencing protocols for viruses, whereas vacuum filtration (VF) is widely used for bacterial studies.

Objectives: To evaluate the bacterial taxonomy composition of river water treated with SMF and VF by 16S metagenomics.

Methods: An urban river sample was divided into two equal portions. For SMF, sterile skim milk was added at 0.05% final concentration. The sample pH was adjusted to 3.5-4.0 with 1M HCl, agitated for two hours, distributed into tubes, centrifuged and DNA extracted from pellets. The other portion was filtered through 0.22µm sized pore nylon filters, which were cut into pieces and added to extraction tubes. Four commercial kits and one in-house protocol were evaluated for DNA extraction in duplicates. ZymoBIOMICS microbial test standard (MTS) was included. 16S rRNA V3-V4 region was amplified and sequenced on MiSeq V3 (2x300bp). Taxonomic analysis was accomplished with EzBioCloud and Qiime2 with SILVA v138.1.

Results: With both tools, genus-level accuracy was achieved for the eight species in MTS for each extraction kit. The observed relative frequencies of the in-house protocol and the FastDNA kit samples most closely resembled the theoretical composition. Dissimilarity analysis revealed that beta diversity between SMF and VF replicates was high in each extraction kit, indicating significant taxonomic variations. The abundances of *Saprospiraceae* and *Polaromonas* increased 3 to 7-fold in the SMF samples. Kit-to-kit taxonomic composition variations were less than SMF-to-VF. Skimmed milk utilization by protein-degrading bacteria such as *Saprospiraceae* was hypothesized.

W342 - A proteomics perspective into recurring bacteria during algal blooms in the North Sea

Presenting Author - *Vaikhari Kale, University Greifswald, Germany*

Author/s – *Vaikhari Kale, G. Y. Grace Ho, Sandra Maaß, Bernhard Fuchs, Dörte Becher*

Abstract Content

Microalgae flourish in marine ecosystems along coastlines due to seasonal changes. Algal species contribute to at least half of the global photosynthetic carbon fixation. During and following the algal bloom, the nutrients released in the seawater provide favourable conditions for bacterioplankton to grow. Using carbohydrate-active enzymes specific to algal polysaccharides, these bacteria have the metabolic potential to strive in the environment. First insights into the samples revealed a dynamic change of bacterioplankton composition in response to spring diatom bloom, which largely consists of a diverse array of Flavobacteriia. This study focuses on the key proteins involved in algal polysaccharide degradation in an environmental bacterium, *Polaribacter* sp. KT25b which was isolated from Helgoland in the North Sea. As this species represents only a small fraction of the biomass in the marine ecosystem, we test a proteomic sample preparation workflow for low cell numbers to explore the experimental limits of analyses dedicated to Polaribacter sp. For an in vitro analysis of proteins from 1 million *Polaribacter* sp. KT25b cells, we employed 2-step in solution digestion protocol for sample preparation. Liquid chromatography with tandem mass spectrometry analysis reveals identification of 1508 proteins in 3/3 sample replicates in data independent acquisition mode in comparison to 381 proteins in data dependent acquisition mode. In the future, this workflow can be combined with cell-sorting experiments targeting flavobacterial genera known to be involved in polysaccharide degradation during spring diatom blooms in Helgoland.

W343 - Microbiome of hospital surfaces independent of human occupation

Presenting Author - *Pedro Daniel Farias, University of Coimbra, Portugal*

Author/s – *Xuanji Li, Søren Sørensen, Paula V Morais*

Abstract Content

Background: In a man-made environment, the colonizing microbiome is dependent on the materials of the surface tested, their humidity, light availability, and in particular their utilization, both purpose and users. Handling equipment and surfaces has an impact on the microbial community associated with these. In a clinical environment, knowing the space's microbiome is important for controlling the dissemination of pathogens between spaces and users.

Objective: This study aims to evaluate the microbiome of a clinical environment before human occupation to determine the factors impacting microbial diversity before regular use by human activity.

Methods: The health care unit was sampled after construction while fully equipped to receive patients and staff. Thirty-three samples were collected among three floors and 10 distinct rooms. All samples were characterized for physical parameters such as humidity, dust, and light exposure. DNA from each sample was collected, and the bacterial diversity was determined based on Illumina sequencing of the 16SrRNA V3-V4 for each sample. All diversity indicators were determined by bioinformatic analysis.

Results: Only two showers' samples were dissimilar from all others according to α-diversity indices. The bacterial community is more related to the equipment than to the space. Correlation analysis clustered surfaces from bathrooms opposing most other surfaces and pieces of equipment, humidity levels have a higher impact on microbial community variability. *Geobacillus* sp. is a dominant genus, 20 to 60% of the identified genera, in most samples analysed followed by *Acinetobacter* sp and *Pseudomonas* sp.

W344 - Increased Use of Quaternary Alkylammonium Compounds during the SARS-CoV-2 Pandemic: Multi-resistance Development in Bacteria

Presenting Author - *Sanjana Balachandran, Justus-Liebig University Giessen, Germany*

Author/s – *Sanjana Balachandran, Sophie Lennartz, Bernd Göckener, Jan Koschorreck, Ines Mulder, Stefanie P. Glaeser*

Abstract Content

Increased use of disinfectants containing quaternary alkyl ammonium compounds (QAACs) during the SARS-CoV-2 pandemic may have increased the occurrence of QAAC resistant bacteria in wastewater, thereby increasing the spread of QAAC resistant bacteria into the environment. Since QAAC resistance genes are often co-located on genetic elements with antibiotic resistance genes, co-selection processes may increase the abundance of multidrug resistant (MDR) bacteria as a consequence to QAAC exposure. Suspended particulate matter (SPM) are solids with diameters greater than 0.45 µm, suspended in water bodies such as rivers and can be separated through filtration, centrifugation or settling (ISO 5667-17, 2008). The composition of SPM particles is influenced by land use, urbanisation, wastewater treatment technologies prevalent in surrounding areas, making them favourable indicators for measuring QAAC resistance gene spread and MDR development in faecal and environmental bacteria. In the current study, SPM samples from three major rivers in Germany with different wastewater impacts were analysed. Samples collected by the German Environmental Specimen Bank from 2006 to 2021 were used to monitor the effects of increased QAAC release since the onset of the pandemic. Total DNA was extracted from SPM samples and the abundance of QAAC resistance genes is currently being studied in comparison to the total bacterial load. Cultivation dependent studies were successfully performed to culture QAAC resistant *Pseudomonas* spp., *Aeromonas* spp. and *Enterobacteriaceae* including *Klebsiella* spp. and *Citrobacter* spp. and will be characterized thoroughly especially with respect to their MDR status which is expected to increase since the onset of the pandemic.

W345 - Illuminating the balance between drift and kinetics in biofilm formation using an agent-based model to manipulate luck

Presenting Author - *Joseph Weaver, Newcastle University, United Kingdom*

Abstract Content

Background: Biofilm communities are shaped by both deterministic and stochastic forces, but the relative importance of those forces varies. Understanding why that balance shifts is desirable, but experimentally manipulating (rather than controlling for) random processes driving stochastic assembly is difficult.

Objectives: We wished to determine the relative importance of deterministic factors (i.e., kinetics) vs. randomness (i.e., luck) during biofilm formation by manipulating both within agent-based models. Further, we wanted to determine how crowding intensity (initial competition population size and spacing) affected that balance.

Methods: Luck was manipulated via 120 seed values controlling random number generation, which determined biomass allocation and division. In the initial simulations, every organism was exactly identical and success or failure was exclusively due to those random factors. The least-successful lineage for each run was identified, assigned different kinetics, and the simulations were re-run using the same random seeds (130680 simulations). This enabled quantifying the change to a kinetic value required to overcome 'bad luck'.

Results: A non-trivial improvement (10-20%) to maximum specific growth rate or substrate affinity is required for a failing lineage to have a 50% chance of overcoming 'bad luck' and transition to thriving. Further, at moderate spacings (5 bacterial diameters) there were large ranges were neither deterministic nor stochastic forces dominated. Those ranges shrank with extremes, drift and growth were respectively favoured by close and loose crowding. There were strong interactions between experimental factors which could not be adequately captured by simple linear models but which were reproducible using generalized additive models.

W346 - Assessing the risk of biocide susceptibility changes: The effect of culture density

Presenting Author - *Layali Jadaan, University of Manchester, United Kingdom*

Author/s – *Christopher Knight, Gavin Humphrys, Thomas Wilmot, Andrew J McBain*

Abstract Content

Evaluation of the risk of microbial resistance to biocidal compounds is frequently done by exposing microorganisms grown in pure culture at high cell-density to sub-lethal aqueous solutions of biocides. The deployment of biocides however frequently involves i. Formulation of biocides with additional compounds that may increase biocidal potency; ii. Relatively low microbial cell-density, particularly in preservation and iii. High biocidal concentrations such that exposed microorganisms may be rapidly inactivated. We have assessed the effect of cell-density on the outcome of such exposures. Eleven microbial strains were exposed to three cationic biocides along with bisphenol biocide triclosan as a comparator. Test microorganisms were diluted 1:1000 or applied directly to agar plates upon which concentration gradients of biocide had been established, and each passaged ten times. Susceptibility of the resulting strains to the four biocides and 12 antibiotics was determined and compared to control organisms. For bacteria exposed to the cationic biocides at low cell-density, 3/33 combinations resulted in over 2-fold decreases in biocide susceptibility. By comparison, 9/33 susceptibility decreases occurred for high cell-density exposures. Furthermore, 34-fold decrease in triclosan (TCN) susceptibility in *E. coli* was observed after exposure at high cell-density compared to no changes at low density. Increases (34/380) and decreases (40/380) in susceptibility to antibiotics occurred after exposure to biocides but only at high cell-density. Susceptibility changes also occurred in non-biocide passaged controls (increase= 21/130, decreases = 16/130). This highlights the importance of cell-density as a relatively little-studied factor in biocide risk assessment.

W347 - Insights into the fungal pathobiome associated with walnut dieback in France

Presenting Author - *Marie Belair, LUBEM, Univ. Brest, France*

Author/s – *Flora Pensec, Cyrielle Masson, Marie-Neige Hébrard, Yohana Laloum, Gaétan LeFloch, Adeline Picot, Agnès Verhaeghe, CTIFL, Centre opérationnel de Lanxade, Prigonrieux, France*

Abstract Content

Background: Since 2013, new unseen symptoms including branch dieback, fruit necrosis and blight have appeared in French walnut orchards, yet widespread in Mediterranean-climate countries, with members of *Botryosphaeriaceae* (mainly *Botryosphaeria*, *Diplodia* and *Neofusicoccum*) and *Diaporthe* reported as main causal agents.

Objectives: This study aimed to unravel the pathobiome associated with walnut dieback symptoms in France and their interactions, by culture-dependent (CD) approach and metabarcoding.

Methods: Environmental samples of symptomatic walnut husks and twigs were collected in 12 orchards (6 in each main production area) in four sampling campaigns (summer 2020 & 2021 for husks; spring 2021 & 2022 for twigs).

Results: Six main phytopathogenic fungal species (*B. dothidea*, *N. parvum*, *D. eres*, *Colletotrichum fioriniae, C. godetiae* and *Fusarium juglandicola*) represented 60% of total isolates (n=1986) by CD approach. Husks were dominated by B*. dothidea, N. parvum, D. eres* and *C. godetiae*, with prevalence ranging from 29 to 69%, depending on years and orchards. In contrast, *D. eres* and *F. juglandicola* were more frequently associated with twigs (prevalence ranging from 45 to 75%). Those species were also predominant by metabarcoding while this approach also revealed a higher degree of diversity. To go further, co-occurrence network analysis based on CD data and/or metabarcoding, showed that *N. parvum* was negatively associated with *D. eres* (r=-0.72) or *F. juglandicola* (r=-0.65) while *F. juglandicola* was positively associated with *C. godetiae* (r=0.78). Altogether, our results allowed to thoroughly describe the fungal pathobiome associated with emergent symptoms in French orchards and explore potential interactions within their members.

W348 - Time series analysis reveals shifts in bacterial soil microbiomes during ginseng cultivation

Presenting Author - *Paul Wan, Western University, Canada*

Author/s – *Vera Tai, Mark Bernards, Greg Thorn, Anka Colo, Rachel Rajsp*

Abstract Content

American ginseng, *Panax quinquefolius L*., is a root crop used in traditional Chinese medicine due to its main bioactive compounds called ginsenosides. Due to its high value in this regard, *Panax quinquefolius* is locally a high-value cash crop. Continued cultivation faces a major challenge in the form of ginseng replant disease (GRD) - a condition which causes root rot and a significant reduction in yield when ginseng is planted in the same soil as a previous crop. Fungal and oomycete pathogens contribute to GRD, but the exact mechanisms that cause soils to become GRD-prone are not well understood. To investigate how soils become GRD-prone, changes to the soil microbiomes were investigated in three commercial ginseng gardens over 3 years of cultivation starting from initial seeding. The V4 region of the bacterial 16S ribosomal gene was used for metabarcoding. The cultivation of ginseng resulted in a significant shift in the soil microbiome compared to control samples, starting from the second year of growth. The bacterial community shift is being further examined to identify specific microbial taxa that correlate with a shift toward GRD-prone soils. The overall goal is to produce diagnostic tools to assist in assessing soil health for ginseng farmers, as well as identifying causal mechanisms of GRD.

W349 - What could Charles Darwin not see? Exploring the belowground biodiversity of the Galápagos Islands

Presenting Author - *Sahra Riviere, Free University of Bozen-Bolzano, Italy*

Author/s – *Sahra Riviere, Seçil Ugur Yavuz, Aart van Bezooijen, Edoardo Pasolli, Manuela Dasser, Veronica Martini, Elisabeth Tauber, Stefano Cesco, Katharina Keiblinger, Martin Gerzabek*

Abstract Content

The Galápagos archipelago is one of the most remarkable hot spots of endemic plant and animal species on Earth. Although many ecological studies have been carried out on plants and animals, there is almost no information about the belowground diversity, especially the microbial communities. Here , we aim to unveil soil biodiversity and its interactions with biotic and environmental conditions in Galápagos soils. Samples collected from Santa Cruz and Isabela islands include agricultural, forest and young volcanic soils (Sierra Negra). Soil taxonomic biodiversity is being investigated through DNA metabarcoding of the bacterial 16 rRNA gene, fungal ITS2 region, protozoa, and animalia CO1 gene. The biodiversity functionality of younger volcanic soils is studied through the whole shotgun metagenome approach. Soil biodiversity and soil physicochemical data are integrated through multivariate statistical methods. Last but not least, this project is conducted by a multidisciplinary team, in which designers and anthropologists have been involved in finding strategies to raise awareness of this hidden belowground biodiversity of the Galápagos Islands among a broader audience, specifically youth and children.

W350 - Biodegradation: a sustainable fate of graphene enabled technologies

Presenting Author - *Purvi Jain, Helmholtz Institute Freiberg fur resourcetechnologie, Germany*

Abstract Content

Graphene and its derivatives (e.g., graphene oxide, GO) are looked upon as next wonder material due to their unique physiochemical properties and endless potential applications in electronics, aviation, medicine and much more, leading to Nobel prize for this discovery. We acknowledge the useful properties of new material, but we should also consider life cycle analysis, the fate and safety assessment to the environment and human health of Graphene based material (GBMs). The over exploitation of wonder material of 20 th century, plastic; is now biggest pollutant on earth, should be the case in point. Biodegradation of GBM is very relevant topic to study further for maximizing its societal beneficiary use. Many environmental bacterial strains have been isolated and characterized from hydrocarbon and metal contaminated site. We hoped to utilise such strains to break down GBMs into less harmful by-products. We have set up the culture condition to grow bacterial strain from contaminated site in flask, in minimal buffer medium in presence of GO as carbon source for several days with regular investigations for viability of bacteria as well as analysis of GO by particle size analysis (dynamic light scattering), optical microscopy, scanning electron microscopy (SEM), and Raman spectroscopy. We have identified a particular strain from coal-tar contaminated site, is able to transform physical structure of GO, observed under SEM, such transformation was absent when the bacterial strain was not inoculated. The overall finding of the research will help in more prosperous and sustainable development of graphene-enabled technologies.

W352 - Microbial response to future climate characteristics in the Greenland Arctic

Presenting Author - *Gabriele Tosadori, Czech Academy of Sciences, Czech Republic*

Author/s – *Jennifer Haskell, Camelia Algora, Alžběta Novotná, Louise Hindborg Mortensen, Lorrie Maccario, Bo Elberling, Anders Priemé, Petr Baldrian*

Abstract Content

Due to climate change, Arctic regions are expected to warm two to four times faster than the global average. The risk is that soil microbial activities, once adapted to warmer temperature and increased winter snowfalls, may cause arctic soils, known carbon pools, to become carbon sources instead. Therefore, microbial communities' responses to warming must be evaluated. To this goal, an experimental site was established in Narsarsuaq, Greenland in 2015. The experimental plot design simulated increased snow cover using passive snow accumulation, and summer warming using open top-chambers. These two treatments were combined in full-factorial design and laid out in six blocks. Top soil samples for microbial analysis were collected, at three different depths, in summer 2020. Samples were then evaluated for their microbial biomass content, enzyme activity, and subjected to amplicon sequencing of 16S rDNA and ITS regions, to assess changes in bacterial and fungal community composition. Preliminary results show that enzymatic activities and fungal biomass decrease with increasing soil depth. Fungal biomass and activity of several measured enzymes were lower in treatment combination (increased snow cover + summer warming) compared to control sites which was particularly pronounced in the uppermost soil layer. Our results thus suggest that increased snowfall together with higher summer temperatures has a negative impact on presence and activity of soil microbial community.

W353 – Microbial community in industrial salted-hides

Presenting Author - *Alicia Campos López, University of Alicante, Spain*

Author/s – *Francisco Nadal Molero, Ana Belen Martin Cuadrado*

Abstract Content

Leather-making industry is an age-old industry and desiccation with NaCl (salt) has been one of the most used methodologies during centuries to obtain valuable skins. However, halophilic prokaryotes present in the salt granules may proliferate and degrade the hides collagen structure besides to render the so called “red-heat” damage. In addition to some bacteria, several haloarchaea have been described as “contaminants” involved in these deteriorations at the salting process. To understand the basis of this process, it is essential to determine accurately the microbial community and changes along time. This study aims also to determine the degradative enzymes that cause huge losses of this valuable raw-material together the possibility to use green-controllers as substitutes of the chemicals used together the salt.

Amplicon (16S rRNA gene) sequencing of more than 30 samples has been carried out together with six metagenomes (60 Gb each) obtained from damaged and no-damaged hides. Assembly of those metagenomes was performed with Megahit, their annotation has been done and several collagenases and keratinases are being analyzed. Also, 4 genomes of contaminants were sequenced together with one Alkalibacillus which presented growth inhibition against several halophiles.

Metagenomes analysis showed higher species biodiversity in non-damaged hides than reddish ones and proteases numbers were higher in metagenomes from “red-heat” hides. Absence of collagenase activity by some *Alkalibacillus* (with inhibitory effect), makes them good putative biocontrol agents. Further laboratory experiments will continue along these lines.

W354 - Searching for the microbial consortium key for the peat-free substrate lock

Presenting Author - *Fachhochschule Erfurt, University of Applied Sciences of Erfurt, Germany*

Author/s – *Julia Brandes, Sebastian Pietschmann, Julius Dawydow, Miriam Bradl, Dirk Möcker, Erika Kothe, Philipp Franken*

Abstract Content

In the face of the implementation of the climate protection program until 2030, in practical horticulture, the development and improvement of peat-free substrates becomes increasingly important. In frame of one of the current projects, defined microbial consortia are selected to replace the functional properties of peat.

In a first step, the contribution of the microbiota derived from compost, from plants and from the greenhouse environment to microbial communities in substrates and on plant roots of the model plant petunia (*Petunia axillaris, Petunia exserta*) was investigated. Substrate and root microbiomes were monitored and potential endophytic microorganisms were isolated and molecularly identified. The results indicated that Proteobacteria dominate the bacterial endophytic community and that representatives of *Basidiomycota* from compost prefer to colonize the roots of *P. exserta* with time. Microorganisms from the greenhouse surrounding establish a complex microbial community in the substrates and in the roots, which differs substantially from the input from the compost.

From the established strain collection, bacterial consortia were used to investigate their plant-growth-promoting (PGP) potential on *Petunia hybrida* cv. ‚Mitchell' and *Ocimum basilicum* in a sterile/non-sterile peat-free substrate. The white rot fungus *Schizophyllum commune* was added to the system to improve the physical and chemical properties. First results showed PGP effects of one of the selected bacterial consortia and the fungal biomass as well as due to the sterilization process. Additional studies are intended to further complete the microbial consortia, to test the benefits of the fungal mycelium supplements, and to evaluate the effect of sterilization.

W355 – Nigerian oil exploration in Iko River Estuary and the remedial microbial communiy consortium

Presenting Author - *Augustine Unimke, University Of Calabar, Nigeria*

Author/s – *Phillip Okerentugba, Abiye Ibiene, Bassey Ubi*

Abstract Content

Background: The gross contamination of the aquatic and terrestrial ecosystems resulting from the continuous input of petroleum-based and other industrial contaminants along with heightened navigational activities in the inland and coastal regions has contributed immensely towards the contamination of the aquatic environment.

Objectives: To assess the microbial community structure present during bioremediation procedure and to ascertain the microbial synergistic capabilities.

Methodology: Standard analytical methods were employed in sample collection, storage, processing and analysis.

Results: The results obtained revealed the mean values of Total Heterotrophic Bacteria [THB] for tidal water 1.46±0.20 (x107), 1.44±0.62 (x107)and 1.84±0.61 (x107)for upstream, midstream and downstream respectively. More so, the mean values for Total Fungi (TF) and crude oil-utilizing fungi (CUF) were 1.08±0.12 (x106), 1.13±0.21 (x106), 1.18±0.20 (x106) and 8.2±0.78 (x104), 9.2±0.20 (x104), 8.8±0.26 (x104) for upstream, midstream and downstream respectively. While the values for benthic sediment were 1.56±0.38 (x108), 1.67±0.32 (x108), 2.24±0.34 (x108) for THB, 1.14±0.32 (x107), 1.24±0.88 (x107), 1.48±0.90 (x107) for CUB, 1.12±0.31 (x107), 1.20±0.52 (x107), 1.40±0.16 (x107) for TF and 8.2±0.12 (x105), 6.2±0.43 (x105), 1.01±0.12 (x106) for CUF. The results showed that there was no significant difference (p> 0.05) in the mean values of each physicochemical parameter across the different micro-ecosystems and stations. This result revealed the intense and degrading impacts of anthropogenic gradients on the biology and physicochemistry the estuary.

W356 - Fungal co-inoculation of the rhizosphere improves the growth of rosemary under nutrient limited conditions

Presenting Author - *Carlos García Gálvez, Institute Of Agrifood Research and Technology (IRTA), Spain*

Author/s – *Carmen Biel Loscos, Francesc Xavier Prenafeta Boldú, Cinta Calvet Pinós, Amèlia Camprubí Nieto, Belén Fernández García*

Abstract Content

We examined the effects of the combined fungal inoculation of rosemary potted plants subjected to nutrient-stress with arbuscular mycorrhiza, dark septate endophytes (DSE), and Trichoderma species. These fungi are known to form stable symbiosis at the roots that increase the fitness of the host plants by improving the bioavailability of soil nutrients, inducing changes in the hostplant metabolism, and controlling phytopathogens. The purpose of this study was to gain a deeper knowledge of the plant-fungal interactions, especially with the lesser-known DSE, and to evaluate their potential for the development of innovative biofertilizers.

Eighty plants (10 per treatment) from rosemary (*Salvia rosmarinus*) rooted cuttings were planted in 1 L containers filled with sterilised sandy soil. Daily sprinkling irrigation was applied but no nutrients were supplied. DSE strains BY13 (*Cladosporiun pseudochalastoporoides* aff.) and BY15 (*Cl. endophyticum*), as well as Trichoderma aureoviride (teleomorph *Hypocrea Aureoviridis*) were inoculated by inserting pre-grown agar discs (5 mm) and 10 mL of liquid culture per pot, respectively. The mycorrhizal inoculum (10 g of bulk cultures of *Rhizoglomus* irregulare obtained from a mycorrhized leek) was applied under the root system. After 125 days of cultivation the shoot and root biomass, and fungal colonisation were measured.

The combined inoculation of R. irregulare with either H. aureoviridis or the two tested *Cladosporium* spp. showed a significant synergistic effect on the development and growth of rosemary, in relation to both the non-inoculated control and the sole inoculation of R. irregulare.

W357 - Expression and activity of antioxidant enzymes in endophytic bacteria: Pseudomonas fluorescens BRZ63 and *Serratia quinivorans* KP

Presenting Author - *Katarzyna Hupert-Kocurek, University Of Silesia, Poland*

Author/s – *Bożena Nowak, Daria Chlebek*

Abstract Content

Endophytic bacteria are microorganisms that inhabit the internal tissues of plants, stimulating their growth and fitness and protecting them from invasion by phytopathogens, including fungi. When present in the plant during infection, these bacteria are exposed to a number of different compounds secreted simultaneously by plants and phytopathogens. These environmental stressors induce defence mechanisms in bacteria, ensuring their survival in the plant and more effective protection of the host from pathogens. The expression of essential antioxidant enzymes such as catalases and superoxide dismutases is crucial to bacterial survival.

The study aimed to investigate the effect of fungal filtrates on the gene expression level of katB and sodB in Pseudomonas fluorescens BRZ63 and katG and sodB in *Serratia quinivorans* KP32. Additionally, the activity of intracellular catalases (CAT) and superoxide dismutase (SOD) was determined.

Each fungal pathogens was cultured separately for two weeks in a liquid medium. Then the supernatant from each culture was sterilised by filtration and used for further studies. Total RNA was isolated from the test (bacteria and filtrates) and control (bacteria) samples and purified prior to cDNA synthesis. The generated cDNA was used as a template in qPCR reactions. CAT and SOD activities were measured spectrophotometrically.

In response to fungal filtrates, BRZ63 showed increased expression of superoxide dismutase while KP32 - catalase. In addition, the response of BRZ63 was significantly higher. Differences in the expression levels of tested genes and the activity of antioxidant enzymes depended on the pathogen.

W358 - Novel and unusual genes for nitrogen and metal cycling in Planctomycetota and KSB1 genomes from a marine subsea tunnel

Presenting Author - *Paula Dalcin Martins, University Of Groningen, Netherlands*

Author/s – *Paula Dalcin Martins, Thomas Hackl, Britt-Marie Wilen, Frank Persson, Mike Jetten, Carolina Suarez*

Abstract Content

Background: The Oslofjord subsea road tunnel is a unique environment, in which the typically anoxic marine deep subsurface is exposed to oxygen. In areas of saline water seepage, concrete and steel biodeterioration is observed likely due to growth of iron and manganese-oxidizing biofilms. Surprisingly, previous 16S rRNA gene surveys of biofilm samples revealed microbial communities dominated by sequences affiliated nitrogen-cycling microorganisms (Karačić et al., 2018).

Objectives: The aim of this study was to identify microbial genomes with metabolic potential for novel nitrogen- and metal-cycling reactions, representing biofilm microorganisms that could link these cycles and play a role in concrete biodeterioration.

Methods: Biofilm DNA was extracted and sequenced on an Illumina NovaSeq6000 platform, generating 150 bp paired-end reads that were processed, assembled and binned as previously described (Karačić et al., 2018; Suarez et al., 2022).

Results: We reconstructed 34 abundant, novel metagenome-assembled genomes (MAGs) affiliated to the phyla Planctomycetota and KSB1. We identified novel and unusual genes and gene clusters in these MAGs related to anaerobic ammonium oxidation, nitrite oxidation, and other nitrogen-cycling reactions. Additionally, 26 of 34 MAGs had also potential for iron, manganese and arsenite cycling, suggesting that bacteria represented by these genomes might couple these reactions. These results expand the diversity of microorganisms putatively involved in nitrogen and metal cycling, and contribute to our understanding of potential biofilm impacts on built infrastructure in the subsurface and marine environments.

W359 - Bacterial antagonisms as nutrient acquisition strategy

Presenting Author - *Astrid Stubbusch, ETH Zurich, Switzerland*

Author/s – *Glen D'Souza, François Peaudecerf, Lucas Paoli, Marek Basler, Cara Magnabosco, Olga Schubert, Martin Ackermann*

Abstract Content

Microbial interactions shape the composition and function of microbial communities with major implications for the ecosystem ecology and biogeochemical cycles. Antagonistic interactions are commonly perceived as means to reduce the growth rate or the cell number of competing community members, however, our understanding of their ecological impact in natural environments remains limited. To address this knowledge gap, we investigate antagonistic interactions mediated by the type VI secretion system (T6SS), a bacteriophage-tail-like nanomachine widespread among Gram-negative bacteria to translocate toxins into neighbouring cells. We study simple marine communities made up of both Vibrio spp. antagonists and non-antagonists using microfluidics and time-lapse microscopy. Our findings suggest a novel role of antagonistic interactions in acquiring nutrients from lysing cells in nutrient-limited conditions, while quickly eliminating competitors in nutrient-rich conditions. To understand if nutrient acquisition from antagonized cells is a general principle, we search for genomic adaptations towards this opportunistic predatory lifestyle in T6SS-harbouring Vibrio spp. within ~3,300 Vibrio spp. high-quality genomes. Additionally, we mine global metagenomic and metatranscriptomic samples of natural microbial communities to investigate whether the abundance of T6SS-encoding bacteria and their expression profile in nature support the new role of the T6SS in opportunistic predation. These findings will help develop an understanding of the ecological role of the T6SS in marine ecosystems and contribute to a quantitative understanding of antagonistic interactions in natural environments.

W361 - Isolation of extracellular vesicles in *Blattella germanica*, a model for the study of host-symbiont communication in insects

Presenting Author - *David Saiz Martínez, University Of Valencia, Spain*

Author/s – *Christian M Sánchez-López, Joaquín Baixeras, Antonio Marcilla, Rosario Gil*

Abstract Content

Organisms do not live in isolation but rather interact with individuals from other species. Yet, mutualistic stable relationships require an “entente cordiale” among the partners leading to a better fitted life form in which all of them benefit.

Symbioses are widespread in eukaryotes, especially in insects. Many of them live in obligate relationship with different ecto- and endosymbiotic bacteria needed to maintain host fitness. It is the case of the cockroach *Blattella germanica*, with two symbiotic systems in separated compartments: the endosymbiont Blattabacterium in specialized bacteriocytes located in the fat body, and a complex microbiota in the gut lumen.

The presence of small RNA molecules (sRNA) has been systematically reported in extracellular environments including extracellular vesicles (EVs), and has been associated to cell-to-cell communication, as an additional layer of regulatory complexity.

Our goal is to investigate whether sRNAs produced by the insect and/or by the endosymbiont exert a regulatory role related to endosymbiosis, either locally (in the bacteriocyte) or in distant target tissues, reached through the hemolymph inside EVs. In this sense, we have isolated EVs from insects hemolymph and characterized them by Nanoparticle Tracking Analysis (NTA) and Transmission Electron Microscopy (TEM). Current studies of their cargo by -omics technology will be also presented and their possible role in bacteria-insect communication will be discussed.

W362 - Antibiotic resistance genes in onsite wastewater treatment systems

Presenting Author - *Gábor Györki, University Of Public Service, Hungary*

Abstract Content

Wastewater treatment systems are important point sources of micropollutants, such as antibiotics and toxic metals. Continuous exposure to these substances may cause microorganisms to develop antibiotic resistance, which can spread among bacterial pathogens with the help of mobile genetic elements. Onsite wastewater treatment systems treating the wastewater of a single or a few households provide alternative solutions to centralized systems and are becoming increasingly popular. Domestic wastewater, however, may contain antibiotics and other drivers of antibiotic resistance, and while little is known about their performance, on-site treatment systems can essentially act as a source of antibiotic resistant genes and bacteria. In our study, we have analysed the chemical and microbiological composition of raw and treated wastewaters from on-site wastewater treatment systems to assess the presence and removal of micropollutants and antibiotic resistance genes. We have found that though composition of raw wastewater as well as the performance of these systems differed, pharmaceutical compounds as well as antibiotic resistance genes were present in OWTS. Although more analyses are needed to draw conclusion regarding factors driving the selection of resistance genes in these environments, our results highlight the importance of monitoring small domestic wastewater treatment systems.

W363 - The black soldier fly as a source of anti-Pseudomonal peptides

Presenting Author - *Laurence Van Moll, University of Antwerp, Belgium*

Author/s – *Linda De Vooght, Jeroen De Smet, Mik Van Der Borght, Paul Cos*

Abstract Content

Background: As effector molecules of the innate immune system with strong antimicrobial activity, antimicrobial peptides (AMPs) have gathered interest as potential next generation antibiotics. The black soldier fly (*Hermetia Illucens*) disposes of one of the largest AMP repertoires ever recorded in insects. Their expansive AMP library has been linked to their eminent survival in microbially contaminated substrates, including food waste and manure. Exploration of these AMPs and their antimicrobial profile could play a role in fighting the ongoing antimicrobial resistance crisis.

Objectives: In earlier research, we found that the black soldier fly produces a remarkable amount of cecropin AMPs with strong activity against gram-negative pathogens including P. aeruginosa, and low cytotoxicity [3]. Our current research aims to fully elucidate the mechanism of the anti-Pseudomonal activity of cecropin HC1.

Methods: To characterize the antimicrobial activity against P. aeruginosa, membrane permeabilization experiments using n-phenyl-naphtylamine were carried out. In addition, a Bodipy-TR cadaverine experiment was used to study LPS-binding effects of HC1, and qPCR and ELISA techniques were used to examine the effect on LPS-induced cytokine production. Lastly, a serial passage experiment was used to examine the rate of HC1 induced resistance development.

Results: HC1 has a rapid onset of action with membrane-permeabilizing effects, and low propensity towards resistance development. In addition, HC1 also shows endotoxin-neutralizing properties, which leads to lower cytokine production by LPS-stimulated mouse macrophages. Next, this anti-Pseudomonal activity will be verified in an in vivo mouse model.

W364 - Impact of genetic traits on microbiota recruitment and defense against a specialist herbivore in *Nicotiana attenuata*

Presenting Author - *Pooja Mehta, Max Planck Institute for Chemical Ecology, Germany*

Author/s – *Chidambareswaren Mahadevan, Elham Karimi Dorcheh, Jürgen Wierz, Rayko Halitschke, Ian Baldwin, Martin Kaltenpoth*

Abstract Content

Microorganisms play a crucial role in plant and insect fitness. *Nicotiana attenuata* seeds lack inherited microbes and have long seed dormancy periods (up to 150 years). In natural ecosystems, however, it is still unclear which plant traits regulate beneficial microbiome recruitment during germination and maintenance of a balanced microbiome. Using plant genotypes altered in functionally essential genes linked to growth traits, signaling, phytohormones, and secondary metabolites production via RNA interference, we assessed their impact on microbiome recruitment through axenic systems with a direct synthetic microbial community quantitation approach, followed by a semi-natural glasshouse experiment and germination in native environments. These three systems demonstrated that genes involved in nicotine biosynthesis, carbon allocation, ethylene production, lignin synthesis, and defense signaling impact early microbiome recruitment. Later on, genes involved in root architecture, signaling, and strigolactone productions are found to be critical players in microbiome maintenance. We also investigated whether these genetic traits influence the fate of Trichobaris mucorea, a stem-boring endophytic herbivore, by affecting its intracellular symbiont and/or the gut microbiome. This will be investigated by characterizing the metabolome of pith samples, assessing the nutritional fate of ingested pith via frass-omics, and analyzing variation in the abundance of symbionts and in the community structure of the gut microbiome of larvae. This study will help us to understand the plant traits involved in recruiting beneficial microbes and their impact on the infestation of economically damaging stem borers.

W365 - Detection of microbiota in samples of water, oyster and faeces of habitants of Chachalacas, Veracruz.

Presenting Author - *Jose Mijail Campos, Universidad Autónoma Metropolitana, Mexico*

Author/s – *Ana María Fernández, María del Carmen Monroy, José Félix Aguirre, Aida Hamdan, Jaime Bustos*

Abstract Content

Background: Gastrointestinal diseases are one of the main public health problems in Mexico, they are transmitted by the consumption of contaminated food and water. Studies on the microbiota in water and food are essential to understand the epidemiology of diseases that affect human populations in different geographical areas.

Objective: To determine by metagenome the eukaryotic and prokaryotic microbiome present in samples of water and oyster (*Crassostrea virginica*) from the Actopan river and samples in the feces of the inhabitants of Chachalacas, Veracruz.

Methods: Water samples from the Actopan River, Crassostrea virginica and fecal samples from the population were collected. DNA extraction was performed on them and used for metagenome determination by amplifying the 18S rDNA and 16S rDNA gene, from MiSeq sequencing at the Integrated Microbiome Resource (IMR) at Dalhousie University in Halifax, Nova Scotia, Canada. Bioinformatic analyzes were performed using MOTHUR software, version 1.48.0.

Results: The presence of the genus *Cryptosporidium*, which is a pathogenic protozoan for humans, was found in water samples and mainly *Crassostrea virginica*. In the population of a single family, the presence of the genus *Entamoeba* was found with a low prevalence, in addition to this, a great variety of eukaryotic microorganisms were found, such as Trichodina, Ichthyosporea, Perkinsus, Candida, among others.

Pathogenic organisms were found within the genus Vibrio which was observed in water samples, *Crassostrea virginica* and in a family like the genus *Shigella*.

W366 - Mitomycin C-induced effects on aerobic methanotrophs in a landfill cover soil - implications of a viral shunt?

Presenting Author - *Tanja Heffner, Leibniz University Hannover, Germany*

Author/s – *Thomas Kaupper, Mara Heinrichs, Hyo Jung Lee, Nadine Rüppel, Marcus Andreas Horn, Adrian Ho*

Abstract Content

Bacterial viruses (phages) are abundant members of the soil community, but their impact on the carbon, and in particular the methane cycle, is poorly understood. Once temperate phages enter the lytic cycle, host cells are lysed, releasing virions, as well as cellular components into the environment, enhancing nutrient availability (i.e., viral shunt). Mitomycin C is a commonly used agent to induce a viral shunt. Here, we investigated the effects of mitomycin C on aerobic methanotrophs and their activity in a complex community from a landfill cover soil, and in pure cultures (*Methylogaea* and *Methylocystis* species). In the landfill cover soil, an increased number of virus-like particles in relation to bacterial cells, enhanced nutrient concentrations (ammonium, succinate), as well as notable effects on microbial activity (i.e., methane uptake and carbon dioxide production) provided evidence of a mitomycin C-induced viral shunt. Mitomycin C addition significantly altered the potentially active bacterial community after 11 days based on 16S rRNA and *pmoA* transcript analysis, indicating differential effects on the bacterial community. Overall, we provide first insights into the effects of mitomycin C on the soil methane uptake and associated methanotrophs, likely mediated by induction of a viral shunt.

W367 - A genome wide analysis of heat survival mechanisms of *Escherichia coli*

Presenting Author - *Muhammad Yasir, Quadram Institute Bioscience, United Kingdom*

Author/s – *Keith Turner, Megan Truong, Sarah Bastkowski, Ian Charles, Mark Webber*

Abstract Content

Background: *E. coli* is a cause of many food or waterborne infections and heat is a common method of protecting foodstuffs from being contaminated. We examined how *E. coli* can adapt to higher temperature and whether it can be 'trained' to develop increased temperature tolerance.

Methods: We used 'TraDIS-Xpress' which assays a large transposon library incorporating outward-transcribing inducible promoters allowing both gene inactivation and modulation of transcription to be scored. We tested responses to four different temperature levels ranging from 37C to 50C and we also assayed genes allowing growth at 47C and 50C after initially sensitising cells at 44C.

Results: We identified genes encoding the cell envelope, DNA repair and chaperone proteins involved in protein misfolding and aggregation as important for survival at high temperatures. Besides known mechanisms, we also found mutations in metabolic genes that are not reported before for high temperature tolerance. We found genes with mutations in protein export, secretion system and nucleoside metabolism were important at 47C and 50C but after sensitisation, mutations in amino acid metabolism genes are important which were not otherwise identified.

Conclusion: This data shows multiple mechanisms of survival at high temperature including the known mechanisms and that how temperature stress is applied are both important for *E. coli* to adapt to heat stress.

W369 - Microbial community composition and temporal dynamics of leaf biofilms *in vitro*

Presenting Author - *Sabina Karacic, University Hospital Bonn, Germany*

Author/s – *Brianne Palmer, Carole Gee, Gabriele Bierbaum*

Abstract Content

Background: In natural freshwater ecosystems, microorganisms including bacteria, archaea, and fungi colonize and form biofilms on leaves and soft tissues. Complex microbial communities involved in biofilm development are influenced by various abiotic and biotic factors. However, the mechanisms determining the composition and development of the biofilm communities on leaves are poorly understood.

Objectives: The aim of this study was to develop leaf biofilms *in vitro* on the leaves of three plant genera and elucidate the microbial community composition under different oxygen conditions. To identify leaf biofilm composition at specific stages of decay, we examined microbial community temporal dynamics over a period of three weeks.

Methods: We used 16S rRNA and ITS to measure microbial diversity and temporal changes of microbial communities of Acer sp., Hedera sp., and Lonicera sp. Leaves were immersed in the pond water under aerobic and anaerobic conditions in vitro for three weeks. Sampling was performed weekly, and samples were investigated by scanning electron microscopy.

Results: The leaf biofilms growing in aerobic conditions varied from biofilms in anaerobic conditions. The alpha diversity of prokaryotes increased over time in aerobic assays, whereas it decreased under anaerobic conditions. In contrast, fungal richness remained stable throughout the experiment. After three weeks, we observed a clear compositional shift in the bacterial and fungal communities. The genera Vogesella, Tolumonas, Rhodoferax, and Aeromonas were initially abundant and decreased over time. Bacteroidales, Ocillospirales and Clostridiales were more abundant in anaerobic conditions. Biofilm leaf composition changes over time and is shaped by availability of oxygen.

W370 - A two-step duplex PCR for rapid detection of *Vibrio harveyi* strains hazardous to fish

Presenting Author - *Javier Barriga-Cuartero, University of Valencia, Spain*

Author/s – *Carla Hernández-Cabanyero, Pablo Ibáñez-Payá, Arnau Pérez Roig, Belén Fouz, Carmen Amaro*

Abstract Content

Vibrio harveyi (Vh) is a marine pathogen that causes vibriosis (*Vh-vibriosis*) in multiple aquatic animals, including species of interest in aquaculture. Global warming is causing an increase in the number of epizootics and outbreaks of *Vh-vibriosis* and, in parallel, an increase in the virulence of some clones. These clones possess two genes that in *V. vulnificus* encode a resistance system to the fish innate immunity. The objective of this study was to develop a duplex PCR that would identify Vh and, at the same time, distinguish strains possessing this resistance system. The chosen targeted genes were toxR and fpcrp (fish phagocytosis and complement resistance protein) for species and virulent clones identification, respectively. This PCR showed 100% specificity and a detection limit of 10^5 CFU/ml. The combination of PCR with a previous enrichment step in alkaline peptone water for 8h reduced the detection limit to 10^2 CFU/ml. The entire protocol was validated in the laboratory with artificially contaminated splenic tissue and real environmental and clinical samples. Consequently, the implementation of this protocol in environmental/clinical testing laboratories would allow early detection and identification of Vh strains hazardous to fish, thus helping to minimize economic losses in the aquaculture industry.

W371 - Which is the role of climatic variables in the dissemination of antibiotic resistance in agroecosystems?

Presenting Author - *Fernando Ruiz Torrubia, Neiker, Spain*

Author/s – *Lur Epelde Sierra, Carlos Garbisu Crespo, Mikel Anza Hortala, Aitor Anitua Martínez*

Abstract Content

Climate change and the emergence of antibiotic resistance are two major threats to human and environmental health that have been addressed mostly separately. Related to this, the use of organic amendments of animal origin represents an important route of resistome entry into agricultural soils.

This work aimed to unravel how different soil temperature and humidity regimes influence the emergence and dissemination of antibiotic resistance in organically amended soils.

We established a microcosm experiment with a total of 16 treatments under different incubation temperatures (4, 12, 21 and 30C) and soil moisture levels (20, 40, 60 and 80 % of the water holding capacity). Cow manure supplemented with oxytetracycline, copper and glyphosate were added in every treatment. Genotypic changes in soil bacterial communities were addressed by droplet digital-PCR of 9 genes associated with antibiotic resistance and 2 genes associated with mobile genetic elements. Phenotypic changes were assessed through the determination of minimum inhibitory concentrations using the microdilution method. In addition, soil microbial biomass and respiration were measured.

The climatic conditions tested in this experiment affected the emergence and dissemination of antibiotic resistance. To decrease the risk of antibiotic resistance in agroecosystems, a proper management of soil amendments needs to be carefully implemented, especially in the current context of climate change.

W372 - Effects of plastic surfaces on colonization and interactions within a biofilm community

Presenting Author - *Lena Preuß, Universität Hamburg, Germany*

Abstract Content

Background: Plastic production and associated with it the pollution of plastic waste within the oceans is increasing rapidly (1). Considering the total amount of plastics produced in total annually polyethylene and polyethylene terephthalate constitutes almost 50% (38% PE and 11% PET) (2). While we now have a fairly good understanding of the colonization of those plastic surfaces, our knowledge of the signal exchange during colonization and its effects on the microbial community is rather sparse (3).

Objectives: In order to shed light on this, we pursue different strategies. First, marine biofilms and marine model organisms (*Vibrio* spp.) are cultivated in the laboratory on different types of plastics like PE and PET and in addition on plastics having modified surface properties starting with previously performed plasma treatment.

Methods: For the characterization of the biofilms grown on different plastic surfaces LSM imaging was performed. To analyze the gene expression during surface colonization in detail RNA seq was used. Genes of interest were characterized using promoter fusions with fluorescence proteins.

Results: Microscopic analyzation has shown that especially the early attachment of Vibrio gazogenes on previously plasma modified plastics like PE as well as PET is significantly increased compared to untreated plastics.

Regarding the results of the transcriptomic analysis of *V. gazogenes* also previously treated surfaces induce significantly different gene expression concerning regulation of whole gene clusters. Especially pathways involved in iron uptake and transport are upregulated when *V. gazogenes* is grown on polyethylene or polyethylene which was plasma activated.

W373 - Microbial communities' interactions in subaerial biofilms inhabiting stone heritage

Presenting Author - *Maria Landolfi, Free University of Bozen-Bolzano, Italy*

Author/s – *Maria Landolfi, Jacopo Melada, Tanja Mimmo, Francesca Cappitelli, Luigimaria Borruso, Federica Villa*

Abstract Content

Ecosystems are ecological levels of organization that are more fundamental than the organisms themselves. In this context, biofilm can no more be seen as an assembled community of member taxa but should be considered as microorganisms that establish complex relations with the surface and the environment they inhabit. The aim of this multidisciplinary work is to characterize the microbial communities of subaerial biofilms inhabiting several lithic substrates and to explore their response to different microclimatic conditions, by studying the metabolically active communities using a metatranscriptomic approach. Furthermore, the effects of the microbial interactions on the substrate material are evaluated by physical and chemical analyses of the substrate. Biofilm samples used in this work were collected in a cemetery masterpiece of 19th-century architecture, which hosts a variety of carbonatic and silicatic lithotypes. The findings of this study will provide a better understanding of the interactions encountered within biofilm communities exposed to different conditions and will shed light on the impacts of these interactions on lithic materials.

W374 - Effect of contrasting atmospheric pollutant gases on cultivable air microbial communities

Presenting Author - *Dinka Mandakovic, Center for Genomics and Bioinformatics, Chile*

Author/s – *Karina Díaz, Madelaine Mejías, Romina Madrid, Rodrigo Pulgar, Dinka Mandakovic*

Abstract Content

Background: The aerobiome (airborne microorganisms) vary according to environmental conditions such as pollution. One way to study the communities of microorganisms is by means of cultivable communities, since this strategy allows evaluating changes in the community through controlled interventions.

Objectives: The aim of this study was to determine the effect of contrasting levels of atmospheric pollutant gases on the diversity, taxonomic composition, and co-occurrence patterns of cultivable air microbial communities. Methods. Active air collections in three culture media Petri dishes were obtained from two zones of the Metropolitan Region of Chile with significantly contrasting concentrations of ozone and nitric oxide. The plates were covered by gas-permeable membranes, and while some were grown in their place of origin, others were exchanged between zones. After seven days of growth, we evaluated the effect of gases on the microbial cultivable community by high-throughput sequencing the bacterial and fungal members. Results. Beta diversity measured by Bray-Curtis dissimilarity showed significant differences between the samples that were exchanged to those that remained in their zone of origin, both for bacteria and fungi. The taxa most sensitive to changes in abundance due to differential gas concentrations were the bacterial phyla Firmicutes and *Actinobacteriota* and the fungal phyla *Ascomycota* and *Basidiomycota*. Co-occurrence network analysis showed that the microbial communities that were exchanged were more complex (greater number of nodes and edges) than the communities that remained in their zone of origin. Therefore, we conclude that pollutant gases modify the overall structure of the culturable communities of the aerobiome.

W375 - Roles of microbiomes to plasticity of *Lobaria pulmonaria* in response to excess nitrogen, light and transplantation experiments

Presenting Author - *Anteneh Tamirat Bogale, University of Greifswald, Germany*

Author/s – *Niclas Kuck, Ulf Schiefelbein, Martin Grube, Daniela Zühlke, Jörg Bernhardt, Mia M. Bengtsson, Maria Braun, Katharina Riedel*

Abstract Content

Lobaria pulmonaria is an epiphytic model lichen composed of a mycobiont, a green-alga and cyanobacterial photobiont, and associated microbiomes. Although the lichen is sensitive to pollutants such as high nitrogen loads and excess light, it is also widely distributed in Europe, Asia, North America, and Africa.

The aim of the current study was to investigate the roles of the microbiome for plasticity of the holobiont in response to nitrogen deposition stress in combination with light stress in a field transplantation experiment in Austria. In addition, we compared microbiomes between distant geographical areas, Austria, and Kilimanjaro, Tanzania. We employed rRNA gene amplicon sequencing analysis and metaproteomics to explore the structural and functional plasticity of the holobiont.

Preliminary ordination results of the 16S rRNA gene amplicons of the microbiome showed differences between nitrogen treated and transplanted groups, and between the two geographic regions. Relative abundances of gene amplicons and proteins related to diverse groups of stress-tolerance were also high among the treatment groups.

Our overall results of metaproteomics and amplicon sequencing analysis indicated that microbiomes could play vital roles in plastic responses of the Lobaria pulmonaria holobiont in response to adaptation to environmental changes and climatic conditions.

W376 - The effect of environmental microbiota on biofilm formation and concentration of *Listeria monocytogenes* in formed biofilms

Presenting Author - *Jasna Kovac, The Pennsylvania State University, United States*

Author/s – *Jasna Kovac, Laura Rolon, Olena Voloshchuk*

Abstract Content

Formation of complex multi-species biofilms by environmental microbiota may increase the survival and persistence of pathogenic *L. monocytogenes* residing in food processing environments.

This study aimed to determine the effect of selected environmental microbiota on the concentration of total microorganisms and *L. monocytogenes* in biofilms.

Studied microbiota included bacterial families Pseudomonadaceae, Xanthomonadaceae, Microbacteriaceae, and Flavobacteriaceae, which were previously shown to co-occur with *L. monocytogenes* in monitored tree fruit packing facilities. The biofilm formation ability and the concentration of total microorganisms and of *L. monocytogenes* was measured in single- and multi-family assemblages. A total of 8, 8, 6, 3, and 6 strains of Pseudomonadaceae, Xanthomonadaceae, Microbacteriaceae, and Flavobacteriaceae, and *Listeria monocytogenes*, respectively, were used in the experiments. Assemblages were comprised of single families and all combinations of families, with *L. monocytogenes* included in each assemblage. Biofilms were grown statically on pegs submerged in a R2A broth in microtiter plates for 3 days at 15°C. Biofilm formation was quantified using a crystal violet assay and confocal laser scanning microscopy. The concentration of total microorganisms in formed biofilms was determined by spread plating. The concentration of *L. monocytogenes* in biofilms was quantified using the most probable number method.

Biofilms formed by Pseudomonadaceae, Xanthomonadaceae, and all families combined had a significantly higher concentration of total microorganisms and *L. monocytogenes* compared to biofilms formed by just *L. monocytogenes*. Furthermore, *L. monocytogenes* was able to attach and/or grow significantly better in multi-family assemblage biofilms, compared to biofilms formed by *L. monocytogenes* alone.

W377 - Isolation and characterization of PAH-degrading bacteria for air purification applications

Presenting Author - *Max Dekeukeleire, University of Antwerp, Belgium*

Author/s – *Preben Van Overmeiren, Kristof Demeestere, Christophe Walgraeve, Filip Willocx, Sarah Lebeer, Dieter Vandenheuvel*

Abstract Content

Polyaromatic hydrocarbons (PAHs) and oxygenated PAHs (oxy-PAHs) are pollutants originating from a variety of sources including incomplete combustion. These airborne pollutants are of very high concern because of their carcinogenicity. Mechanical and chemical methods generally used to remove PAHs from contaminated sites have limited effectiveness and are expensive. A more cost-effective and ecological alternative is bioremediation, which uses microorganisms to degrade PAHs into non-toxic compounds. In this study, we aim to map the air quality of indoor environments typically contaminated with PAHs and oxy-PAHs, and to isolate and identify beneficial environmental bacteria for air purification applications to bioremediate airborne PAHs and oxy-PAHs.

Therefore, we have set up an air sampling campaign in fire stations, mapping the indoor concentration using a novel air sampling method based on low volume sampling of closed environments to collect gaseous and particle-bound PAH and oxy-PAH up to ultra-low concentrations in 3 different fire stations.

Overall, phenanthrene was the most abundant compound, followed by fluorene and acenaphthylene. Based on the air sampling data, new bacterial strains are being isolated from soil, phyllosphere, and abiotic surfaces, capable of metabolizing the most prominent and harmful (oxy-)PAHs found in these fire stations. This is done through an enrichment, isolation, and identification pipeline. So far, more than 200 micro-organisms have been isolated, which are being analysed for their bioremediation capacity. Successful strains are further characterized through bioremediation experiments and whole-genome sequencing to determine their biosafety, and the genetic and molecular basis of their bioremediation capacity for applications in fire stations.

W379 - A novel reporter system to identify natural products mediating cross-kingdom microbial interactions

Presenting Author - *Maira Rosin, Leibniz Institute for Natural Product Research and Infection Biology, Germany*

Author/s – *Mario Krespach, Maria Stroe, Kirstin Scherlach, Volker Schroeckh, Christian Hertweck, Axel Brakhage*

Abstract Content

In all habitats on earth microorganisms form consortia with many different species closely living together in the soil. The interspecies communications in these communities are decisive for function of microbial communities and further lead to the induction of otherwise silent natural product biosynthesis gene clusters. One prominent example is the interaction of the bacterium *Streptomyces rapamycinicus* with the fungus *Aspergillus nidulans*. Upon co-cultivation, the streptomycete is able to activate the otherwise silent ors biosynthesis gene cluster in *A. nidulans*. The trigger, however, remained obscure. To test the ability of microorganisms and compounds to induce the ors gene cluster we generated an *A. nidulans* reporter strain which has the orsA gene fused to a nanoluciferase and GFPs. With this reporter strain we were able to identify several bacterial species randomly collected from soil that induced green fluorescence in the fungus. Further analyses discovered the compound group of arginoketides including azalomycin F produced by *S. rapamycinicus*/*S. iranensis* which serve as the long sought-after bacterial signals for this induction . Interestingly, extracted soil also led to an increased nanoluciferase activity indicating that arginoketides are indeed present in the soil. Arginoketides can be found around the world and seem to play an important role in mediating microbial interactions in the soil.

W381 - Assessing biotransformation of TNT by three different bacterial species using Compound-Specific Isotope Analysis

Presenting Author - *Swati Gupta, The Ben-Gurion University of the Negev, Israel*

Author/s – *Hagar Siebner, Zeev Ronen*

Abstract Content

The explosive 2,4,6-trinitrotoluene is a hazardous pollutant that contaminates groundwater and soil. Various microbial strains, such as Stenotrophomonas strain SG1, Rhodococcus strain YH1, and Diaphorobacter strain DS2, were found to transform TNT under aerobic conditions through various reaction pathways. In this study, we used Compound-Specific Isotope Analysis (CSIA) to compare the isotopic effects of TNT transformation and to gain knowledge of its mechanism in various treatments under aerobic conditions. An apparent kinetic isotope effect (AKIEN) of 1.011 ± 0.0013, 1.045 ± 0.0024, and 1.035 ± 0.0009 were found for strains SG1, DS2, and YH1, respectively, during TNT transformation. The corresponding ^N/C values were 1.5 ± 0.1, 8.5 ± 0.8, and 8.0 ± 0.6, implying the involvement of oxidative mechanism for SG1, while the reduction was the main path for the others. The analysis of the metabolites supported the above findings of oxidative mechanism for SG1 while more reductive for the others. In all strains, type I nitroreductases (oxygen-insensitive) were present in the strain's genomes. Our finding suggests that CSIA allows the detection of the major TNT biotransformation pathway in these strains. The analysis of 13C and 15N isotopes in TNT could be used to identify the TNT biotransformation processes in contaminated environments based on the isotope enrichment pattern presented in this research work.

W382 - Detection of *Escherichia coli* and disinfectant-tolerant bacteria potentially transmitted by irrigation to *Capsicum annuum*

Presenting Author - *Elona Tahiri Vela, Justus-Liebig University Giessen, Germany*

Author/s – *Rreze M. Gecaj, Arben Mehmeti, Stefanie P. Glaeser*

Abstract Content

Irrigation water potentially contaminated by the discharge of untreated wastewater into water bodies can contaminate fresh produce with water-borne pathogens (antibiotic-resistant and disinfectant-tolerant bacteria) that can be transferred to consumers, particularly when consumed raw.

This study aimed to assess the impact of bacterial pollution on the aquatic ecosystem and its receiving environment caused by the discharge of untreated wastewater in Kosovo. Therefore, irrigation water, soil, and Capsicum annum from five vegetable-growing areas were examined for the presence of total and extended-spectrum beta-lactamase (ESBL) *Escherichia coli* (*E. coli*) and potential pathogenic and quaternary alkylammonium compound (QAAC)-tolerant bacteria.

Two different strategies were applied for this purpose, a direct plating and non-selective pre-enrichment cultivation. *E. coli* were cultured on Tryptone Bile X-glucuronide (TBX) agar, whereas antibiotic-resistant ESBL *E. coli* were cultured on TBX supplemented with antibiotic cefotaxime (CTX). Potential pathogenic bacteria were cultured on Mueller-Hinton (MH) agar and QAAC-tolerant bacteria were cultured on MH supplemented with benzalkonium chloride disinfectant C-12 (BAC-C12).

A total of 315 bacteria were isolated from water (n=262), soil (n=33), and Capsicum annum (n=20) samples and phylogenetically identified at the genus level by 16S rRNA gene sequencing. Sixty-nine and 50 isolates examined from TBX+/-CTX were identified as *E. coli* and ESBL *E. coli*, respectively, whereas 108 disinfectant-tolerant bacteria examined from MH+BAC-C12 were phylogenetically identified as members of *Providencia*, *Morganella*, *Pseudomonas*, and *Aeromonas*.

This study identified several risks that contaminated irrigation water through untreated wastewater discharge constitutes a source of multidrug-resistant bacteria that may enter the food chain.

W383 - Fengycin and TasA of *Bacillus subtilis* stimulates the growth and immunization of plants by targeting the seed storages

Presenting Author - *María Victoria Berlanga-Clavero, IHSM-UMA-CSIC, Spain*

Author/s – *Carlos Molina-Santiago, Antonio de Vicente, Víctor J. Carrión, Pieter C. Dorrestein, Diego Romero*

Abstract Content

Beneficial microbes are known to stimulate the germination of the seeds; however, the exact mechanisms mediating these interactions are only beginning. *Bacillus subtilis* is a commonly detected member of the plant holobiont and provides multifaceted traits to the plant health. In this work, we demonstrated that *B. subtilis* triggered genetic and physiological responses in seeds that resulted in changes in the metabolic and developmental status of adult plants.

A multidisciplinary approach based on microscopy, transcriptomics and metabolomics demonstrated that the chemically diverse extracellular matrix of *Bacillus* structurally cooperate in bacterial colonization of the seed storage tissues. The amyloid protein TasA and fengycin, two components of the extracellular matrix differentially stimulated levels of ROS inside seeds after imbibition and targeted the oil bodies of the seed endosperm, provoking specific changes in lipid metabolism or accumulation of glutathione-related molecules that resulted in two different plant growth programs: the development of seed radicles or major growth and immunization of adult plants. Our findings prove the versatility of the bacterial ECM in establishing a mutualistic interaction with plants.

W384 - Synergistic antimicrobial potential of lactoferrin and oregano extract on potentially pathogenic ported bacteria

Presenting Author - *Emoke Pall, USAMV CLUJ, Romania*

Author/s – *Diana Olah, Aurel Vasiu, Emilia Trif, Vasile Cosma, Marina Spinu*

Abstract Content

The widespread and uncontrolled use of antibiotics has led to the emergence of multi-drug resistant microorganisms. Thus, the use of substances with antimicrobial potential can represent reliable alternatives, thus contributing to the reduction of the load of multiresistant pathogens in the veterinary field. Lactoferrin is an iron binding glycoprotein present in exocrine secretions, with multiple biological functions, including antimicrobial potential. This study aimed to assess the antimicrobial capacity of lactoferrin alone and in combination with alcoholic extract of oregano (*Origanum vulgare*) on *Staphylococcus* spp. strains (n=5) isolated from nasal cavity of healty swine raised on low-input outdoor farms from North Western and Central Romania. The antibacterial activity was tested by the agar-well diffusion (Kirby–Bauer assay) and broth microdilution methods. The study findings confirmed the hypothesized enhanced antimicrobial properties of lactoferrin in combination with oregano extract against Staphylococcus spp. strains, compared to the two products tested alone and control antibiotic. Our results indicate a synergistic antimicrobial potential for two natural products thus may contribute to the improvement of animal welfare by reducing the load of potentially pathogenic, antibiotic-resistant bacteria.

W385 - MADAME: enhancing and automating data and metadata retrieval in microbiome analysis

Presenting Author - *Sara Fumagalli, University of Milan-Bicocca, Italy*

Author/s – *Giulia Soletta, Giulia Agostinetto, Manuel Striani, Maurizio Casiraghi, Antonia Bruno*

Abstract Content

Capturing interactions of microbial networks and their entire diversity requires the integration of multiple datasets, pushing current research toward microbiome meta-analysis. To date, huge amounts of data and metadata from microbiome studies are stored in publicly available repositories, representing a valuable resource.

To facilitate and automate the process of data and metadata retrieval, we designed MADAME (MetADAta MicrobiomE), a bioinformatic user-friendly tool. Starting from a free-text query or a provided accessions list, user can download data and metadata, and retrieve the publications related to the bunch of identified projects. In addition, to allow a sensible choice before the actual download of the sequences, the user can generate a report including informative statistics about data.

We applied MADAME to the specific case-study of skin microbiome. Thanks to MADAME we were able to download 34 projects and related metadata, and retrieve related publications. Through the generation of report, statistics and plots, MADAME revealed a great heterogeneity in the identified projects, some of which were far from the queried topic. This output was not related to the functionality of MADAME, but rather due to the lack of data harmonization in metadata description. For this purpose, we propose a shared coordinated effort in metadata compilation consistency, and raw data fairness. Thanks to our report, user can download only adequate projects and detect the misplaced ones, saving time and resources.

Our work results in an easy-to-use tool that can assist a greater understanding of microbial diversity, facilitating the first step of meta-analyses.

W387 – Isolation of fungal specimens from endemic snails feces in a threatened coastal ecosystem

Presenting Author - *Angela Ampuero, Universidad Peruana Cayetano Heredia, Peru*

Author/s – *Andre Ampuero, Carlos Martel, Fatima Rivera, Sarita Olortegui*

Abstract Content

Background: Lomas are seasonal vegetal formations that distribute in certain areas along the Pacific South American coast. Soil in this arid ecosystem is influenced positively by biological crust, a surface thin layer composed by several microorganisms such as fungi. Maintenance of this layer could be vital to this ecosystem, given its relevance in erosion prevention. Several abiotic and biological factors have been attributed to the spore dispersal in fungi. Organisms like small vertebrates and arthropods are relevant as vectors at different spatial scales. However, mollusks have been overlooked as a potential taxon for this mechanism, with a few studies that have showed fungi are able to survive the gut. In this study we show the dispersion of fungi carried by two species of native snails in a Peruvian Lomas Ecosystem.

Objectives: Isolate fungal species from feces of snails *Succinea peruviana* and *Bostryx conspersus*. METHODS Fresh feces were observed in the microscope to confirm the presence of fungal structures. Samples were resuspended and seeded in Sabouraud plates supplemented with chloramphenicol. Selected colonies, posterior to the incubation time, were seeded again in plates of Sabouraud agar and Potato agar. Isolated strains were in process of identification through observations of fungal structures and biochemical tests.

Results: From 52 feces samples, we confirmed the presence of yeasts mostly in the Bostryx snail samples and microalgae in all the samples. Also, 75 strains were isolated, between yeasts and filamentous fungus. Part of these fungal strains were recognized as members of the *Cladosporium* and *Penicillium* genus.

W388 - Soft bioleaching as a process to enrich low-grade alkaline mining waste

Presenting Author - *Romeu Francisco, University of Coimbra, Portugal*

Author/s – *Beatriz Rito, Leonor Matos, Paula V. Morais*

Abstract Content:

Background: The world faces global challenges which require, among other measures, the use of sustainable methods for the recycling of waste. Extraction of metals by the mining industry, refineries or recycling centers requires the use of high-grade materials to be economically viable. Low grade ores or waste present a challenge since the quantity of interfering compounds overshadow the valuable resources and prohibitively increases extraction costs. Although not as strong acid producers as chemolithoautotrophic microorganisms such as *Acidithiobacillus ferrooxidans* (used in Cu bioleaching processes), heterotrophic microorganisms can still cause soft weathering of minerals through the production of organic acids, enzymes, and metallophores.

Objectives: The objective of the current study was to demonstrate the action of heterotrophic microorganisms isolated from bauxite and magnesite mining residues on those same residues, in order to remove low value elements such as Fe or Ca and increase the relative abundance of valuable elements in the solid waste.

Methods: The strains were incubated with bauxite or magnesite mining residues (1%), at different starting pHs and fed with different carbon sources. The metals present in the liquid fraction were quantified by ICP-MS, and the final solid residues were analyzed by XRF.

Results: The strains showed different capacities for the removal of Al, Fe, Mg, Zn, Ca, in 4 days. The process was more efficient at neutral pH, demonstrating that acids do not play an important role in the process. Overall, this study contributes to the understanding of the function of autochthonous heterotrophs on metal mobility in alkaline residues.

W390 - Design of biofactories expressing synthetic PETases and MHETases for polyethylene terephthalate degradation.

Presenting Author - *Elias R. Olivera, University of León, Spain*

Author/s – *Luis Getino, Alejandro Chamizo-Ampudia, Sonia Garrido-Chamorro, Carlos Barreiro, José María Luengo*

Abstract Content

Background: Our modern everyday life is inconceivable without the use of petrochemical plastics. High-level production, extensive use and rapid disposal have resulted in their large-scale accumulation as waste discharged to the environment.

Polyethylene terephthalate (PET) is mainly used for production of bottles, containers, films, and fibers. PET is a linear polyester of repeating units of terephthalate and ethylene glycol. The PET monomer is designated bis(2-hydroxyethyl) terephthalate (BHET). Only a few bacteria and fungi have been described for the degradation of PET to oligomers or monomers.

Ideonella sakaiensis 201-F6 has become a model bacteria able to use PET as energy and carbon source. This ability to use PET as carbon source is based in the existence of a PET hydrolase (PETase) and a second enzyme capable of degrading mono(2-hydroxyethyl) terephthalate (MHET). PET hydrolase as a secreted enzyme produces the intermediate MHET, which is internalized by the cell and hydrolyzed by MHETase. The resulting monomers (ethylen glycol and terephthalate) are then used for bacterial metabolism.

Objectives: PET and BHET degradation enzymes from I. sakaensis, and alternatives engineered have been included in biotechnological chassis: *Pseudomonas* and *Rhodococcus* strains. Modified strains are being tested for PET degradation.

Methods and Results: Strains derived from *Pseudomonas putida* and *Rhodococcus* sp. HE24-12 have been genetically modified with replicative plasmids containing different versions of PETase and MHETase from *I. sakaensis*. These strains have been tested for the modification of BHET and PET showing biotransformation of BHET in METH and terephthalate as well as deterioration of PET films.

W391 - Phosphate solubilizing Rhizobacteria confer a stronger influence on wheat root traits and aboveground physiology through the expression of their genes responsible for P solubilization

Presenting Author - *Mahreen Yahya, National Institute for Biotechnology and Genetic Engineering, Pakistan*

Author/s – *Ejaz ul Islam, Maria Rasul, Claudia Breitkreuz, Thomas Reitz, Mika Tarkka, Sumera Yasmin*

Abstract Content

Applying phosphate solubilizing bacteria (PSB) as bio-fertilizers has enormous potential for sustainable agriculture. Despite this, there is still a lack of information regarding the expression of key genes related to P-solubilization (PS) and efficient formulation strategies. In this study, we investigated rock phosphate solubilization by *Ochrobactrum* sp. SSR (DSM 109610) by relating it to bacterial gene expression and searching for an efficient formulation. qPCR primers were designed for PS marker genes glucose dehydrogenase (gcd), pyrroloquinoline quinone biosynthesis protein C (pqqC) and phosphatase (pho). SSR inoculated soil supplemented with rock phosphate (RP) showed 6-fold higher expression of pqqC and pho compared to inoculated soil without RP. Additionally, an increase in plant P (2%), available soil P (4.7%) and alkaline phosphatase (6%) activity was observed in PSB-inoculated plants supplemented with RP. Root architecture improved by SSR, with higher root length, diameter and volume.

Positive correlations were observed between the PSB solubilization in presence of different insoluble P sources, and soil available P, soil phosphatase activity, seed P content and grain yield of field grown inoculated wheat, when di-ammonium phosphate fertilizer application was reduced by 20 %. The present study reports for the first time marker gene expression of an inoculated PSB strain and provides a valuable groundwork to design field scale formulations that can maintain inoculum dynamics.

W392 - Algae blend affected bacterial composition of lambs digesta microbiome

Presenting Author - *Timur Yergaliyev, University of Hohenheim, Germany*

Author/s – *T. Yergaliyev, C.S.C. Mota, V.A.P. Cadavez, U. Gonzales-Barron, A.R.J. Cabrita, A.J.M. Fonseca, M.R.G. Maia, A. Camarinha-Silva*

Abstract Content

Background: Diet greatly affects lambs' meat quality, with pasture leading to a healthier profile than concentrate. Algae have been reported to promote meat quality and decrease methanogenesis directly or through microbiome modulation.

Objectives: Evaluate the effect of micro- and macroalgae blend supplementation to concentrate on lambs' gut microbiome, compared to pasture and concentrate fed lambs.

Methods: Three groups of 10 Bordaleira lambs were fed 1) pasture, 2) concentrate or 3) concentrate + 5% algae blend (Algaessence®, Portugal). After 2 months, lambs were sacrificed and digesta from the rumen, abomasum, and colon was sampled. DNA was sequenced targeting bacterial and archaeal communities. Bioinformatical analysis was performed in Qiime2 [3] and ALDEX2 [4].

Results: Pasture diet was the most diverged according to microbial profiles. Archaea Methanobrevibacter and Methanomethylophilus were more abundant in concentrate diets, while Methanomethylophilaceae was associated with pasture. Bacteria Butyrivibrio, Clostridia, Pseudobutyrivibrio, Quinella, Succiniclasticum and Saccharimonas were more represented in pasture, while Acetitomaculum, Bifidobacterium, Limosilactobacillus, Ruminobacter, Succinivibrio, Blautia, Anaerostipes, Coprococcus and Dialister in concentrate diets. Microbial alpha diversity of pasture lambs was higher than those of animals fed concentrate or algae. Regarding beta diversity, PCoA plots separated pasture samples from others. For archaea, PERMANOVA analysis revealed differences only between pasture diet and others, while for bacteria, all diets were different.

Lambs fed on pasture hosted different microbiomes compared to the concentrate diets in the rumen, abomasum, and colon. Algae supplementation to concentrate diet affected only bacterial composition, while alpha diversity and archaeal composition were not affected.

W393 - Biodiversity and biological activities of *Actinobacteria* from Algerian Arid Lands

Presenting Author - *Nadjette Djemouai, University of Ghardaia, Algeria*

Author/s – *Nadjette Djemouai, Atika Meklat, Sid Ahmed Saadi, Asma Nacer, Carol Verheecke-Vaessen*

Abstract Content

In this study, we report the diversity of actinobacteria that inhabit the roots and the rhizosphere of Artemisia herba-alba Asso. The antagonistic and enzymatic activity as well as the PGP traits of the obtained strains are studied. In total, 92 actinobacterial isolates were obtained. Based on their identification, the isolated actinobacteria were assigned to different genera with a dominance of Streptomyces in the rhizospehere and Nocardioides in the roots of A. herba-alba Asso. Molecular characterization of all endophytic isolates showed that the majority of the endophytes are related to Nocardioides albus with similarity percentages ranging from 99.30 to 99.86%. On the other hand, the molecular study involved only 13 strains of *Streptomyces* from the rhizosphere and showed the presence of 10 different species that share similarities of 99.38 to 100% with the type species of this genus. The results of the antagonistic activity showed that only 17.02% of the endophytic strains had antimicrobial activity. However, 73.33% of the rhizosphere strains showed more interesting antimicrobial activities especially the strains BTS40 and BKS30. The screening of PGP traits in vitro revealed that the majority of endophytic and rhizospheric strains are capable of ammonia production (84.78%), siderophores (72.83%), nitrogen fixation (68.48%) and having 1-aminocyclopropane-1-carboxylate deaminase activity (66.30%). The rhizosphere of Artemisia herba-alba is a rich reservoir of diverse and important actinobacterial species with potential antibacterial and antifungal properties that can further benefit agricultural industry and could be applied in helping plants to withstand harsh conditions and biological aggressions.

W394 - Location of N2O metabolizing microbes in above the ground vegetation in the transect from temperate to sub-Arctic region

Presenting Author - *Krishnapriya Thiyagarasaiyar, University of Eastern Finland, Finland*

Author/s – *Dhiraj Paul, Johanna Kerttulla, Reti Ranniku, Kaido Soorsaar, Katerina Machácová, Henri Siljanen*

Abstract Content

Nitrous oxide (N_2O) is an important greenhouse gas in the troposphere controlling ozone concentration in the stratosphere through nitric oxide production. Nitrification and denitrification are the two main microbial processes that release N_2O. Most of the studies were focusing on N_2O metabolism by soil and sediment microbes. However, a very limited study is available on microbes involved N_2O metabolism in above ground vegetation. Therefore, in the present study N_2O metabolism of above ground vegetation across the temperate to sub-arctic regions was conducted. In order to quantify microbes capable of N_2O reduction, we conducted quantitative polymerase chain reaction (qPCR) assay targeting the nosZ gene encoding the catalytic subunit of the nitrous oxide reductase. Around 91 snap frozen with liquid nitrogen in situ samples were analyzed which includes Hornbeam, European beech, Downy birch, Norway spruce, and some below ground shrubs. To identify the abundance of N_2O consuming microbes, plant parts i.e., leaves, stems and tree cores at the high of 1.5 m were used in the present study. Abundance of nosZ gene copies varied from 100 to 1000000 target copies/g of plant samples and highest was observed in temperate forest phyllospheric samples and especially in hornbeam branches (5200000 copies). Ammonia, nitrite, and nitrate contents of plant tissues were investigated through spectrophotometric analysis. Concentration of inorganic nitrogen varies according to regions and plant samples, which might indicate microbial role in nitrification and denitrification process. The study showed that microbes present in above ground vegetation may play major role in N_2O metabolism forests.

W396 - Examining the impact of microbial-based cleaning products on human pathogens

Presenting Author - *Chidinma Lynda Akaihe, University of Manchester, United Kingdom*

Author/s – *Christopher Knight, Aline Metris, Barrett Paul, Mullen Kerys, Andrew J. McBain*

Abstract Content

Microbial-based cleaning products (probiotic cleaners) are believed to improve the hygiene of kitchen worktops, floors, and other surfaces through mechanisms that include antimicrobial activity of germinated Bacillus spores. We have microbially characterised a selection of probiotic cleaners and have assessed inhibitory activities of the cleaner vehicle and the associated microorganism(s). Seven probiotic cleaners were analysed for the presence of bacteria/microbial spores. An agar well diffusion test was used to evaluate the antimicrobial effects of these products against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Each probiotic cleaner was centrifuged to produce a bacteria-free product (BFP) and pelleted spores if present. Antimicrobial activity of the BFP and the spores after germination were tested against S. aureus, *E. coli*, and P. aeruginosa. Amplified DNA of isolates from MBCPs were identified using 16S ribosomal DNA sequencing. Five out of 7 MBCPs contained Bacillus spores. Four Bacillus isolates had direct antimicrobial effects in agar well diffusion tests against S. aureus only. None of the germinated spores from the products tested was able to produce an inhibitory substance against the growth of *E. coli* and P. aeruginosa. All the BFP tested inhibited the growth of S. aureus. BFP (6/7) inhibited the growth of *E. coli*, and BFP (3/7) inhibited the growth of P. aeruginosa. The probiotic products contain a combination of two or more microbial species. MBCPs had antibacterial effects primarily attributable to antimicrobials in the product formulation. Further work will investigate the safety of MPCP use with respect to changes to the Human microbiome.

W397 - High Salt Levels Reduced Dissimilarities in Root-Associated Microbiomes of Two Barley Genotypes

Presenting Author - *Asma Nacer, Université Des Sciences Et De La Technologie Houari-boumédiène, Algeria*

Author/s – *Zhichun Yan, Xu Chang, Amina Bouherama, Said Amrani, Rene Geurts, Ton Bisseling*

Abstract Content

Plants harbor in and at their roots bacterial microbiomes that contribute to their health and fitness. The microbiome composition is controlled by the environment and plant genotype. Previously, it was shown that the plant genotype-dependent dissimilarity of root microbiome composition of different species becomes smaller under drought stress. However, it remains unknown whether this reduced plant genotype-dependent effect is a specific response to drought stress or a more generic response to abiotic stress. To test this, we studied the effect of salt stress on two distinct barley (*Hordeum vulgare L.*) genotypes: the reference cultivar Golden Promise and the Algerian landrace AB. As inoculum, we used soil from salinized and degraded farmland on which barley was cultivated. Controlled laboratory experiments showed that plants inoculated with this soil displayed growth stimulation under high salt stress (200 mM) in a plant genotype-independent manner, whereas the landrace AB also showed significant growth stimulation at low salt concentrations. Subsequent analysis of the root microbiomes revealed a reduced dissimilarity of the bacterial communities of the two barley genotypes in response to high salt, especially in the endophytic compartment. High salt level did not reduce α-diversity (richness) in the endophytic compartment of both plant genotypes but was associated with an increased number of shared strains that respond positively to high salt. Among these, Pseudomonas spp. were most abundant. These findings suggest that the plant genotype-dependent microbiome composition is altered generically by abiotic stress.

W398 - Microbial hydrogen cycling in agricultural systems – plant beneficial or detrimental?

Presenting Author - *Zahra Islam, The University of Melbourne, Australia*

Author/s – *Chris Greening, Hang-Wei Hu*

Abstract Content

Soil microorganisms have long been recognised as key players in biogeochemical nutrient cycles, particularly within the carbon, nitrogen and phosphorus cycles, with more recent studies highlighting their effects on the cycling of atmospheric trace gases such as hydrogen (H2) and carbon monoxide. Research into the diversity and metabolic potential of plant-associated bacteria has revealed that numerous taxa harbour H2 scavenging genes capable of mediating atmospheric H2 oxidation, including those associated with *Arabidopsis thaliana*, *Oryza sativa* and in numerous plant-associated bacteria that can fix nitrogen, suggesting that the H2 produced is recycled by closely associated microorganisms or internally if they harbour a high-affinity uptake hydrogenase. Moreover, previous research has indicated that both exogenous H2 gas and hydrogen-oxidising bacteria have a net positive effect on legumous plants, though the effects on non-legumous plants are less clear. Using a multidisciplinary approach, we aim to combine traditional microbial culturing methods with soil physicochemical analysis, greenhouse studies, gas chromatography phylogenetics and meta-omics techniques to characterise the trace gas oxidation capacity of plant-associated microorganisms within diverse Australian agricultural settings, determine whether this process can benefit agricultural crops and whether agricultural practices such as usage of fertilisers effect the aerobic H2 scavenging capacity of plant-associated bacteria. Understanding the role atmospheric trace gas cycling plays in agricultural soils and how they are affected is paramount for developing agricultural management practices that effectively harness the inherent properties of indigenous soil microorganisms.

W399 - A cell-direct quantitative PCR based method to monitor viable genetically modified microorganisms

Presenting Author - *Bumkyu Lee, Jeonju University, Korea, Republic of*

Author/s – *Bumkyu Lee, Yang Qin, Yang Ha Kim*

Abstract Content

The development and commercialization of industrial genetically modified (GM) microorganisms is actively progressing worldwide, highlighting an increased need for improved safety management protocols. We aimed to establish an environmental monitoring method, using real-time PCR and propidium monoazide (PMA) treatment to develop a quantitative detection protocol for living GM microbial strains: *Escherichia coli*, *Corynebacterium glutamicum*, and *Saccharomyces Cerevisiae*. Direct PCR, whereby the microbial strain was placed directly in the PCR reaction solution for analysis, was applied for the DNA materials used in the experiment. The target genes were the endogenous genes in each microbial strain. Quantitative analysis based on real-time PCR led to a detection limit of up to 10-4 of the cell culture solution for *E. coli*, while the limit of detection (LoD) was 10-4 and 10-1, respectively, for *C. glutamicum* and *S. cerevisiae*. Real-time PCR had an approximately 10-fold lower LoD for the group with PMA treatment than the group without PMA treatment. This can be presumed to be because only the DNA of viable microorganisms had been amplified in the PCR, while PMA exposure of the DNA of dead cells prevented it from being amplified. The dPCR for the three microbial strains had an approximately 10-fold higher LoD compared to the real-time PCR. Compared to DNA-based qPCR methods, cell suspension direct PMA-qPCR analysis provides reliable results and is a quick and accurate method to monitor living GM microbial cells that can potentially be released into the environment.

W400 - Diversity and activity of nitrous oxide regulating metabolism in the above-ground process in the spruce forests in the boreal

Presenting Author – *Dhiraj Paul, University Of Eastern Finland, Finland*

Author/s – *Inga Paasisalo, Anuliina Putkinen, Chris Jones, Sara Hallin, Mari Pihlatie, Henri Siljanen*

Abstract Content

Nitrous oxide (N2O), strong greenhouse gas (GHG), sink strength of boreal and arctic peatlands and forests in a warming climate is a key question for climate change mitigation. Currently, vegetation in the climate change assessment and GHG flux models, are lacking information on microbiological mechanisms consuming atmospheric N2O within above-ground vegetation. Therefore in the present study we first time investigated the microbial role in N2O consumption in above-ground processes using novel captured metagenomics techniques. We have collected phyllospheric samples from spruce upland forest in Finland Viikki, Kuopio and Pallas. All samples consumed N2O in small microcosm experiment, were five times higher concentration of N2O than in the atmosphere were introduced for the plant tissues samples under anoxic conditions. However, the samples closer to the city environments consumed more than those from the pristine environment. Optimization of microbial DNA extraction followed by captured metagenomics and bioinformatics analysis was done to identify the abundance of functional genes involved in the process. Functional gene diversity analysis indicated abundance of nosZ gene in most of the samples. It was also noted that nosZ clade I abundance was much higher compared to the nosZ clade II. Bacterial genera *Bradyrhizobium*, *Acidovorax* belong to clade II were detected whereas bacterial genera *Thauera*, *Azoarcus*, *Rubrivivax*, *Leptothrix* belong to clade I were observed. Abundance of nifH, narG indicated that presence of microorganisms related to nitrogen fixation and denitrification activities in the phyllospheric samples. Therefore, our finding indicates the importance of microbial interactions in above-ground systems and their N2O metabolism.

W401 - Soil fungi as biomediator in silver nanoparticles formation and antimicrobial efficacy

Presenting Author - *Hana Sonbol, Princess Nourah Bint Abdulrahman University, Saudi Arabia*

Author/s – *Afrah E Mohammed, Shereen M Korany*

Abstract Content

Introduction and Objectives: Biogenic agents in nanoparticles fabrication are gaining great interest due to their lower possible negative environmental impacts. The present study aimed to isolate fungal strains from deserts and assess their ability in silver nanoparticles (AgNPs) fabrication and evaluate their antibacterial effect.

Methods: Soil fungi were identified using 18s rDNA, and their ability in NPs fabrication was assessed as extracellular synthesis, then UV-vis spectroscopy, dynamic light scattering (DLS), energy-dispersive X-ray spectroscopy, and transmission electron microscopy were used for AgNPs characterization. The antibacterial activity of fungal-based NPs was assessed against one Gram-positive methicillin-resistant S. aureus (MRSA) and three Gram-negative bacteria (*E. coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*). Ultrastructural changes caused by fungal-based NPs on *K. pneumoniae* were investigated using TEM along with SDS-PAGE for protein profile patterns.

Results: The three fungal isolates were identified as *Phoma* sp. (MN995524), *Chaetomium globosum* (MN995493), and *Chaetomium* sp. (MN995550), and their filtrate reduced Ag ions into spherical P-AgNPs, G-AgNPs, and C-AgNPs, respectively. DLS data showed an average size between 12.26 and 70.24 nm, where EDX spectrums represent Ag at 3.0 keV peak. G-AgNPs displayed strong antibacterial activities against *Klebsiella pneumoniae*, and the ultrastructural changes caused by NPs were noted. Additionally, SDSPAGE analysis of treated *K. pneumoniae* revealed fewer bands compared to control, which could be related to protein degradation.

Conclusion: Present findings have consequently developed an eco-friendly approach in NPs formation by environmentally isolated fungal strains to yield NPs as antibacterial agents

W402 - Remicult: A Metagenome Engineering Approach for Culturing the 'Unculturable'

Presenting Author - *Ole Hylling, Technical University of Denmark, Denmark*

Author/s – *Mads Frederik Hansen, Jonas Stenløkke Madsen, Barth Smets*

Abstract Content

Microbiologists have only obtained <1% of Bacteria (and Archaea) species in pure cultures while the remaining species are aptly coined as the 'Microbial Dark Matter'(MDM). Evidently, accessing MDM culturability will pave the way for new avenues in microbial ecology and biotechnology, and we believe one reason to this unculturability is harmful generation of reactive oxygen species (ROS) at laboratory conditions. Many environmental communities experience low fluxes of electron donors, thus generating low amounts of ROS. By contrast, laboratory conditions present a high flux of electron donors, leading to high generation of ROS, while simultaneously isolating members from one another. We hypothesize that some members of environmental communities lack ROS defenses. Thus, any attempt to culture said members fails at standard conditions as ROS accumulates and hampers growth. The aim of the Remicult project is to develop a biological, gene delivery system for metagenome engineering of environmental communities. We intend to remediate culturability of members that lack ROS defenses. This delivery system employs vector constructs that harbor genes for ROS-scavenging (H2O2) and a fluorescent marker for cell sorting. We have a now constructed broad-host vectors harboring an operon insert, consisting of a fluorescent marker gene fused with an *E. coli* katG gene. Furthermore, we have established an *E. coli* model for H2O2 MIC in katG/katEG (catalase) mutants and observed recovered growth along fluorescent signal in complementation experiments of mutants harboring the vectors. The Remicult project is now mature enough to venture in to remediating culturability of the proposed (unculturable) species.

W403 - Natural temperature gradient of geothermal area in Iceland on microbially driven CO2 and CH4 dynamics

Presenting Author - *Johanna Kerttula, University Of Eastern Finland, Finland*

Author/s – *Johanna Kerttula, Henri Siljanen, Cristina Biasi*

Abstract Content

Natural temperature gradients within geothermal areas provide a platform for studying the effects of warming on microbially driven greenhouse gas (GHG) dynamics in natural environment. Sitka spruce forest (FN) in Iceland is a study site as a part of “ForHot” research network that was established in 2011 to study soil temperature effects on ecosystem processes. The FN site has a natural geothermal soil temperature gradient that, at the depth of 10 cm, ranges from ambient temperature to +65 °C. Soils in Northern latitudes, especially boreal forests and permafrost, store most of the global soil organic carbon. Soil organic carbon is at high risk to be released to the atmosphere as carbon dioxide (CO2) and methane (CH4), due to temperature induced microbial activity.

Objective of the study is to quantify the temperature effects on biotically produced CO2 and CH4 in natural environment.

We measured CH4 and CO2 dynamics along the natural temperature gradient at sitka spruce forest in Iceland during peak growing season in June 2022, and connected the GHG fluxes to simultaneous RNA expression and the presence of functional genes of methanogenesis with novel targeted metagenomics -tool. We measured both soil surface flux with static chambers and soil depth profile with soil gas probes at 5, 10, 15, and 20 cm. Stable isotopes (13C-CO2 and 13-C CH4) were measured to source partition and to exclude geothermal bias. Parallel to GHG measurement soil RNA and DNA were sampled at ambient, +5 and + 40C soil temperatures. Results will be discussed.

W404 - Occurrence of *Cryptosporidium* in game of Brandenburg, Germany

Presenting Author - *Claudia Jäckel, German Federal Institute For Risk Assessment, Germany*

Author/s – *Claudia Jäckel, Anne Mayer-Scholl, Jens Andre Hammerl, Carl Gremse, Karsten Nöckler, Martin Richter*

Abstract Content

Cryptosporidiosis is a widespread diarrheal disease of humans/animals, caused by *Cryptosporidium*. The protozoan is mainly ingested by the consumption of water or raw food, contaminated by the contact with feces of infected persons/animals. However, there is only insufficient information on the worldwide distribution of *Cryptosporidium*. For a better understanding of the risk of human cryptosporidiosis by consumption of contaminated food, this study investigated the occurrence in game of Brandenburg, Germany.

In this study, 562 wildlife samples from 14 districts in Brandenburg taken during three hunting years (2017-2020) were tested for the presence of *Cryptosporidium*. Extracted samples were molecularly checked for the presence of two gene sequences (18S rDNA, COWP) using nested PCR and specified by sequencing/RFLP.

Compared to other European studies the results indicate a high molecular prevalence of *Cryptosporidium* in game of Brandenburg (wild boar: 23.28%, red deer: 9.68%, roe deer: 17.24%, fallow deer: 33.33%), whith huge differences between the years and districts. Young animals are more affected than older ones. Additionally to the C. sp. deer and vole specific genotype, human pathogenic species *C. scrofarum*, *C. suis*, *C. parvum* and *C. ubiquitum* were also identified.

Cryptosporidium seems to be autochthonous in German game. For the assessment of the potential risk, which may arise from the consumption of food a monitoring of raw consumed food and game should be established.

W405 - Exploration of the chemoreceptor repertoire of plant pathogens

Presenting Author - *Roberta Genova, Zaidín Experimental Station, Spain*

Author/s – *Roberta Genova, Miguel Matilla, Tino Krell*

Abstract Content

Background: Plant pathogens contain many more chemoreceptors than the bacterial average. Sequence analysis reveal that many chemoreceptor families are specific for plant associated bacteria. However, there is only very scarce information available on the function or ligands recognized by plant pathogens.

Objectives: The aim of this study is filling these gaps of knowledge on bacterial sensing. We identify chemoreceptor function, using *Pectobacterium atrosepticum* SCRI1043 as model organism, which is among the top 10 most relevant bacterial phytopathogens. SCRI1043 contains 36 chemoreceptors, mostly of unknown function.

Methods: The individual chemoreceptor sensor domains were overexpressed and purified by affinity chromatography. Purified protein was submitted to differential scanning fluorimetry based thermal shift assays to screen different compound arrays for potential ligands. The compounds were tested by isothermal titration calorimetry to derive binding constants. Chemotaxis assays were performed to test bacterial attraction or repulsion to the compounds.

Results: We identify the function of three chemoreceptors encoded in a gene cluster containing four paralogous genes. PacG (*Pectobacterium atrosepticum* chemoreceptor G), PacH and PacI sensor domain bound, among other ligands, plant-derived compounds like salicylic acid, vanillin and agmatine derivatives. Chemotaxis studies reveals P. atrosepticum attraction to vanillin and 4- hydroxibenzoic acid; further studies will reveal whether and to which degree *P. atrosepticum* responds to the ligands identified and whether these responses are associated with virulence.

W406 - Differential capacity of invasive and native algal holobionts for control of their microbiomes

Presenting Author - *Marjan Ghotbi*

Abstract Content

Microbiomes are important functional interfaces that play a key role in hosts physiology and ecology. The seaweed microbiome, in the form of an epiphytic multikingdom biofilm, influences the host development and acts as a second skin regulating physical and chemical defense against pathogens. Thus, seaweeds' ability to control the composition of their microbial biofilm can affect their health and resilience. To test whether the alga's potential to control its biofilm differs between native and invasive species, we experimentally studied microbial biofilms (prokaryotes and microalgae) formed on a porous proxy surface in the vicinity of three seaweeds, including one invasive and two native species (*Gracilairia vermiculophylla*, *Fucus serratus, Fucus vesiculosus*) and compared them with mature epiphytic biofilms from the same seaweeds. Although mature biofilm on native seaweeds showed higher prokaryotic diversity, the invasive seaweed had a higher diversity in its core microbiome, i.e. ability to maintain a higher diversity of persistent microbes. Microalgae diversity exhibited no significant differences among seaweeds. Substrate type imposed a stronger force in shaping composition of microbial communities rather than algal treatments or time of exposure. At the community level, no significant compositional differences were found among biofilms on proxy surfaces adjacent to native vs. invasive holobionts and empty control bottles. However, at taxon level the highest similarity was seen between the invasive holobiont and its adjacent biofilm. We observed the highest contrast between control biofilms and invasive seaweeds adjacent biofilm. This may represent the higher capacity of invasive seaweed for control of their microbiome.

W408 - Isogenic heterogeneity in lifespan follows an evolutionary trade-off in response to amino acid identitya

Presenting Author - *Kiyan Shabestary, Imperial College London, United Kingdom*

Abstract Content

In the wild, microorganisms mainly operate at suboptimal growth conditions with fluctuations in nutrient abundance. Constrained by finite resource allocation principles and subject to fitness pressure, adaptation to suboptimal conditions often takes the form of a strategic choice between two conflicting tasks: growth or survivability maximisation. Here, we systematically study the impact of single amino acid on cellular metabolism and report isogenic macro-heterogeneity in response to a change in nitrogen quantity and quality conserved in *Saccharomyces Cerevisiae* strains. Cells exposed to a nitrogen down-shift differentiate into subpopulations of different sizes, chronological lifespans and growth resumption capabilities. We monitor the metabolic response of the subpopulations using a protein-tagged GFP library coupled to high-throughput microscopy and single-cell tracking. We show that depending on the nitrogen source and quantity available, cells can choose to either maintain or abort this differentiation process. We propose that this macro-heterogeneity is a case of bet-edging where subpopulations operate at distinct spaces of the growth-survivability trade-off depending on the amino acid present. These results establish amino acids as important signalling molecules for chronological lifespan and growth rate determination.

FP1/1 - Virus-host interactions in atmosphere-close aquatic ecosystems of the Central Arctic

Presenting Author - *Janina Rahlff, Linnaeus University, Sweden*

Author/s - *Karin Holmfeldt*

Abstract Content

Background: Aquatic viruses are abundant biological entities acting as key players in shaping microbial communities. In polar environments, viruses face challenges like limited host availability and harsh conditions. Due to restricted ecosystem accessibility, little is known about viruses from aquatic ecosystems at high latitudes.

Objectives: The aim was to investigate viral abundance, diversity, and host interactions at the air-water interface of aquatic ecosystems of the Central Arctic.

Methods: Aquatic samples for virus-host analysis were collected from ~60 cm depth including the submillimeter surface microlayer during the Synoptic Arctic Survey on icebreaker Oden (1) in summer 2021. Water was sampled from a melt pond (MP) and open water (OW) before undergoing size-fractioned filtration. Metagenomics and cultivation were applied to investigate prokaryotic and viral communities.

Results: Alpha-diversity for prokaryotes in the MP was much lower compared to OW. The MP was dominated by a single Flavobacterium sp., while surface water from the Arctic Ocean contained a variety of Flavobacteria, Alpha- and Gammaproteobacteria, as well as Marine Group II Archaea. From 1331 recovered viral OTUs, ~12% were in silico linked to a Pelagibacter metagenome-assembled genome as the host. Viral diversity on the host fraction (5 - 0.2 µm) of the MP was strikingly limited compared to OW, but the few, mainly Flavobacteria phage-related vOTUs, proliferated strongly in the MP based on read mapping. Ten vOTUs encoded for glycerol-3-phosphate cytidylyltransferase (tagD), which could serve cryoprotection of the host (2). The results suggest that viruses have elaborate strategies to endure in extreme and host-limited environments.

FP1/2 - Viral and bacterial diversity during grass silage preservation

Presenting Author - *Johan Sebastián Sáenz, University of Hohenheim, Germany*

Author/s - *Bibiana Galicia, Bianca Rehkugler, Jana Seifert*

Abstract Content - The conservation of forage feed by ensilaging is one of the most common fermentation processes and its success is mainly driven by microorganisms. Even though the role of bacteria and fungi in the silage preservation have been broadly described, no information on the virome and its interaction with the silage microbiome is known. We used 18 metagenomes and 16S rRNA amplicon libraries to describe the composition and structure of the bacterial and viral community during a 40-day grass silage preservation. We observed a rapid decrease in the pH and a shift in the bacterial and viral composition within the first two days of the preservation. Besides, the viral populations were more diverse within the first two days compared to the end of the preservation. Also, the predicted putative host of the recovered viral populations during each sampling time resembled the changes in the bacterial community. The viral population in grass silage is unknown because only 10% of the total recovered viral populations clustered with a reference genome and ~30-40% of total phages could not be assigned to a known viral family. Several defence mechanisms were found across the recovered bacterial MAGs, being the restriction modification the most common system. CRISPR-Cas arrays were not commonly found in the bacterial genomes but those found in *Lentilactobacillus diolivorans* and *Levilactobacillus brevis* showed a pre-existing interaction with the viral populations. The data suggest that viral populations are enriched during grass ensiling and they could have a role in the establishment of the bacterial community.

FP1/3 - Identification of the cellular factor UHRF1 as a new epigenetic repressor of Human T-Lymphotropic Virus type 1 transcription

Presenting Author - *Estelle Plant, Département de Biologie moléculaire, Faculté des Sciences Campus de Charleroi Université Libre De Bruxelles (ULB), Belgium*

Author/s - *Estelle Plant, Laure Vreux, Mathilde Galais, Lorena Nestola, Maryam Bendoumou, Jean-Marie Péloponèse, Carine Van Lint*

Abstract Content

Background: Human T-lymphotropic Virus type 1 (HTLV-1) is characterized by a long period of latency leading in a few cases to an aggressive lymphoproliferative disease, adult T-cell leukemia/lymphoma (ATLL) (1), or into a neurological degenerative syndrome known as HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (2). The mechanisms leading to the development of HLTV-1-associated diseases are still unclear. The latency state is a viral strategy to escape the host immune system contributing to tumor development. A better understanding of the mechanisms driving HTLV-1 into latency could allow the development of new therapeutic strategies against HTLV-1 infection and its associated diseases.

Objectives: Our present study aimed at determining the role of the cellular factor UHRF-1 (Ubiquitin-like containing PHD and RING Finger domains 1) in HTLV-1 transcriptional regulation and latency.

Methods & Results: We demonstrated that UHRF1 repressed HTLV-1 transcription by transient transfection and RNA interference assays using latently-infected cell lines. We showed the binding of UHRF1 in vitro to the Tax-responsive element 1 of the viral promoter by electrophoretic mobility shift assays, and the recruitment of UHRF1 in vivo to the HTLV-1 latent promoter in infected cell lines. This recruitment was accompanied by the recruitment of epigenetic remodelers known to interact with UHRF1. We assessed the mRNA levels of UHRF1 in cells from HAM/TSP and ATLL patients and showed an inverse correlation between UHRF1 and Tax transcripts levels. Finally, UHRF1 interacted with Tax without affecting the stability of Tax. Altogether, our results identified UHRF1 as a new critical epigenetic repressor of HTLV-1 transcription.

FP1/4 - The role of I203M and I208L on raltegravir resistance in Ugandan HIV-1

Presenting Author - *Paul Solis-Reyes, Western University, Canada*

Author/s - *Emmanuel Ndashimye, Mariano Avino, Cissy Kityo, Art Poon, Immaculate Nankya, Eric Arts*

Abstract Content

Background: Integrase strand-transfer inhibitor (INSTI) use in sub-Saharan Africa has increased rapidly in recent years, but little is known about INSTI resistance in the non-subtype B HIV-1. Uganda has among the highest burdens of HIV-1, but no studies have analyzed INSTI resistance specific to this region.

Objective: To identify novel INSTI resistance pathways specific to Ugandan HIV-1.

Methods: We recently sequenced the integrase gene of 51 clients failing treatment with and 328 clients naïve to raltegravir. We identified two novel mutations, I203M and I208L, which often appeared together and were strongly associated with raltegravir failure. To determine the role of these mutations on raltegravir resistance, we first generated I203M, I208L, and double mutant viruses with either subtype A or B integrase. We then selected for high-level raltegravir resistance in these viruses, as well as in wild-type viruses, via a long-term (~200 days) drug-dose escalation experiment. We tracked the development of raltegravir resistance and accumulation of mutations within integrase in each virus before and throughout this experiment.

Results: I203M and I208L, both alone and in combination, are not sufficient for INSTI resistance. However, during raltegravir escalation, I203M/I208L mutants developed primary INSTI-resistance mutations faster on average compared to wild-type. This corresponded with quicker and stronger development of phenotypic INSTI resistance on average in the I203M/I208L mutants.

Conclusions: We provide early evidence that the newly described I203M and I208L mutations observed in Ugandan raltegravir-experienced clients may influence raltegravir resistance by decreasing the fitness costs associated with primary drug resistance mutations.

FP2/1 - Resistance to host antimicrobial peptides mediates resilience of gut commensals during infection in *Drosophila melanogaster*

Presenting Author - *Igor Iatsenko, Max Planck Institute for Infection Biology, Germany*

Author/s - *Igor Iatsenko, Aranzazu Arias-Rojas, Dagmar Frahm*

Abstract Content

The microbiota is exposed to host immune effectors triggered by the pathogens during intestinal infection. Yet, unlike pathogens, healthy gut microbial communities remain stable through the infection (1,2). How commensals stably persist in the gut during infection-induced immune response remains mostly unexplored. Here, we used *Drosophila melanogaster* and its natural pathogen *Pectobacterium carotovorum* as a model to investigate how infection shapes the commensal communities in the host gut. First, using 16s rRNA sequencing, we found no significant effect of infection on microbiota species richness and diversity. Next, focusing on a major *Drosophila* commensal *Lactiplantibacillus plantarum* (3), we showed that it remains stable throughout the time-lapse of infection and is resistant to *Drosophila* antimicrobial peptides (AMPs), unlike pathogens. By a transposon screening, we identified L. plantarum mutants sensitive to AMPs. These mutants were impaired in peptidoglycan or teichoic acid modifications, resulting in increased negative cell surface charge and higher affinity to cationic AMPs. AMP-sensitive *L. plantarum* mutants were cleared from the gut after infection and aging-induced gut inflammation in wild-type, but not in AMP-deficient flies, suggesting that the resistance to host AMPs is essential for commensals to persist in an inflamed gut environment. These results together with our previous findings (4) illustrate the importance of microbial resistance to AMPs in host-pathogen and host-commensal interactions.

FP2/2 - The role of mexZ in infection beyond antibiotic resistance

Presenting Author - *Pablo Laborda, Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Denmark*

Author/s - *Pablo Laborda, Signe Lolle, Sara Hernando-Amado, José Luis Martínez, Søren Molin, Helle Krogh Johansen*

Abstract Content

Antimicrobial resistance (AMR) currently constitutes a major health problem. *Pseudomonas aeruginosa* is one of the microorganisms with high-risk regarding AMR, since having low intrinsic susceptibility to many antimicrobials and an overwhelming capacity to acquire AMR, mainly by mutations when establishing persistent lung infections. Mutations in mexZ, encoding the local negative regulator of the MexXY efflux pump encoding genes, have been reported as the most frequently acquired mutations during such infections. Although traditionally related to resistance to the first-line drug tobramycin caused by overproduction of MexXY resulting in extrusion of this antibiotic, mutations in mexZ surprisingly contribute to only low levels of AMR, and in fact are rarely selected for when exposing bacteria to tobramycin in vitro. These facts, together with the frequent appearance of mexZ mutations in the clinic, suggest additional consequences of these mutations in connection with the infection process. Searching for alternative phenotypes associated with mexZ mutations, we have investigated the colonization strategy of a mexZ mutant in an in vitro cell culture infection model comprising all the characteristic differentiated cell types normally encountered in human airway epithelia in vivo. We observed that mutated mexZ bacteria, in contrast to wild-type bacteria, accumulate inside the epithelial cell layer, where a more protective environment might be found. The altered invasiveness was caused by a disrupted equilibrium between the overproduced MexXY and MexAB, an efflux pump able to extrude quorum-sensing molecules. These findings offer an alternative explanation for antibiotic treatment failure rooted in host-microbe interaction dynamics collaterally altered by AMR development.

FP2/3 - Bacterial single-cell RNA-seq through advanced MATQ-seq

Presenting Author - *Christina Homberger, University of Wurzburg, Germany*

Author/s - *Regan J Hayward, Lars Barquist, Jörg Vogel*

Abstract Content

RNA-sequencing technologies have provided important insights into the transcriptional and regulatory networks in many bacteria and their hosts. Yet, these approaches typically report average expression profiles in populations and fail to provide information on the single-cell level. Heterogeneity at the single-cell level has been mostly explored in eukaryotic cells; however, the phenomenon of phenotypic heterogeneity is also commonly observed in genetically identical bacteria and thought to help bacterial populations to adapt faster to changing environments and allow for division of labor. However, it has been difficult exploring this in bacteria due to the scarcity of global single-cell approaches.

Recently achieved technical advances have paved the way for true single-cell transcriptomics (1). Here, we report our improved MATQ-seq protocol for bacterial single-cell RNA-seq (2) that provides a much higher efficiency of cDNA synthesis, a very low dropout rate, and pioneering implementation of Cas9-based rRNA depletion compared to our previously published protocol (3). Using the advanced approach, we capture many more genes, allowing us to investigate gene expression heterogeneity of *Salmonella enterica* serovar Typhimurium under different growth conditions and reported a higher gene detection limit as well as an improved overall gene coverage compared to the previous protocol. Evaluating our approach, previously described heterogeneity within *Salmonella* associated with flagellar expression and genes encoded in pathogenicity islands was confirmed. We have also achieved to capture expression profiles of small regulatory RNAs. Due to the high sensitivity and the low cell loss, our approach is especially suited for studies with limited input material.

FP2/4 - Neurotransmitter-producing bacteria interact with adrenergic receptors

Presenting Author - *Friedrich Götz, Interfaculty Institute for Microbiology and Infection-medicine Tübingen (IMIT), Germany*

Author/s - *Arif Luqman, Moushumi Purkayastha, Samane Rahmdel*

Abstract Content

Background: Trace amines (TA) are neurotransmitters endogenously produced in mammals. However, they are also produced by bacteria belonging to the human microbiota. In certain staphylococcal species TAs are produced by an enzyme named staphylococcal aromatic amino acid decarboxylase (SadA). The TAs were secreted and could interact with the host.

Objective: Which role do TAs of commensal bacteria play in infection and wound healing?

Result: SadA has a broad spectrum of activity because it decarboxylates tryptophan, tyrosine and phenylalanine to tryptamine (TRY), tyramine (TYM), and phenethylamine (PEA), in a pyridoxalphosphate (PLP)-dependent reaction, it also decarboxylates dihydroxy phenylalanine (L-DOPA) and 5-hydroxytryptophan (5-HTP) to the neurotransmitters DOP and serotonin. Metagenomic analysis of the human skin microbiota revealed that SadA homologs are widespread particularly in the phyla Actinobacteria, Proteobacteria, Firmicutes, and Bacteroidetes. Many of the genera that have a SadA homolog belong to the classical skin and gut microbiota. The potential interaction of TA-producing bacteria with the host has been studied with *Staphylococcus epidermidis* and *Staphylococcus pseudintermedius*. Their secreted TA trigger the internalization by human cells by activation of the α2-adrenergic receptor (α2-AR). Moreover, TA alone and a TA-producing S. epidermidis strain accelerate wound healing by antagonizing the □2-adrenergic receptor (□2-AR) in keratinocytes. Since, at least in staphylococci, aromatic amino acids are almost completely converted to TAs which are secreted, a comparatively high concentration accumulates in the environment where they can exert presynaptic "amphetamine-like" effects. Currently, the impact of bacterial-derived TAs on certain neurological diseases is investigated.

FP2/5 - RsaL promotes heterogeneity and bimodality of quorum sensing activation in *Pseudomonas aeruginosa*

Presenting Author - *Marta Mellini, University Roma Tre, Italy*

Author/s - *Morgana Letizia, Lorenzo Caruso, Alessandra Guiducci, Carlo Meneghini, Livia Leoni, Giordano Rampioni*

Abstract Content

Background: In its original formulation, quorum sensing (QS) is regarded as an intercellular communication system that allows bacterial cells to synchronize gene expression in response to cell density. However, growing evidence reveals that cell-to-cell variation in the QS activation state can occur, often resulting in coexisting subpopulations of quorate (QS ON) and non-quorate (QS OFF) cells sharing the same environment. A certain degree of heterogeneity and bimodality has been recently observed also in the activation state of las QS system in the opportunistic pathogen *Pseudomonas aeruginosa*. However, the molecular mechanism(s) underlying this phenomenon remain unknown.

Objectives: To provide a mechanistic explanation to *P. aeruginosa* QS heterogeneity and bimodality.

Methods: Single-cell level analyses on ad hoc designed biosensor strains in which las system activation results in fluorescence emission were performed through confocal microscopy imaging.

Results: We found that activation of the las QS system did not occur synchronously in all cells of the population, with different fractions of quorate and non-quorate subpopulations co-existing even at high cell density. In addition, we demonstrated that heterogeneous and bimodal activation of las system arose at the transcriptional level, through the action of the RsaL negative regulator. In accordance, we observed an inverse correlation between rsaL expression levels and las QS activation in single cells. Interestingly, activation of the rhl QS system, that is not controlled by RsaL, showed reduced low heterogeneity and no bimodality, reinforcing the importance of RsaL in determining cell-to-cell variation in las QS activation.

FP2/6 - Novel mechanisms of fimbriae-mediated bacterial adhesion and colonisation of host tissues

Presenting Author - *Anton Zavialov, University Of Turku, Finland*

Author/s - *Anton Zavialov, Henri Malmi, Natalia Pakharukova, Minna Tuittila, Sari Paavilainen*

Abstract Content

Pathogenic bacteria express fibrous adhesive virulence organelles that mediate targeting to the sites of infection. In Gram-negative bacteria, the most common adhesive organelles are fimbriae (aka adhesive pili) assembled via the dedicated chaperone-usher pathway (CUP). Functionally, CUP fimbriae can be divided into monovalent and polyvalent adhesins that use different strategies to recognize cells of infected hosts by binding to specific receptors exposed on these cells. Here, we show that the largest and most widespread class of CUP systems, archaic or sigma CUP fimbriae, use an entirely novel mechanism that allows bacterial adhesion and biofilm formation on both biotic and abiotic surfaces. X-ray crystallography analysis and AlphaFold modeling of fimbriae from different archaic CUP systems show that instead of receptor-binding pockets these adhesins use a unique hydrophobic tip-finger structure to bind to a wide range of surfaces. Cryo-EM structure of *Acinetobacter baumannii* archaic Csu pili, reveal the novel ultrathin zigzag architecture of pilus rods that provides the pilus with high mechanical stability as well as superelasticity that allow bacterial attachment in highly turbulent environments. Furthermore, we show that this unique architecture enables formation of 3D-biofilms of *A. baumannii*, making this important nosocomial pathogen highly resistant to stress and antibiotic treatment.

FP2/7 - *Candida albicans* and *Staphylococcus aureus* reciprocally promote virulence

Presenting Author - *Raymond Pasman, University of Amsterdam, Netherlands*

Author/s - *Stanley Brul, Bastiaan Krom, Bas Zaat, Jianbo Zhang*

Abstract Content - *Candida albicans* is a prevalent oral commensal which can become pathogenic during immune deficiencies. *C. albicans* pathogenesis involves upregulation of various virulence factors (e.g. biofilm formation, switching yeast to hyphal growth, invasion, and secretion of lytic enzymes and peptides) usually leading to localized and superficial infections. However, occasionally lethal bloodstream infections (BSI) may develop (candidemia). Interestingly, at least 20% of candidemia cases are known to coincide with bacteraemia. *Staphylococcus aureus*, the third most co-isolated bacteria during candidemia, is a leading cause of primary BSIs which occur without a known portal of entry. Accordingly, recent studies have suggested that *C. albicans* is able to facilitate *S. aureus* invasion and dissemination. However, the direct influence of co-culturing on virulence has not yet been studied. In this study we aimed to examine the direct effects of *S. aureus* on both *C. albicans* hyphal formation and invasive properties through agar invasion assays and (timelapse) microscopy. Moreover, using qPCR and proteomics we further investigated the effects of co-culturing on growth and secreted virulence factors. Results show that co-culturing significantly increases hyphal formation and invasion while facilitating increased *S. aureus* integration into the biofilm in a more adherend and complex structure. qPCR results indicate that this increase in *S. aureus* is likely promoted by *C. albicans* pH control. Finally, the exoproteome of these biofilms showed significant increases in secreted candidal and staphylococcal virulence factors during co-culturing compared to the respective mono-cultures. Altogether, these results show that *C. albicans* and *S. aureus* reciprocally promote virulence during co-culture.

FP2/10 - Visualization of *Yersinia* type 3 secretion system components down to the molecular level by MINFLUX microscopy

Presenting Author - *Alexander Carsten, Universitätsklinikum Hamburg-Eppendorf, Germany*

Author/s - *Maren Rudolph, Tobias Weihs, Roman Schmidt, Isabelle Jansen, Christian A. Wurm, Andreas Diepold, Antonio Virgilio Failla, Manuel Wolters*

Abstract Content

Type 3 secretion systems (T3SS) are essential virulence factors of numerous bacterial pathogens and inject effector proteins into host cells. The needle-like T3SS machinery consists of more than 20 components, has a length of around 100 nm and its different sections along the length axis are up to 30 nm broad. A temporally and spatially resolved visualization of the T3SS using fluorescence microscopy techniques has been challenging, as its intrabacterial components are highly dynamic and in permanent exchange with other bacterial structures. By labeling single T3SS components with self-labeling enzymes or nanobodies and employing super-resolution microscopy techniques such as (Live-)STED, STORM and MINFLUX, we succeeded to visualize and resolve single components in different sections of the T3SS machinery. Using MINFLUX microscopy we achieved resolutions down to the molecular scale of T3SS components such as YscL and the pore protein YopD. In addition, 3D MINFLUX nanoscopy allowed us to determine the three dimensional distribution of sorting platform protein YscL within a single bacterium. Two-color MINFLUX nanoscopy allowed visualizing both YscL and YopD in parallel during a cell infection.

Continuation of this work will allow us to investigate T3SS structure and function with unprecedented resolution and therefore gain new insights into the infection process of human pathogens in order to develop novel treatment and prevention strategies.

FP2/11 - Discovery of a new secreted DNase in the opportunistic human pathogenic fungus *A. fumigatus*

Presenting Author - *Simone Edenhart, Leibniz Institute for Natural Product Research and Infection Biology, Germany*

Author/s - *Marie Goldmann, Juliane Macheleidt, Olaf Kniemeyer, Axel Brakhage*

Abstract Content

The saprophytic fungus *Aspergillus fumigatus* occurs ubiquitously and contributes to the degradation of organic matter. However, several virulence factors and the capability to grow at 37 °C enable the fungus to switch to a pathogenic life style. Since its conidia (fungal spores) become airborne easily, they are part of the air we breathe. When inhaled, conidia in the respiratory tract of healthy people are usually quickly eliminated by epithelial cells and resident alveolar macrophages. However, for immunocompromised individuals, they pose a serious risk and can lead to various diseases, ranging from invasive, to locally-restricted forms of infection and allergic disorders. If the first line of defense of the human immune system fails, conidia can start to germinate and form hyphae. The growing hyphae are too large to be engulfed by macrophages and thus are killed by recruited neutrophils which have an array of extracellular killing mechanism. One of those mechanisms includes the formation of neutrophil extracellular traps (NETs)1, which mainly consist of nuclear DNA decorated with in part fungicidal proteins. Using a serological proteome analysis (SERPA) to investigate new *A. fumigatus* protein antigens recognized by human IgGs of patient sera, we identified 44 new immunogenic proteins. Amongst these proteins, we discovered a so far uncharacterized, secreted DNase. This DNase was found to be expressed during murine *A. fumigatus* infection2 and we now investigate its potential as putative virulence factor, helping hyphae to degrade NETs during infection.

FP2/12 - A comparison of Micro-CT scanning with traditional histology to assess Clostridial damage in the larval model of *Galleria*

Presenting Author - *Ronald Dixon, University of Lincoln, United Kingdom*

Author/s - *Susan Aldridge, Joseph Edwards, Joseph Brown, Fernando Montealegre-Zapata, Sammy Kay*

Abstract Content

Background: *Clostridium perfringens* associated diseases (CPADs) are complex and multifactorial infections. Models of CPADs in mammals has led to poor understanding of the underlying pathogenesis and has limited the field. Cost effective and high-throughput alternatives, such as the *G. mellonella* larval infection model, as they withstand incubation at 37°C, require no specialist equipment and exhibit an innate immune system that is analogous to mammals. Although the larval model is increasingly used for the study of bacterial/viral pathogenesis and treatment strategies it relies on mortality rates and qualitative melanisation scoring as indicators of disease progression.

Objectives: To determine for the first time whether microCT analysis is practical and improves pathological interpretation in larvae.

Methods: Larvae were challenged with 105 CFU of *C. perfringens* reference strain ATCC13124 and clinical strain JBCNJ055. At 0, 24, 48 and 72h post infection larvae were fixed in 10% NB formalin and either processed by traditional histological techniques or imaged by micro-CT.

Results: Histopathology identified distinct morphological patterns of tissue destruction with JBCNJ055, but were not identified in ATCC 13124 challenged larvae. Micro-CT scans of the former were rendered into a 3D model which revealed gut inflammation, distinct areas of necrotic-like tissue and evidence of gas production, elements lost in traditional 2D histological sections. Interestingly, micro-CT scans also revealed areas of nodulation within larvae challenged with ATCC 13124. In conclusion, we have shown that non-invasive micro-CT reveals characteristics that were not visible by traditional histology and although lacking resolution more comparative studies are indicated.

FP2/13 - *Drosophila* versus *Mycobacteria*: Using host-pathogen interactions to study physiology and pathogenesis of *Mycobacterium abscessus*

Presenting Author - *Eleanor Marshall, Imperial College London, United Kingdom*

Author/s - *Sophie Burbaud, Jasper Sangen, Jake Jacobson, Andres Floto, Marc Dionne*

Abstract Content

Mycobacterium abscessus is a highly drug-resistant emerging pathogen which poses a major threat to susceptible individuals, particularly people with cystic fibrosis. Little is known about the infection process and intracellular behaviours used by *M. abscessus* to survive and proliferate within the intracellular environment, nor about the innate host immune responses that may detect and respond to *M. abscessus* infection and how these host responses impact the behaviour of this bacterium. A previous GWAS identified deletion mutations within a putative type IV flp pilus operon in *M. abscessus* that were associated with more persistent infection in cystic fibrosis patients(1). We targeted two of the genes in this operon for knockout using a CRISPR-Cas9 system. These knockout mutants were characterised using a *Drosophila melanogaster in vivo* infection model to provide insight into the role of this *M. abscessus* operon during infection and intracellular survival of the bacteria. Both mutants exhibited strong defects in their ability to proliferate within and ultimately kill the host. By exploiting the genetic tractability of *D. melanogaster*, we have identified that the host immune signalling pathway downstream of TNFα is targeted by this *M. abscessus* operon. We hypothesise that this putative type IV system may trigger inhibition of macrophage apoptosis, allowing *M. abscessus* to grow intracellularly before spreading to further macrophages via necrosis. Current experiments are directed at identifying the specific host target of this *M. abscessus* operon and defining the genetic architecture of the interaction between host and pathogen revealed by these bacterial mutants.

FP2/14 - Uncharted roads, uncertain journeys and outcomes: an antibiotic tolerance evolution story

Presenting Author - *Bram Van den Bergh, VIB-KU Leuven, Belgium*

Author/s - *Thomas Schalck, Jan Michiels*

Abstract Content

Antibiotic-tolerant persisters prolong treatment and increase resistance development. In contrast to resistance, persistence evolution is ill understood. Before, we showed rapid evolution of persistence where persistence levels correlate with treatment frequency. Recently, we delineated how such evolution leads to cytoplasmic acidification in one set of high-persistence mutants. While the mutational target size for persistence is assumed to be larger than for resistance, I initially only identified five targets in *E. coli* that acquired gain-of-function mutations and are contingent on a functional stationary phase response and stringent response. With such unique starting point, we wondered what untapped potential remains hidden and how evolution would proceed in absence of the prime targets. Based on evolutionary theory, we expect evolution to be more slowly and diverse.

We combined genomic engineering (removing prime targets and/or regulators), laboratory evolution and population-wide sequencing with antibiotic tolerance and resistance tests. While evolution proceeds slower with more variation in the knockouts, all ancestors with/without prime targets and regulators, still readily reach high antibiotic tolerance. Without targets and/or regulators, however, the evolved clones are less robust for experimental variation and thus less fit. New evolutionary targets are identified and more diverse, and are examined for their fitness and background specificity. Even more so than for resistance, evolution of high tolerance is inevitable upon frequent antibiotic treatment. Our results indicate that it can be slowed down while new targets can be used to improve future therapy.

FP2/15 - Clumpy adhesion to human cells increases *Escherichia coli* antibiotic tolerance

Presenting Author - *Muhammad Moman Khan, Brandenburgische Technisch Universitat, Germany*

Author/s - *Muhammad Moman Khan, Katarzyna Sidorczuk, Peter Schierack, Rafal Kolenda*

Abstract Content

Escherichia coli infections result in the formation of adhesion patterns which are hallmarks of pathogenic actions by major intestinal pathotypes. This study characterizes and identifies factors contributing to a novel and previously uncharacterized biofilm-like phenotype, known as Clumpy adhesion by the *E. coli* strain 4972. Adhered clumpy structures by this *E. coli* on human epithelial cells, especially bladder cell line 5637, could tolerate the antibiotic stress of up to 16mg/ml ampicillin and 2µg/ml gentamicin. Initial analysis revealed that adhered bacteria diverged from unattached bacteria in the supernatant by their transcriptomic and proteomic signatures. Transcriptomic analysis unveiled differential expression of 622 genes, including 148 unannotated genes, between bacteria forming clumps and in the supernatant. Seven genes deletion mutants i.e., spy, flgH, fimH, ffp, pilV, spnT and yggT, were generated and analysed for motility, adhesion, prevalence and antibiotic stresses. ΔflgH demonstrated the loss of adhesion up to 80%. ΔpilV and ΔyggT adhesion significantly increased to 151% and 145.5%, respectively, and upon complementation, adhesion significantly reduced to 53% and 13%, respectively. Clumps produced by Δffp and ΔspnT were more resistant and protected the bacteria with ΔspnT showing the best clump formation in terms of ampicillin stress protection. In case of gentamicin stress, ΔyggT depicted the highest susceptibility where the antibiotic stress completely eliminated the bacteria. Overall, we investigated the clump formation on the epithelial cell surface and the effects on the antimicrobial tolerance along with the contribution of several novel factors crucial to clump formation on the susceptibility to the selected antibiotics.

FP2/16 - High-resolution metatranscriptomics disclose *in vivo* host-pathogen interactions in cystic fibrosis airways

Presenting Author - *Elio Rossi, University Of Milano, Italy*

Author/s - *Mads Lausen, Antonella Colque, Bibi Uhre Nielsen, Rikke Møller, Marianne Skov, Tacjana Pressler, Søren Molin, Novo Nordisk Foundation Center for Biosustainability, Lyngby, Denmark*

Helle Krogh Johansen, Rigshospitalet, Copenhagen, Denmark

Abstract Content

Background: Cystic fibrosis (CF) is a multi-organ disease with heterogeneous clinical outcomes. Recurrent airway infections and an intense inflammatory response characterise CF patients from early childhood, leading to premature death. Immune cells' defects have been detected, but other factors, such as the active lung microbiome are known to play a role. Yet a thorough picture of the ongoing host-pathogen interactions at the site of infection is missing.

Objectives: Using meta-transcriptomics, we investigate the activity of the host immune system and the active microbiome in human CF sputum.

Methods: CF sputum is collected from a cohort of 52 CF patients. RNA sequencing is used to reconstruct the active microbial community, immune cell types and to analyse gene expression of human cells.

Results: CF patients clustered in three groups based on the active microbiome. Groups are slightly associated with age and characterised by the presence of specific microorganisms. Younger patients show a microbiome dominated by oral taxa, while older patients differentiated in two groups based on the abundance of *Aspergillus*, *Mycobacterium* and *P. aeruginosa*. Heterogeneous immune responses and modulation of several immunological pathways can be linked with the different groups. For example, presence of *Aspergillus* and *Mycobacterium* is particularly associated with expression of genes involved in type 2 innate lymphoid cells (ILC2), which have been proposed to be linked with type 2 immune responses and poor outcomes. Overall, our results highlight the heterogeneity in host-pathogen interaction in CF patients warning for the need of personalised monitoring and interventions.

FP2/17 - Journey of *Vibrio cholerae* outer membrane protein OmpU to the host cell mitochondria

Presenting Author - *Arpita Sharma, Indian Institute Of Science Education And Research, Mohali, India*

Author/s - *Shashi Prakash Yadav, Shelly Gupta, Arunika Mukhopadhaya*

Abstract Content

Mitochondria is central to the caspase-dependent and -independent programmed cell death processes. Various bacterial toxins target host cell mitochondria to cause cell death. Our lab has earlier shown that *V. cholerae* outer membrane porin protein OmpU induces target cell death in a programmed manner with mitochondria playing a central role. Translocation of OmpU into the host cell mitochondria is instrumental in the induction of mitochondrial membrane permeability transition leading to caspase-independent cell death (Gupta et al., *J. Biol. Chem.*, 2015). However, how OmpU is translocating to the mitochondria was not known. In this study, we aimed to investigate how OmpU traffics inside the host cell and integrates into the mitochondrial membrane. We have observed that OmpU localizes to the outer membrane of the mitochondria in HEK293 human epithelial cells and integrates as an integral membrane protein via TOM22 transporter protein. Further, we have observed that OmpU translocates to the mitochondria of these cells via the clathrin-independent caveolin-mediated endocytic pathway. Both the early and the late endosomal compartments along with the lysosomes are involved in the trafficking of OmpU inside the cells. Our study has generated a crucial insight about how an outer membrane protein of an extracellular bacterium can translocate to the host cell mitochondria and integrate into the mitochondrial membrane.

FP3/1 - Exploring the dog gut microbiome within a large-scale investigation of animal gut metagenomes

Presenting Author - *Anna Cusco, Fudan University, China*

Author/s - *Anthony Noel Fullam, Pamela Ferretti, Shaojun Pan, Oleksandr Maistrenko, Sebastian Schmidt, Peer Bork, First name: Luis Pedro*

Abstract Content

Background: Most pet owners consider their animals family members and care about their health. However, most studies on dog microbiomes focus on dogs from colonies rather than pets in households.

Objective: By exploring public databases, we aim to characterize the dog gut microbiome within a global context.

Methods: We first assembled a comprehensive collection of dog gut metagenomes (n=444), and manually curated the associated metadata. We then computed taxonomic abundances, retrieved metagenome-assembled genomes (MAGs), and compared them to animal gut metagenomes (from the Global Microbial Gene Catalog [1], n=5,519).

Results: *Prevotella*, *Bacteroides* (Phocaeicola), and *Blautia* are highly prevalent and abundant in dogs and most mammals' guts. *Sutterella* and *Collinsella* are abundant and prevalent in Canids' and Felids' guts but are found less frequently and in lower proportions in other mammals' guts. *Fusobacterium* is one of the most abundant genera in dogs' guts, which is not observed in other mammals.

We obtained high- and medium-quality MAGs for the most abundant taxa within a sample. For Prevotella genus, most of the MAGs belong to *Prevotella copri*. Although it was the most abundant species in some samples, getting high-quality MAGs rather than medium-quality MAGs was not the norm. For *Bacteroides*, *Blautia*, and *Fusobacterium*, recovered MAGs are from multiple species. Individual dogs harbour up to eight different *Bacteroides* species, seven *Blautia*, and five *Fusobacterium*.

Following steps include collecting novel pet dog samples and applying long-read metagenomics sequencing to recover high-quality MAGs for hard-to-assemble genomes and lower abundant species, and explore pangenomes.

FP3/2 - The evolution of a bacterial tRNA gene set by within-genome duplication events

Presenting Author - *Wing Yui Ngan, Max Planck Institute for Evolutionary Biology, Germany*

Author/s - *Florence Bansept, Arne Traulsen, Jenna Gallie*

Abstract Content - The transfer RNA (tRNA) content of cells affects the efficiency of protein synthesis. To study how organisms can adapt to novel translational demands, our laboratory uses two engineered strains of *Pseudomonas fluorescens* SBW25, each lacking one or more tRNA genes. Previously, we have shown that these slow-growing strains rapidly recover fitness in serial transfer evolution experiments by duplicating large segments of the chromosome (up to 1 Mb), each containing a small (~100 bp) compensatory tRNA gene. However, while adaptive, these large duplications are mechanistically unstable and hence are unlikely to persist over longer evolutionary time scales. Here, we investigate the evolutionary fate of the duplications and the new tRNA gene copies that they contain. We extend the evolution experiment to 100 transfers (~700 generations) and characterize the evolving lineages. We find that, within each evolving population, various duplication fragments rapidly arise and compete. Over time, progressively smaller – and hence, mechanistically more stable – duplication fragments arise and dominate in all lineages. The smallest of these is a duplication fragment of only 236 bp, encompassing the compensatory tRNA gene and promoter. Our results provide a detailed, real-time example of a bacterial tRNA gene set evolving in response to translational challenges

FP3/3 - Novel Antisense Overlapping Genes in EHEC and Other Pathogens Expand their Gene Reservoir

Presenting Author - *Klaus Neuhaus, Technical University Munic, Germany*

Author/s - *Franziska Graf, Barbara Zehentner, Michaela Kreitmeier, Abele Miriam, Christina Ludwig, Zachary Ardern, Siegfried Scherer, Technical University of Munich, Freising, Germany*

Abstract Content

Background: The existence of overlapping genes (OLGs) with significant coding overlaps revolutionizes our understanding of genomic complexity. The abundance of long overlapping genes in prokaryotic genomes is likely to be significantly underestimated. To date, only a few examples of such genes are fully established.

Objectives: The number of examples of prokaryotic overlapping genes currently is limited, but constantly growing. Establishing the existence of genes in alternative reading frames may help explain taxon-specific evolutionary novelty.

Methods: RNA sequencing, ribosome profiling, phenotype testing of strand-specific knockouts, and in some cases mass spectrometry was used to verify protein-coding from overlapping genes.

Results: Signals of novel overlapping genes were found in RNA seq and ribosome profiling data. We confirmed that the overlapping candidate genes have typical structural elements for their expression. Strand-specific knock out mutants showed clear phenotypes. In the case of *P. aeruginosa*, proteins were found via mass spectrometry. These taxonomically-restricted genes provide evolutionarily novel functionality.

FP3/4 - Whole-genome transcriptional analysis of yttrium stress in strain *Mesorhizobium quingshengii* J19

Presenting Author - *Carina Coimbra, University of Coimbra, Portugal*

Author/s - *Carina Coimbra, Rita Branco, Paula V. Morais*

Abstract Content

Mesorhizobium quingshengii strain J19 was isolated from the surface of prospecting gold galleries from the Jales mine and tolerates high Y concentrations.

This work aims to study the gene expression profiles associated with Y exposure, identifying genes involved in Y tolerance or Y detoxifying processes. The genome transcriptional profiling was used to identify genes in strain *M. quingshengii* J19 that were differentially expressed after 3h of exposure to 0.2 mM Y.

The genome draft of strain J19 was sequenced, assembled into 63 contigs and revealed a size of 6.52 MB. A total of 6341 Coding DNA Sequences (CDS) regions were identified, and the draft genome's GC content was 63.2%. The strain J19 genome was used to map RNAseq data obtained after Y stress. We found that 127/6343 (2%) CDS were significantly differentially expressed (SDE) under Y exposure, with 36.2% and 63.8% up-regulated and down-regulated CDS, respectively. Among the SDE, a significant amount of CDS involved in biological processes and molecular functions were identified (15.8% and 5.5%, respectively). Within the biological processes, the expressed CDS were mostly involved in metabolic processes (48.1%), cellular processes (18.4%) and biological regulation (17.5%).

Our data suggest that Y mostly affects the cellular homeostasis of iron and zinc, and activates the arsenic detoxifying mechanisms. One gene, coding for a TonB transporter was particularly up-regulated, over a 7.5-fold change, under Y stress. This transporter is being assessed to validate its role in the Y detoxification process.

FP3/5 - Functional and evolutionary characterization of unknown genes from uncultivated taxa

Presenting Author - *Alvaro Rodriguez del Rio, Centro de Biotecnologia y Genomica de Plantas, UPM-INIA, Spain*

Author/s - *Alvaro Rodriguez del Rio, Joaquin Giner-Lamia, Carlos P. Cantalapiedra, Jorge Botas, Ziqi Deng, Ana Hernandez Plaza, Lucas Paoli, Department of Biology, Institute of Microbiology and Swiss Institute of Bioinformatics, ETH Zürich, Switzerland*

Thomas S.B. Schmidt, Structural and Computational Biology Unit, European Molecular Biology Laboratory, Heidelberg, Germany

Abstract Content

Metagenomics has provided the genomes of thousands of uncultivated bacterial and archaeal lineages, revealing an enormous amount of previously unknown genes. Even though recent studies have highlighted the apparent importance of these novel genes, their overall significance is largely unexplored.

Here, we applied a comparative genomics approach to identify high-quality novel protein families exclusive from uncultivated species, and characterized them from a functional, ecological and evolutionary point of view.

For such a purpose, we computed protein families from a large multi-habitat metagenomic dataset covering 400M genes from 170k prokaryotic genomes, and applied strict quality and novelty filters to avoid families formed by incomplete genes, assembling artifacts, distant homologs and potential pseudogenes. This led us to uncovering 400K high-quality gene families exclusive from uncultivated taxa.

This vast number of high-quality novel gene families are widespread within and across habitats, indicating their ecological value. They also spread out across the microbial phylogeny, are notably overrepresented in recently discovered taxa, and a fraction can accurately distinguish entire uncultivated phyla, classes and orders, likely representing synapomorphic traits.

Moreover, 18% of the novel protein families are present in phylogenetically conserved operon regions, suggesting they may take part in central microbial processes. In order to facilitate their characterization, we developed http://novelgenefamilies.compgenomics.org, which shows the genomic neighbors of any of our novel gene family predictions.

Overall, our work provides a global phylogenomic analysis of the largely uncharted repertoire of unique genes from uncultured prokaryotes, serving as a base resource for investigations on their roles.

FP3/6 - Comparative analysis of antibiotic resistance dynamics by high-throughput laboratory evolution of 9 bacterial species

Presenting Author - *Tomoya Maeda, Hokkaido University, Japan*

Author/s - *Tomoya Maeda, Kumi Tanabe, Atsushi Shibai, Hazuki Kotani, Chikara Furusawa*

Abstract Content

Background: Widespread of antimicrobial resistance (AMR) is a growing concern for global public health. Since the emergence of AMR is based on evolutionary dynamics, quantitative analysis of antibiotic resistance evolution as well as genome analysis of clinical isolates has been extensively performed using representative pathogens. However, the contribution of such identified mutations in certain species to the AMR among other species remains elusive.

Objectives: In this study, we focus on the commonality and individuality of AMR dynamics among different species.

Methods: Using a laboratory automation system, we conducted high-throughput laboratory evolution of nine bacterial species including both gram-negative (*Escherichia coli*, *Klebsiella variicola*, *Enterobacter kobei*, *Citrobacter amalonaticus*, *Acinetobacter bouvetii*, and *Pseudomonas putida*) and gram-positive bacteria (*Bacillus subtilis*, *Lactobacillus plantarum*, and *Staphylococcus auricularis*) against three antibiotics doxycycline, meropenem, and ciprofloxacin. 216 independent culture lines (9 species × 4 conditions × 6 replicates) were subjected to laboratory evolution for about 200 generations. Next, we performed genome resequencing analysis of 144 evolved strains. The effects of representative mutations found in the evolved strains of *E. coli*, *A. bouvetii*, *P. putida*, *and B. subtilis* on antibiotic resistance were further confirmed by the reconstructed mutant strains.

Results: The comparative mutational analysis identified that a mutation in rne encoding RNase E is commonly identified in the evolved gram-negative bacteria. Deletion of the C-terminus of RNase E of *E. coli*, *A. bouvetii*, and *P. putida* showed increased antibiotic resistance. These results suggest the importance of rne mutations in gram-negative bacteria for AMR.

FP3/7 - Evolutionary trajectories of secondary replicons in bacteria

Presenting Author - *Olga Bochkareva, Institute of Science and Technology Austria, Austria*

Author/s - *Natalia Dranenko, Mikhail Gelfand*

Abstract Content

Most bacterial genomes have a single chromosome that may be supplemented by a few smaller, dispensable plasmids. Their sizes range from 100 kb in obligate parasites to over 15 Mb in free-living species. However, to date, approximately 10% of the bacteria with completely sequenced genome, mostly pathogens and plant symbionts, have more than one stable large replicon. Some secondary replicons are species-specific, carrying pathogenicity or symbiotic factors. Other replicons are kept on genus-level taxonomy (e.g. in *Vibrio* and *Burkholderia* genera), carry house-keeping genes and may rich several Mb.

In our work, we analyzed replicons' abundance and sizes in the genomes of different species and interpreted the observations in the context of species evolution. We identified two patterns of multipartite genomes' evolution. The first, more common one, demonstrates a stable size of secondary replicon that is likely characteristic of 'megaplasmid'. In turn, in 8 genera of 3 families: Pseudoalteromonadaceae, Burkholderiaceae and Vibrionaceae we observed positive correlation between the chromosome and the secondary replicon sizes. This observation indicates an integration of both replicons in genome evolution of these genera via gene gain/loss supporting the classification of these secondary replicons as 'chromids'. Assumption of the dependency by linear regression revealed that the chromids fluctuated faster that chromosomes. Higher rates of gene gains/losses in the chromids supports the hypothesis that multipartite genome organization allows bacteria better adopt to changeable conditions via accumulation of niche specific genes over-expressed in certain environments in additional replicons.

FP3/8 - Building the world's largest phage-host interaction atlas using proximity ligation technology

Presenting Author - *Ivan Liachko, Phase Genomics, United States*

Author/s - *Jonas Grove, Benjamin Auch, Yunha Hwang, Christopher Staley, Peter Girguis, Alexander Khoruts, Emily Reister, Phase Genomics, Inc., Seattle, WA, United States*

Abstract Content

Bacteriophages, Earth's most abundant biological entities, interact with all life and shape the global ecosystem through their impacts on microbial community composition and horizontal gene transfer. However, phage-host relationships are challenging to identify without the use of culture-based experiments to generate unambiguous evidence for a phage's presence in a given host. This restricts the scope and microbial diversity that can be surveyed.

Proximity ligation sequencing is a powerful genomic method for associating viruses with their hosts directly in native microbial communities. Proximity ligation captures, in vivo, physical interactions between the host microbial genome and the genetic material of both lytic and lysogenic phage. Similar to culturing experiments, these linkages offer direct evidence that phage sequences were present within an intact host cell, thereby establishing a phage-host pair. Unlike culturing experiments, proximity-ligation methods do not require the propagation of living bacterial cells and unlike single-cell sequencing experiments, only capture phage-host interactions inside cells. The combination of intra-phage and phage-host signal enables us to simultaneously deconvolve viral genome bins (vMAGs) directly from metagenomes and to assign microbial hosts to large numbers of vMAGs without culturing.

Applying this technology to hundreds of complex microbiome samples has yielded thousands of novel phage and archaeal virus genomes with host assignments, as well as large numbers of new microbial genomes. Through broad-scale application of proximity ligation sequencing, we are creating a global-scale atlas of highly diverse phage-host interactions. We will present published and unpublished work highlighting the power of this approach in metagenomic discovery.

FP3/9 - Importance of DNA-methylation in entomopathogenic bacteria with similar lifestyle

Presenting Author - *Julien Brillard, Inrae, France*

Author/s - *Nadege Ginibre, Ludovic Legrand, Amaury Payelleville, Victoria Bientz, Anne Lanois, Jean-Claude Ogier, Sylvie Pages, INREA, Montpellier, France*

Alain Givaudan, INREA, Montpellier, France

Abstract Content - DNA methylation plays various roles in bacteria and can contribute to bacterial interactions with their hosts, through diverse mechanisms such as genome maintenance or epigenetic regulations. The bacteria *Xenorhabdus* and *Photorhabdus* are pathogenic for insects and are found in the gut of soil nematodes with whom they are symbiotically associated. The roles of a conserved DNA-Methyltransferase (MTase) were investigated in these bacteria using deregulated strains and methylomic approaches.

Great diversity was observed in the distribution of MTase-encoding genes, and a single MTase (Dam, for DNA-adenine MTase) was identified in all the species of these two genera. Methylome analysis showed that the GATC motifs recognized by Dam reached a high methylation rate (>99%) in the studied strains. Enrichment of unmethylated motifs in promoter regions was observed and may reveal mechanisms of epigenetic regulation. The Dam overexpression was associated with a reduced swimming motility, caused by a downregulation of flagellar genes, in both *Xenorhabdus* and *Photorhabdus*. In contrast, alteration of various major phenotypes such as hemolysis or virulence depended on the species studied.

Our work revealed the diverse roles played by a conserved DNA MTase during the life cycle of entomopathogenic bacteria.

FP3/10 - The impact of *Escherichia coli* genetic background on its ability to evolve antimicrobial resistance

Presenting Author - *Marco Galardini, Twincore GmbH (MHH/HZI), Germany*

Author/s - *Adam Mulkern*

Abstract Content

Background: The rise of antimicrobial resistance (AMR) is a serious threat to global health in the 21st century. Life-threatening multi-drug resistant microbes have emerged and thus new approaches are needed to maximize the “shelf-life” of antimicrobials by reducing the risk of the emergence and spread of resistance.

Objectives: Recent studies have shown how different strains belonging to the same bacterial species possess a different propensity to develop AMR (“evolvability”). The genetic determinants of differences in evolvability have however not been determined in *E. coli*; our aim is to discover them using laboratory evolution.

Methods: We have so far exposed 82 *E. coli* strains to 5 antimicrobials and 6 biological replicates: Amikacin, Ceftriaxone, Colistin, Rifampicin and Tobramycin, using a serial passaging protocol with daily doublings in concentration, from 1/8th of the Minimum Inhibitory Concentration (MIC) until 16X MIC. We have preserved one evolved population for each biological replicate for genome sequencing and further characterization.

Results: Using the passage at which we last observe growth as a measure of evolvability, we observed substantial variability in the ability of each strain to become resistant, which varies by genetic background and drug class, with Ceftriaxone and Rifampicin showing the largest variance. At the genome level, we observed differences in the mutations acquired during the experiment, further underscoring the influence of *E. coli*’s genetic background on evolution. We will show data on more strains and antimicrobials, as well as patterns of cross-sensitivity in the evolved isolates, which have important implications in designing combination therapies.

FP3/11 - A Novel de-novo reference-based guided genome assembly pipeline for environmental isolates

Presenting Author - *Manisha Aswal, Acharya Narendra Dev College (University of Delhi), India*

Author/s - *Neelja Singhal, Manish Kumar*

Abstract Content

Background: Whole genome sequencing (WGS) has become a fast and affordable technique to obtain detailed information about a particular organism. It involves assembly of sequencing reads followed by annotation of assembled genome. There are primarily two methods of genome assembly: de-novo and reference based. De-novo assembly of short reads leaves gaps that leads to incomplete genome assembly. While, reference based require knowledge of a closely related reference genome.

Objective: Environmental isolates are cultured microorganism recovered from any environmental source. For these isolates in reference-based assembly, finding best reference is difficult as databases contain number of closely related isolates. If de-novo approach of assembly is used, generally it results in incomplete assembly. Therefore, we developed a genome assembly pipeline, named as de-novo reference-based guided genome assembly, that uses both approaches.

Method: In this pipeline, pre-processed short-sequence reads was assembled into contigs using the de-novo assembly approach. The assembled contigs are then scaffolded using a best reference genome selected on the basis of lowest Mash value and atleast 75 % alignment value of contigs. Benchmarking showed a significant increase in the N50 value.

Result: Using this pipeline three *Escherichia coli* isolates from river Yamuna, a prominent anthropogenic urban river of northern India were assembled and we obtained maximally 60% increment in N50 value leading towards completeness of genome assembly. Also, this pipeline could be easily applied to study any other bacterial genomes.

Pipeline code https://doi.org/10.6084/m9.figshare.21436392.v1

FP3/12 - Studying the microbiome and resistome in peri-implantitis

Presenting Author - *Lucinda J. Bessa, Egas Moniz School of Health & Science, Portugal*

Author/s - *Carolina Pires, Ricardo Alves, João Botelho, Gil Alcoforado, José J. Mendes, Lucinda J. Bessa*

Abstract Content

Background: Peri-implantitis (PI) is the major peri-implant complication and can lead to dental implant loss. Getting more understanding of the microbiome in dental implants and of its resistome, in both health and disease states, can provide valuable data to improve diagnostic and treatment strategies for PI. Given the complex oral microbiome composition and structure, metagenomic sequencing techniques are required.

Objectives: To study the microbiome and resistome of implant subgingival biofilm and saliva samples from patients with healthy implants and PI-affected implants.

Methods: Both saliva and implant subgingival biofilm samples were collected from patients with healthy implants (n=10) and diagnosed PI-affected implants (n=10). The institutional Ethics Committee approved the study. DNA was extracted from all samples using the DNeasy PowerSoil Pro Kit (Qiagen). Then, a shotgun metagenomic sequencing analysis was accomplished.

Results: The taxonomic profiling (diversity and abundance) as well as insights on the spectrum of function genes, namely antibiotic resistance genes (ARGs) on each sample were obtained. A full analysis is being presently carried out to i) infer detailed differences in the genomic architecture and function between microbial biofilms from PI-affected implants and healthy implants, ii) compare the microbiome and resistome in saliva with those in implant subgingival biofilms, within the same patient.

FP4/1 - Local adaptation and molecular mechanisms of resistance to antifungal compounds in a model organism

Presenting Author - *A. Pedro Gonçalves, National Cheng Kung University, Taiwan*

Author/s - *A. Pedro Gonçalves*

Abstract Content

The domestication of *Neurospora crassa* has been as a major driver of the fields of fungal molecular and cell biology. Not only this filamentous species enjoys modest nutritional requirements and grows swiftly in the laboratory, but also its genome is considerably well annotated, and a near-complete deletion strain collection is available for functional analyses. Furthermore, decades of research with *N. crassa* have been accompanied by the accumulation of wild isolates from different points of the globe, which are an invaluable tool to study local adaptation. Using a panel of antifungal compounds, including standard clinical drugs as well as promising new molecules, we found that drug resistance is naturally heterogeneous in wild populations of *N. crassa*, and chromosomal mapping of the causal loci is underway to unveil the genetic basis of the observed natural diversity. Furthermore, we are interested in the regulatory role played by two Zn2Cys6 transcription factors, CZT-1 and TAH-3, during fungal responses to various drugs. In summary, albeit non-pathogenic, N. crassa offer a plethora of advantages as a model to study antifungal drug resistance. Since the paucity of valid molecular targets in the fungal cell has been hindering the discovery of new antifungal drugs, we consider that the identification and functional characterization of new genes and pathways involved in cell death and drug resistance may inform the adoption of new therapeutic schemes.

FP4/2 - Genetic basis underlying compensation of fitness cost of in gene amplification-mediated heteroresistance

Presenting Author - *Ankita Pal, Uppsala University, Sweden*

Author/s - *Ankita Pal, Dan I. Andersson*

Abstract Content - Antibiotic heteroresistance, a common phenomenon in many pathogenic bacteria, is defined as a phenotype wherein a susceptible bacterial population harbors a small subpopulation of cells that are more resistant to the antibiotic than the main population. One mechanism by which bacteria can generate heteroresistance is by tandem amplification of resistance genes. Generally, the amplifications are unstable and carry large fitness costs, phenotypically manifested as reduced growth rates. For the amplifications to be stably maintained in the population, the fitness cost associated must be ameliorated. This study aims to decipher the genetic basis underlying the compensation of fitness costs in gene amplification-mediated heteroresistance strains. We evolved four different clinical isolates that show heteroresistance to tobramycin, gentamicin, and tetracyclines at increasing antibiotic concentrations, several-fold above the minimal inhibitory concentration (MIC) of the main susceptible population. We found that increasing antibiotic concentrations lead to rapid enrichment of resistant isolates with up to 50-fold increases in the resistance gene copy number, severely reduced growth rates and increased MIC. These isolates were further evolved to study the mechanisms that can lead to the amelioration of fitness costs. We observed that growth compensations occur via different chromosomal mutations that confer partial/full resistance together with the reduction in the gene amplifications. These mutations do not affect the loss rate of gene amplification in compensated mutants in absence of selection pressure. By employing a deterministic model, we show that the loss rates are driven by the intrinsic instability and fitness costs of the gene amplifications.

FP4/3 - Fe ions effect on hydrogenase activity of *Ralstonia eutropha* H16 in various growth media

Presenting Author - *Sona Nikolyan, Department of Biochemistry, Microbiology and Biotechnology, Yerevan State University, Armenia*

Author/s - *Sona Nikolyan, Armine Margaryan, Anna Poladyan*

Abstract Content - Nowadays hydrogenase enzymes (Hyds) are considered alternative catalysts in H2-based enzymatic fuel cells. As they are highly active with turnover frequencies for H2-oxidation able to participate in electricity generation. *Ralstonia* spp., including *Ralstonia eutropha* H16, possess O2-tolerant [NiFe] Hyds, which can oxidize H2 in the presence of O2. *Ralstonia eutropha* H16 bacteria were grown heterotrophically in Nutrient Broth (NB) and Fructose–Nitrogen (FN) minimal medium. Bacteria were cultivated aerobically on a shaker at 130 rpm, 33 °C. H2-oxidizing Hyd activity was measured spectrophotometrically, by the reduction of methylene blue under 570 nm. To investigate the effect of Fe3+ ions on Hyd activity, 0.3 mM FeCl3 was added to the NB and FN minimal medium, pH 7.0. Total H2-oxidizing Hyd activity of whole cells of *R. eutropha* H16 was measured after 24, 48, 72, and 96 h of growth. An activity of approx. 0.5-0.2 U mg–1, cell dry weight (CDW) was observed without the addition of iron either NB or FN media throughout 24h and 48h of bacterial growth. While the addition of 0.3mM FeCl3 increased H2-oxidizing Hyd activity by approximately 2-3 fold compared to the control samples without iron addition. Notably, there is no Hyd activity in the NB medium after 72h and 96h, whereas, a negligible Hyd activity (0.1-0.2 U mg–1 CDW) was observed in the FN medium. Taken together, these findings indicate that appropriate iron ions supplementation improves the Hyd activity of *Ralstonia eutropha* H16 in different growth media.

FP4/4 - The inner membrane protein YhdP modulates the rate of anterograde phospholipid flow in *Escherichia coli*

Presenting Author - *Jacqueline Grimm, Faculty of Dental Medicine, Hebrew University of Jerusalem, Israel*

Author/s - *Handuo Shi, Wei Wang, Angela Mitchell, Ned Wingreen, Kerwyn Casey Huang, Thomas Silhavy*

Abstract Content

Background: A long-standing mystery in the field of bacterial outer membrane (OM) biogenesis is how phospholipids (PLs) are trafficked across the periplasm. Interestingly, what little is known points to a mechanism that is very different from all known OM biogenesis pathways: PL transport is both bidirectional and indiscriminate. Because of this, it has been suggested for decades that PL transport occurs via passive diffusion, but the genes involved remain elusive.

Objectives: To identify genes involved in PL transport, we utilized a mutation in the OM protein MlaA, called MlaA*, that causes lysis in stationary phase. Since anterograde PL transport is a critical step in the pathway, we reasoned that disruptions in PL transport genes should slow cell death. Importantly, MlaA* gave us a simple proxy measurement for PL transport, since anterograde transport results in shrinking of the inner membrane (IM) that is easily visualised by microscopy.

Methods and Results: We conducted transposon-directed insertion sequencing to identify mutations that slowed lysis in MlaA* cells. The top hit of our screen was an IM protein of unknown function called YhdP. Using single-cell imaging, we verified that YhdP disruption delays lysis by preventing transport of PLs from the IM to the OM. YhdP is part of a family of IM proteins, the AsmA-like protein family, that are collectively essential and have domains homologous to those of eukaryotic PL transporters. These results suggest that YhdP, along with its protein family, permits passive diffusion of PLs by forming protein bridges spanning the periplasm.

FP4/5 - Microbial respiration without quinones is conserved from early evolution stages in *Dehalococcoides*

Presenting Author - *Lorenz Adrian, Helmholtz Centre for Environmental Research, Germany*

Author/s - *Lorenz Adrian, Darja Deobald*

Abstract Content

The existence of a membrane potential is a universal feature of extant life and must have evolved before the last universal common ancestor. While earliest sessile life may have exploited the natural potential between alkaline hydrothermal vent fluid and the ancient acidic ocean, free-living life stages must have generated their membrane potential by actively translocating protons or sodium ions. In most extant life quinones play a key role in the generation of a membrane potential. However, methanogenic archaea and acetogenic bacteria generate their membrane potential via a conserved quinone-independent membrane-bound methyl transferase and a membrane-bound Rnf complex, respectively. Here we studied if the quinone-free respiration in strictly anaerobic organohalide-respiring Chloroflexi, class Dehalococcoidia, respiring with hydrogen and organohalides using a single metalloprotein complex for proton translocation, is ancestral. Together with ATPase, the respiration complex constitutes the complete catabolism in *Dehalococcoides*.

To obtain a comprehensive semi-quantitative picture of the degree of conservation of ancestral traits in Dehalococcoidia, we combined respiratory characteristics with phylogenetic analysis of genomic traits, metabolic modelling, protein biochemistry and structural analysis of the respiratory protein complex and the cell envelope. Our results indicate a simple mechanism is responsible for proton translocation avoiding electron transfer across the membrane and obviating membrane-integrated cofactors, such as haem-bound cytochromes. We show that quinone-independent organohalide respiration in Dehalococcoidia is directly coupled to proton translocation. Comparison with overall characteristics of the strain suggest this process is ancestral and not derived from genome reduction.

FP4/6 - Decoding the assembly and biogenesis of bacterial metabolosomes

Presenting Author - *Mengru Yang, University of Liverpool, United Kingdom*

Author/s - *Luning Liu, Jay Hinton*

Abstract Content

Many bacteria possess self-assembled proteinaceous organelles, named bacterial microcompartments (BMCs), to segment key metabolic pathways. The 1,2-propanediol utilization microcompartment (Pdu BMC) is one typical example of catabolic BMCs found in Salmonella and other enteric bacteria. Pdu BMCs confine reactions for degradation 1,2-propanediol, an abundant carbon source in the mammalian gut, which is important for enteric pathogenesis. Elucidating how thousands of proteins self-assemble to form functional BMCs is essential for understanding their significance in cellular metabolism and pathogenesis. Here, we seek a comprehensive understanding of the stoichiometric composition, organization and the de novo assembly of Pdu BMCs by using QconCAT-driven quantitative mass spectrometry, genetic analysis, live-cell fluorescence imaging, electron microscopy and growth assays. We obtain accurate stoichiometry of shell proteins and internal enzymes of the natural Pdu BMCs and reveal the stoichiometric and structural remodeling of metabolically functional Pdu BMCs. Moreover, we determine the role of individual shell proteins and identify the proteins that bind the shell and cargo, and the gene product that distributes the Pdu BMCs. We find the Pdu MCP undertakes a concomitant assembly process in which the shell and cargo assemble independently at the cell poles. The internal cargo core is formed through the ordered assembly of multiple enzyme complexes and exhibits liquid-like properties within the metabolosome architecture. These findings provide insights into the organization and assembly principles of Pdu BMCs, and may inform strategies for repurposing natural microcompartments using synthetic biology for biotechnological applications.

FP4/7 - The function of bacterial membrane microdomains is determined by specific flotillin-driven protein interactions

Presenting Author - *Samuel García Poveda, Centro Nacional de Biotecnología (CNB-CSIC), Spain*

Author/s - *Rabea M. Wagner, Julia García Fernández, Sagar U. Setru, Benjamin Machta, Ned S. Wingreen, Daniel Lopez*

Abstract Content

Bacterial membranes laterally organize functional membrane microdomains (FMMs), different in lipid and protein composition from the rest of the membrane. The integrity of FMMs is crucial for the correct function of diverse bacterial processes and is largely mediated by a scaffold protein called flotillin. The FMMs of the model bacterium *Bacillus subtilis* contain two structurally similar flotillin-like proteins, FloA and FloT. Despite their similarity, these two flotillins bind to different protein components and unevenly distribute within the bacterial microdomains. In this work, we use live-cell imaging (Total Internal Reflection Fluorescence Microscopy, TIRFM) in combination with biochemical and molecular approaches to characterize FloA and FloT dynamics in *B. subtilis* cells. We discovered that bacterial actin-like cytoskeleton restricts the movement of FMMs within the membrane, analogously to the observed for lipid rafts in plants. Additionally, we found that differences in the C-terminus domain between FloA and FloT are largely responsible for their uneven distribution and their preferential interaction with specific protein partners. This variability in distribution and dynamics, along with their preferentiality of interacting partners, explain the phenotypic differences that *B. subtilis* floA and floT mutants show under environmentally harsh conditions. In summary, we show that FloA and FloT assemble functionally distinct microdomains by binding to different protein partners, which define their specific contribution to distinct cellular processes.

FP4/8 - Identification of a large family of amine-specific sensors in bacteria and archaea

Presenting Author - *Jean Paul Cerna-Vargas, Estación Experimental Del Zaidín, Consejo Superior De Investigaciones Científicas - Universidad Politécnica De Madrid, Spain*

Author/s - *Jean Paul Cerna-Vargas, Vadim Gumerov, Igor Zhulin, Tino Krell*

Abstract Content

Background: A major bottleneck in microbiology is the lack of information regarding signals recognized by the large majority of cellular receptors. Sensor domains present a high degree of sequence divergence; therefore, ligand specificity cannot be predicted based solely on sequence similarity. We have reported a bioinformatics-based identification of a subfamily of dCache sensor domains that are specific for amino acids.

Objectives: The aim of this study is to determine whether a similar approach permits that identification of sensor domains that recognize hydrophobic signals, such as quaternary amines (QAs).

Methods: Bioinformatics approaches included dataset searches, multiple sequence alignment and phylogenetic tree construction. Structural models were built with Alphafold. Ligand binding was assessed by Isothermal titration calorimetry (ITC).

Results: Based on three available structures of dCache sensor domains with bound QAs (2), the conserved residues of the ligand binding pocket were identified. This information allowed the prediction of thousands of QA-specific dCache domains. Ten target proteins were selected and their ligand profile determined by ITC. Results confirm binding to QA, and in addition several targets also bound other biogenic amines. Amine responsive dCache sensor domains are found in multiple receptor families widely distributed throughout bacteria and archea, highlighting their biological importance.

Taken together, our results show that this computational and experimental approach for the identification of ligand specificity can be applied to different type of signals, advancing significantly our understanding of signals that stimulate receptors.

FP4/9 - Novel methanogenic methyl transferase systems

Presenting Author - *Julia Kurth, Philipps-University Marburg, Germany*

Author/s - *Olivier Lemaire, Tristan Wagner, Cornelia Welte, Peter Fischer, Diana Sousa*

Abstract Content

Methoxylated aromatic compounds are components of lignin and coal and are very abundant on Earth. However, the conversion of these compounds has previously only been described for bacteria and not for archaea. The methanogenic archaeon *Methermicoccus shengliensis* was the first archaeon shown to be capable to convert methoxylated aromatic compounds, also called methoxydotrophic growth. In a recent study we showed by performing genome analysis, tanscriptomics and proteomics that *M. shengliensis* uses an O-demethylation/methyl transfer (Mto) system that is more related to that of acetogenic bacteria than the methyl transferase system of methylotrophic archaea. We were able to elucidate the respective methyl transfer reactions by activity assays and protein crystallization highlighting that tetrahydromethanopterin instead of coenzyme M is the final methyl acceptor, which differs from the conventional methanogenic methyl-transfer systems. Next to the transfer of the methyl group deriving from methoxylated compounds also the methyl transfer from tetramethylammonium, a compound that is present in marine environments, is an intriguing topic and has not been studied in detail. We found that a member of the *Methanococcoides* is able to grow on tetramethylammonium. After purifying the respective proteins either heterologously or via pulldown assay from the host itself, we now aim on characterizing the respective methyl transferase system by UV-vis spectroscopy and enzyme activity assays.

FP4/10 - New view on SbtB signaling: Cross-talks between cAMP, c-di-AMP and redox signaling

Presenting Author - *Khaled Selim, University of Freiburg, Germany*

Abstract Content

PII superfamily consists of widespread signal transduction proteins found in all domains of life1. In addition to canonical PII proteins involved in C/N sensing, structurally similar PII-like proteins evolved to fulfill diverse, yet poorly understood cellular functions. For efficient CO2 fixation at low ambient concentrations, cyanobacteria evolved highly specialized carbon concentrating mechanism, to augment intracellular inorganic carbon (Ci) levels. Recently, we identified the PII-like protein SbtB as Ci sensing module via sensing various adenine nucleotides including the second messenger nucleotides cAMP3, and c-di-AMP4, involved in global cellular homeostasis. We showed that cAMP acts as carbon signal, whereas adenyl-nucleotide binding links SbtB signalling to the energy state of the cells3,5. The c-di-AMP signaling through SbtB turned out pivotal for day-night acclimation of cyanobacteria via regulation of glycogen metabolism. To our knowledge, this is the first signaling protein known integrating both cAMP and c-di-AMP signaling. Moreover, SbtB possess a C-terminal extension with a disulfide bridge, which we call R-loop. We revealed an unusual ATP/ADP apyrase activity of SbtB that is controlled by the R-loop. We followed the sequence of the hydrolysis reactions from ATP to AMP in crystallographic snapshots and revealed the structural mechanism by which changes of the R-loop redox state modulate apyrase activity. This highlights SbtB as a central switch-point in cyanobacterial cell physiology, integrating not only signals from the energy state (adenyl-nucleotide binding) and the carbon supply via cAMP binding, but also from the diurnal status reported by the R-loop redox-switch and c-di-AMP binding.

FP4/11 - CRISPR Screening to determine the molecular basis of plasmid cost in enterobacteria

Presenting Author - *Cristina Herencias, Instituto Ramón Y Cajal De Investigación Sanitaria (irycis), Spain*

Author/s - *Ignacio de Quinto, Laura Jaraba-Soto, David Bikard, Álvaro San Millán, Jerónimo Rodriguez-Beltran*

Abstract Content

Plasmids facilitate horizontal gene transfer between bacteria, promoting their adaptation to a wide range of environmental conditions. Despite the advantages that plasmids provide to their host bacteria, they usually produce a fitness cost —a reduced growth rate and weakened competitiveness of plasmid-bearing strains under non-selective conditions. However, the plasmid-host interactions and the molecular mechanisms driving plasmid fitness costs remain poorly understood.

CRISPR interference (CRISPRi) screenings are a powerful large-scale genetic approach that allows the study of gene function in multiple species and applications. CRISPRi takes advantage of a catalytically dead version of Cas9 (dCas9) that binds to DNA, preventing the expression of targeted genes. The binding of dCas9 is directed by a single guide RNA (sgRNA) that base pairs with target DNA. By using libraries containing thousands of sgRNAs, CRISPRi screens provide information on the fitness effects produced by individually blocking the transcription of all genes within a genome.

Here, by targeting all genes within the *Escherichia coli* genome, we decipher the molecular basis of the fitness costs produced by six diverse plasmids at unprecedented throughput and precision. We uncover multiple plasmid-specific responses that highlight that crosstalk between plasmid and chromosomal genes is pervasive. More importantly, we reveal a source of fitness costs that is conserved across plasmids and involves the cellular periplasmic stress response. Our results shed light on new constraints and opportunities for plasmid evolution while suggesting a common mechanism for plasmid fitness costs that remains to be validated.

FP5/1 - Effects of S. sclerotiorum on the expression of genes encoding non-ribosomal peptides and polyketides in *P. fluorescens* BRZ63

Presenting Author - *Daria Chlebek, University of Silesia, Poland*

Author/s - *Bożena Nowak, Katarzyna Hupert-Kocurek*

Abstract Content

Sclerotinia sclerotiorum is a pathogen of many crop plants. The infected plants show reduced assimilation and nutrient and water transport, resulting in wilting and plant death. An important role in the biological protection of plants can be played by endophytic bacteria, which, through their ability to colonise the interior of plants and through various pathogen biocontrol mechanisms, reduce the susceptibility of plants to disease and ensure their welfare. Among the mechanisms limiting the development of fungal pathogens in bacteria of the genus *Pseudomonas*, we can distinguish the production of siderophores, biosurfactants or polyketides.

This study aimed (i) to investigate the ability of strain BRZ63 to protect oilseed rape against *S. sclerotiorum*, (ii) to investigate the effect of fungal filtrates on the expression of nrpS, pvdL, sidS, vsmA, and pksIII genes potentially involved in this process.

The protective role of the bacteria on oilseed rape was tested in pot cultures for 35 days. Expression of selected genes was assessed by culturing the bacteria in the presence of fungal filtrates. Total RNA was isolated at 24, 48, and 72h and purified prior to cDNA synthesis. The generated cDNA was used as a template in qPCR reactions.

The presence of bacteria alleviated the negative effect of *S. sclerotiorum* on the oilseed rape and increased its biomass. The bacterial response, expressed as altered expression of selected genes, was strongest after 48h exposure to the fungal filtrates. Among others, the expression levels of the pvdL and vsmA genes encoding non-ribosomal peptide synthetases were considerably up-regulated.

FP5/2 - Discovering symbiotic bacteria providing chemical defense molecules for nudibranchs

Presenting Author - *Maria Dzunkova, University Of Valencia, Spain*

Author/s - *Maria Dzunkova, Dafne Porcel Sanchis, Vicente Arnau Llombart, Tanja Woyke, Michael Burkart, Marta Pola Pérez, James J. La Clair, Tomáš Tyml, Devin Doud, Frederik Schulz, Samuel Piquer-Esteban*

Abstract Content

Soft-bodied marine animals, such as sponges, corals and mollusks, are known to use a variety of bioactive compounds, but thousands of them are yet to be discovered. Some of these molecules might be synthesized by yet uncultured symbiotic microbes. Nevertheless, detection of biosynthetic gene clusters (BGCs) in genomes of yet uncultured microbes does not guarantees that these compounds are produced *in vivo*, nor guarantees that the animals use these compounds for their defense. Herein we present nudibranch as a new model system for studying microbes producing bioactive molecules used to fend off its potential predators. Compared to filter-feeder animals, such as sponges and corals, nudibranchs have more complex body and tissue structures, thus there is a higher probability that abundant uncultured bacteria present in their mantle are symbiotic. In order to demonstrate that bacteria in nudibranch mantle actively produce bioactive molecules, we used a fluorescent labeling approach involving custom synthase-targeting probes. By applying targeted single-cell genomics on *Doriopsilla fulva* microbiome, we captured a new bacterial genus harboring a BGCs, encoding a novel bioactive molecule which was then extracted directly from the nudibranch mantle, confirming the role of symbiotic bacteria in nudibranch chemicals defense. Afterwards, we investigated 64 samples of different nudibranchs collected in coastal areas across whole Europe. While some nudibranch specimens contained only free living marine microbes, several specimens contained a high abundance of previously unknown bacterial taxa, suggesting their role nudibranch chemical defense.

FP5/3 - Get-together under the snow: fungal – bacterial interactions in four glacier forefields

Presenting Author - *Edoardo Mandolini, University of Innsbruck, Austria*

Author/s - *Maraike Probst, Anusha Telagathoti, Luis Miguel Rodriguez-Rojas, Ursula Peintner*

Abstract Content

Bacterial-fungal interactions in recently deglaciated ecosystems promote biogeochemical cycles, mineral soil fertility, and pioneer plant growth, but the diversity of keystone microbes and the quality of their interactions remain largely unexplored. Here, we investigated the diversity of both fungal and bacterial communities to predict core microbial networks and to estimate conserved interactions across comparable deglaciated systems.

We studied the soil fungal and bacterial communities at the early stages of soil development (0-25 years) in four receding calcareous glaciers of the Alps (>200 samples). High-resolution marker-gene (16S and ITS) analysis were performed alongside detailed soil geochemical analysis. Then, network analysis (SpiecEasi) was performed for each dataset and core microbial interactions were identified across glacier forefields. Finally, the strength of stochastic versus deterministic processes was evaluated to explore their influence in determining the microbial communities across high-scale spatial distances.

Bacterial and fungal communities differed in a location-specific manner, sharing remarkably few common taxa. We found extremely dense networks in all locations, with fungi clearly dominating the keystone nodes for all major interactive clusters. We speculate that conserved interactions across glacier forefields are rather based on trophic preferences than on phylogenetic diversity.

Our data emphasize (i) the unique diversity of soil microbial communities in glacier forefields likely depending on stochastic processes of dispersion, but provide (ii) evidence for common ecological roles based on conserved microbial interactions.

FP5/4 - Gen-directed re-sensitization of carbapenem-resistant microorganisms in biofilms

Presenting Author - *Damien Tortuel, Laboratory Microorganisms: Genome Environment, France*

Author/s - *Nicolas Charbonnel, Sylvine Batista, Racha Beyrouthy, Richard Bonnet, Ousmane Traoré, Geneviève Bricheux, Christiane Forestier*

Abstract Content

Background: Antibiotic resistance is a major concern in public health. The control of environmental reservoirs is necessary to limit the spread of resistance. Hospital effluents are considered as hotspots of antibiotic-resistant bacteria, mainly within biofilm communities. CRISPR/Cas9 system can be used to target resistance genes and re-sensitize bacteria when the resistance is plasmid mediated, as is the case of the carbapenemase-encoding gene oxa48.

Objectives: The goal of this work is to decrease the burden of carbapenem resistance in complex communities such as hospital effluents using a CRISPR/Cas9-based tool targeting carbapenemase-encoding genes.

Materials-Results: A conjugative plasmid carrying the CRISPR/Cas9 system targeting carbapenemase-encoding genes was engineered, using a plasmid isolated from environment with high conjugation frequency (pB10). Two large regions of pB10 encoding antibiotic resistance genes were removed and replaced by a Cas9 encoding-gene and a single guide RNA targeting oxa48 gene designed using conserved regions of twenty-one oxa48 encoding regions. The re-sensitization capacity of the resulting oxa48-targeting CRISPR/Cas9 tool was then assessed using a recipient strain harboring a pOXA-48 plasmid both as planktonic and sessile bacteria. When mating was performed on filters with planktonic recipients, the mating efficiency was estimated at 54% of the total recipient bacteria and about 99.48% of the transconjugants were re-sensitized. When mating was performed with 24h pre-formed biofilm, only 0.006% of recipients received the engineered plasmid, but 100% of them were re-sensitized. Despite a very high efficiency of re-sensitization shown by the tool, further experiments are necessary to improve its spreading capacities in biofilm communities.

FP5/5 - Ancient gene pools preserved in cryo-environments: a novel tool for climate change microbiology

Presenting Author - *Amedea Perfumo, Alfred Wegener Institute Helmholtz Center for Polar and Marine Research, Germany*

Author/s - *Uğur Çabuk, Kathleen Stoof-Leichsenring, Lars Harms, Ulrike Herzschuh*

Abstract Content

Cryo-environments are repository of ancient DNA (aDNA) molecules thousand- and million-year-old. They store the genetic fingerprint of past environmental microbiomes and how they adapted to climate conditions, thus offering unique insights into microbial dynamics relevant to future climate change. Furthermore, aDNA may be an exceptional source of potentially novel functional molecules that can inspire today's biotechnology.

Using long-term aDNA data across differing ecosystems in glacial-interglacial climates, we aimed at identifying trends of microbial community composition shifts and functional consequences (e.g., on biogeochemical processes) along with ecophysiological adaptive traits of key-taxa. Our prospect is to use this information i) to predict how microbial functions and feedback mechanisms will respond to extreme climate in the future and ii) to help develop innovative microbial technologies to mitigate climate change impact.

To do so, we applied shotgun metagenomics on aDNA (up to 600,000-year-old) extracted from chronosequences of permafrost, ice and sediments from various locations in the Arctic, including the Batagay megaslump and Muostakh island (two emblematic examples of climate-impacted nature).

Based on various case studies, we show the impact of climate on past arctic microbiome composition and feedback response such as increase of carbon cycle under warmer temperature. We also show the effect of vegetation change on nitrogen cycling soil community in arctic tundra during the past 2000 years. Finally, we present results on the application potential of functional genes (e.g., degradative enzymes) mined from such extreme habitats.

FP5/6 - Metaproteogenomic resolution of a cold-water geyser ecosystem enriched in CPR bacteria

Presenting Author - *Till L.V. Bornemann, University of Duisburg-Essen, Germany*

Author/s - *Perla Abigail Figueroa-Gonzalez, Tjorven Hinzke, Sandra Maass, Joern Starke, Carrie Moore, Doerte Becher, Alexander J. Probst (1, 2)*

1: Environmental Metagenomics, Research Center One Health of the University Alliance Ruhr, Faculty of Chemistry, University of Duisburg-Essen, 45151 Essen

2: Centre of Water and Environmental Research (ZWU), University of Duisbu

Abstract Content

Background: Bacteria of the Candidate Phyla Radiation (CPR), constituting 25% of the bacterial biodiversity, are characterized by streamlined genomes without key metabolic pathways such as lipid or protein biosynthesis. Consequently, CPR are mostly considered symbionts. Despite CPR bacteria being ubiquitous, they are poorly characterized, in particular when referring to subsurface dwellers.

Objectives: We studied temporal metaproteogenomic dynamics of the microbial community in the cold-water geyser Wallender Born, and elucidated both symbiotic relationships as well as key community members.

Methods: We coupled genome-resolved metagenomics to metaproteomics to elucidate community composition and determine their activity in a 12-day time-series. Groundwater was collected and sequentially filtered on 0.2µm and 0.1µm filters to fraction CPR bacteria and potential hosts. Using proportionality networks, we investigated whether CPR have specific interaction partners. In-depth analyses of genome and metaproteomic annotations for Gracilibacteria, who represent the most abundant CPR bacteria in the geyser, were used to unravel their lifestyle.

Results: We recovered 751 high-quality genomes, which represented 123 population genomes after dereplication. Co-occurrence networks indicated two sources of groundwater based on two distinct clusters, with one cluster enriched in CPR bacteria. Gracilibacteria were the most abundant CPR but showed no co-occurrence with potential hosts. Annotation showed that Gracilibacteria MAGs encoded for many proteins related to scavenging (e.g., secretion systems type II/IV, transport systems, putative type VI secretion systems). This metaproteogenomic investigation sheds light on the repertoire of proteins of scavenging bacteria and their expression in deeply sourced groundwater.

FP5/7 - *In-situ* fluorometry onboard the future space biology exposure platform, Exocube

Presenting Author - *David Burr, Free University of Berlin, Germany*

Author/s - *Elisa Ravaro, Lucas Bourmance, Louise Gilet de Chalonge, Adrienne Kish, Andreas Elsaesser*

Abstract Content

Given the remarkable ability of some microorganisms to prosper in extreme environments, several species have the potential to survive in space. The growing number of space exploration missions present many interesting questions regarding forward planetary contamination, life detection and life-support technologies. Although some space and planetary conditions can be replicated in the laboratory, the emulation of microgravity or solar and cosmic radiation (and their interaction) is particularly challenging, thus space-based experiments are critical. To address questions regarding biomolecular stability and microbial responses to space conditions, Exocube (Exposure of Organics/organisms) has been selected for implementation by the European Space Agency as part of the new European Space Exposure Platform. This miniaturized laboratory is scheduled to be installed outside of the International Space Station in 2024/2025. Expanding on previous space-exposure platforms, Exocube will perform real-time in-situ observations of microbial growth, survivability and cellular functions by utilizing a sophisticated microfluidics system in combination with miniaturized spectrofluorometric measuring capabilities. Exocube will employ fluorescent marker probes to monitor a variety of cellular processes, such as metabolism, membrane structural integrity and reactive oxygen species accumulation. However, as this is a novel and unconventional use of these fluorescent reporter dyes, extensive pre-flight optimization testing is a necessity. Here we present the current development of Exocube, specifically focusing on the preliminary biocompatibility and long-term stability testing of potential fluorescent stains. This work represents the newest generation of exobiological exposure platforms, aiming to address questions of fundamental radiation biology, microbial survivability and biomolecular mechanisms in space.

FP5/8 - Biomarkers predict the presence of a common intestinal microbial function and the relationship to diet and gut physiology

Presenting Author - *Qing Li, Aarhus University, Denmark*

Author/s - *Hans-Joachim Ruscheweyh, Lærke Hartmann Østergaard, Micael Libertello, Kim Skalborg Simonsen, Alberto Scoma, Clarissa Schwab*

Abstract Content

Functionality of intestinal microbiota depends on diet and gut physiology, how these factors contribute to the occurrence of specific microbiota activities has not been completely revealed. Microbial pdu and cbi-cob-hem clusters encode the key enzyme glycerol/diol dehydratase (Pdu) that mediates the transformation of dietary nutrients glycerol to 1,3-propanediol and 1,2-propanediol to propionate, and enzymes for cobalamin synthesis, a co-factor and shared good of microbial communities. The objective of this study was to investigate whether Pdu related traits of intestinal microbiota relate to diet and gut physiology via metabolic and genetic biomarkers.

Fecal samples from 55 species (n=104) provided from Givskud and Copenhagen Zoo were analyzed using a novel approach combining metagenomics with quantification of metabolic and genetic biomarkers and in vitro fermentations. Our results suggest that activity of pdu/cbi-cob-hem is a common feature of animal microbiota. 1,3-propanediol was detected in 56% of samples, 85% harboured at least one taxon carrying pdu with an abundance ≥ 4.5 log cells · g-1. Pdu was shared by few taxa with 58/5040 metagenomic assembled genomes harboring the operon. Pdu/cbi-cob-hem was more diverse and abundant in microbial communities of hindgut-fermenting omnivores/carnivores, which corresponded to higher amount of 1,3-propanediol and propionate produced in vitro. Taken together, this study suggests gut physiology and diet direct the potential of gut microbiota to utilize glycerol/1,2-propanediol and to produce cobalamin with a higher occurrence in hindgut fermenters. Our approach combining metagenomics with phenotypic analysis enabled the prediction of a common function in animal fecal microbiota by metabolic and genetic biomarkers.

FP5/9 - Land-use change drives changes in soil microbial functioning and carbon utilization across an agricultural mosaic landscape

Presenting Author - *Alexa Byers, Lincoln University, New Zealand*

Author/s - *Alexa Byers, Leo Condron, Steve Wakelin, Eirian Jones, Amanda Black*

Abstract Content - Soil carbon (C) supports a range of essential ecosystem functions and is tightly linked with the ability of ecosystems to withstand abiotic and biotic stress. Intensive land-use change can impact the functioning of soil ecosystems which in turn influences the resilience of soils to withstand the impacts of climate change. As soil microorganisms drive both the decomposition of and formation of soil C, studying their response to land-use change is essential to provide ecologically meaningful assessments of soil C resilience.

Our research aimed to quantify differences in soil microbial activity, functioning, and C utilization patterns across a dense mosaic of different land uses subject to varied disturbance regimes. These land uses included native podocarp forest (mataī & kahikatea), regenerating native scrub (Kunzea robusta (kānuka)), irrigated pasture, grazed pasture, and plantation forest (*Pinus radiata*). We used a range of methods to examine the soil microbiome including measurements of basal respiration, microbial biomass C (fumigation-extraction), microbial necromass (i.e. muramic acid & glucosamine quantification), and functional gene profiling (shotgun metagenomics sequencing).

We identified distinct signatures of each land use on the emergent properties of the soil microbiome, with more intensively disturbed sites exhibiting greater variability in their soil microbiome and C dynamics. The impact of land-use change and disturbance regime on soil microbial C cycling has key implications for informing us how to manage mixed-use agricultural landscapes to protect soil C resilience in light of climate change.

FP5/10 - Glyphosate-based herbicides alter the composition of foliar endophytic microbial communities in potato

Presenting Author - *Suni Anie Mathew, University Of Turku, Finland*

Author/s - *Suni Anie Mathew, Riitta Nissinen, Kari Saikkonen, Marjo Helander*

Abstract Content

Background: Glyphosate-based herbicides (GBHs) are the most globally used pesticides in agriculture. The active ingredient 'glyphosate' inactivates the 'the shikimate pathway', an essential metabolic route in plants. GBHs are considered safe mainly due to their fast degradation process and absence of shikimate pathway in animals. Contrary to the widespread safety claim, studies show that glyphosate and its degradation products are retained in the soil, especially in colder climates. Furthermore, as shikimate pathway is present in several bacteria and fungi the persistence of glyphosate in the soil could risk the existence of microbes interacting with crops planted in fields after GBH application. Hence, it is crucial to understand the effects of GBHs on non-target organisms.

Objectives: We tested the effects of GBH on endophytic bacterial and fungal communities of potato (Solanum tuberosum).

Methods: We analyzed the endophytic microbial community compositions in leaf and root tissues of potato plants grown in controls and glyphosate-treated experimental plots via 16SrRNA and ITS sequencing. Statistical analyses determined changes in microbial community compositions and showed microbial taxa that contributed to dissimilarity between control and glyphosate-treated samples.

Results: GBH altered the overall community composition of endophytic bacterial and fungal communities in leaves of potato and not in the roots. As plants depend on their microbial communities for nutrition, stress tolerance and other survival functions, the changes in the endophytic microbiota due to GBH residues in soil could cause severe long-term effects to the health of agricultural plants.

FP5/11 - The role of cyt bd oxidase in the oxygen response of Methanosarcinales

Presenting Author - *Evgenii Protasov, Max Planck Institute for Terrestrial Microbiology, Germany*

Author/s - *Katja Platt, Anja Poehlein, Rolf Daniel, Andreas Brune*

Abstract Content

Methanogens are considered strictly anaerobic archaea that are sensitive even to traces of oxygen. Nevertheless, certain members of the order Methanosarcinales have been shown to inhabit spatially or temporarily oxygenated environments. Despite several reports that methanogens are able to withstand oxygenation of their environment, the underlying mechanisms are not well understood (Conrad, 2020). We isolated six new species of Methanosarcinales from the digestive tracts of arthropods, an environment that is characterized by high oxygen fluxes (Sprenger, 2000). Comparative genome analysis revealed that all isolates encode homologs of a membrane-associated cytochrome bd oxidase.

Using optical oxygen sensors and gas chromatography, we assessed the response of methanogenic cell suspensions to controlled oxygen fluxes and their ability to recover from oxygen exposure. Transcriptomic analysis revealed that the exposure to oxygen affected the expression of numerous genes, including the upregulation of cydAB and other genes potentially involved in electron transport, suggesting that a high-affinity Cyt bd oxidase is responsible for oxygen consumption. Comparative genome analysis indicated that certain genes involved in oxygen reduction and detoxification were likely transferred from Bacilli to the last common ancestor of Methanosarcinales via lateral gene transfer but were eventually lost in all but a few species.

Our results suggest that the phylogenetically distant Methanosarcinales and Methanobacteriales vary in their oxygen tolerance and exploit different strategies to deal with environmental oxygen exposure. The ability to withstand high oxygen fluxes is particularly pronounced in members of the order Methanosarcinales that colonize the digestive tract of arthropods.

FP5/12 - Widespread signatures of microbial interaction modulation across diverse natural communities

Presenting Author - *Janko Tackmann, University of Zurich, Switzerland*

Author/s - *João F. Matias Rodrigues, Christian von Mering*

Abstract Content

Species interactions are essential determinants for microbial ecosystem structure and functioning. To understand how these systems assemble and shape their environment, knowledge of the underlying ecological relationships is key. While co-culture experiments can elucidate interactions in small communities, computational tools are necessary to guide these efforts and form hypotheses on complex, realistic ecosystems.

Despite their popularity, statistical network approaches typically violate an important insight: microbial interactions are not static, but may flexibly change in a context-dependent manner. Species cooperating in one community may be fierce competitors in another, depending on environmental conditions or the presence of other "modulating" species. Such higher-order interactions (HOIs) may be widespread, but have thus far not been extensively quantified in natural communities.

Here, we present a fast, flexible computational framework that detects signatures of HOIs directly within count tables of complex, natural microbial communities. It utilizes statistical tests that capture context-dependent co-occurrences to predict candidates for modulating species and environmental factors. Furthermore, it features state-of-the-art compositionality correction and reduces the effect of confounders, such as shared-habitat biases.

We screened the Human- and Earth Microbiome Project datasets and found widespread signatures of HOIs across dozens of habitats. Notably, many HOIs are concentrated in uncharacterized modulating hub species, which may have an underappreciated impact on their communities. We furthermore observed striking environment-specific signatures, including a pronounced mitigation of predicted positive interactions within the human gut, indicative of frequent swaps of cross-feeding partners. Our method thus helps elucidate the thus far hidden plasticity of complex, natural microbial networks.

FP5/13 - Fundamental metabolic strategies of heterotrophic bacteria

Presenting Author - *Matti Gralka, Vrije Universiteit Amsterdam, Netherlands*

Author/s - *Shaul Pollak, Otto Cordero*

Abstract Content

Through their metabolism, heterotrophic microbes drive carbon cycling in many environments. These microbes consume (and produce) hundreds to thousands of different metabolic substrates, begging the question of what level of description is required to understand the metabolic processes structuring their communities: do we need to account for the detailed metabolic capabilities of each organism, or can these capabilities be understood in terms of a few well-conserved carbon utilization strategies that could be more easily interpreted and more robustly predicted? Based on the high-throughput phenotyping of a diverse collection of marine bacteria, we showed that the fundamental metabolic strategy of heterotrophic microbes can be understood in terms of a single axis of variation, representing their preference for either glycolytic (sugars) or gluconeogenic (amino and organic acids) carbon sources. Moreover, an organisms position on this axis is imprinted in its genome, allowing us to successfully predict metabolic strategy across the bacterial tree of life. Our analysis also unveils a novel and general association between metabolic strategy and genomic GC content, which we hypothesize results from the difference in C:N supply associated with typical sugar and acid substrates. Thus, our work reveals a fundamental constraint on microbial evolution that structures bacterial genomes and communities and can be leveraged to understand diversity in functional terms, beyond catalogs of genes and taxa.

FP5/14 - Sulfamethoxazole addition increased microbial activity in environmental river water and biofilm samples

Presenting Author - *Sarah Haenelt, Helmholtz Centre for Environmental Research, Germany*

Author/s - *Gangan Wang, Hans Hermann Richnow, Steffen Kümmel, Hryhoriy Stryhanyuk, Jochen Ait Müller, Niculina Musat*

Abstract Content

Biofilm-forming microorganisms are embedded in extracellular polymeric substances and hence well protected against external stressors, e.g. antibiotics. While this issue is well addressed in clinical settings, similar studies on diverse biofilm-forming microbial communities in natural environments are still rare.

Here we raised the question how the metabolic activity of aquatic microbial communities in naturally formed biofilms is impacted by the broad-spectrum antimicrobial sulfamethoxazole (SMX) when compared to planktonic communities thriving in river waters.

We investigated biofilm-forming and planktonic microbial communities from pristine and anthropogenically polluted river water using stable isotope probing with 15N-labelled ammonium as a tracer. Bulk and single-cell techniques were applied to monitor microbial activity of microorganisms exposed to different SMX concentrations (up to 1 mg/L) by means of 15N incorporation into the biomass. Bulk measurements were conducted using an Elemental Analyser coupled with Isotope Ratio Mass Spectrometry (EA IRMS), and single-cell analysis was done using nano-scale Secondary Ion Mass Spectrometry (nanoSIMS). Microbial community structure was studied via 16S rRNA gene amplicon sequencing.

Surprisingly, biofilm-forming as well as planktonic communities show a clear increase in microbial activity upon exposure to SMX. Bulk analysis reveals different responses comparing pristine and anthropogenically polluted river water, which is confirmed by preliminary results on a single cell level. Overall, acquired data indicate that SMX concentrations up to 1 mg/L stimulate microbial activity in aquatic environments, however showing heterogeneity within microbial communities. To what extent microbial composition and community structure play a key role in this metabolic response remains to be determined.

FP5/15 - Insights into landfill fungal and bacterial community structure and plastic biodegradation competencies

Presenting Author - *Memory Tekere, University of Pretoria, South Africa*

Abstract Content

The ever-increasing demand for plastics throughout the world has resulted in increased plastic solid waste pollution threat, which has piqued the curiosity of experts and public. Because of the vast manufacture of plastic, large volumes of plastic garbage are discarded into the environment, damaging natural ecosystems. Plastic wastes are resistant to thermal, biological, and photo-oxidation, causing issues in waste management, blockage of drains and sewage systems, as well as soil pollution. Microbial bioremediation and advances in plastic management are necessary as part of a green solution to plastic pollution. The goal of this study was to unpack plastic degrading bacterial and fungal strains from an urban landfill, screening them for terephthalate, polyethylene, and polystyrene degrading capabilities, constituent enzymes, and developing a potent consortium in optimized lab-scale bioreactors. After 90 days of incubation, the consortium exhibited a total of 26 bacterial and 12 fungal taxa with 22.4-55.6 percent LDPE-degrading capability, with the top prevalent bacteria being Chitinophagaceae, *Planococcus*, Gemmatimonadaceae, *Rhodanobacter*, *Paludisphaera*, and *Achromobacter*. *Galactomyces*, *Trichosporon*, Penicillium, *Aspergillus*, and *Talaromyces* were the top fungi. FTIR investigations of enrichment culture-treated PE revealed structural/functional group alterations such as C-H (2831-2943 cm1) and CH2 (1400 cm1) stretching, C=O and C=C (680-950 cm1) scission, and C=O integration (3320-3500 cm1) into the carbon backbone. The findings of this study, taken collectively, contribute immeasurable insights into the utilization of bacteria, and fungal consortiums in plastic biosynthesis, as well as enzymatic plastic degradation and biosynthesis.

FP6/1 - Metabolomics to decode microbial interactions in kombucha beverage

Presenting Author - *Thierry Tran, UMR Procedes Alimentaires et Microbiologie, France*

Author/s - *Thierry Tran, Chloé Roullier-Gall, François Verdier, Antoine Martin, Philippe Schmitt-Kopplin, Hervé Alexandre, Raphaëlle Tourdot-Maréchal*

Abstract Content

Kombucha is a fermented beverage produced from sugared tea through the activity of a yeasts and bacteria consortium. Those microorganisms are present both in the liquid and the biofilm developing during fermentation. Kombucha consortia exist in multiple microbiological diversities. Interactions taking place among yeasts and bacteria raise questions regarding the control of fermentations. Metabolic interplays involved in the production of organic acid from sugars have already been described, at the expense of other metabolites. Among the two aerobe and anaerobe production phases, the first main one has been investigated.

Interactome between kombucha microorganisms was investigated by reducing an original consortium to three selected microorganisms. Two yeasts (*Brettanomyces bruxellensis* and *Hanseniaspora valbyensis*) and one acetic acid bacteria (*Acetobacter indonesiensis*) were investigated in monocultures and cocultures (dual or trio) in sugared tea after a 7-day aerobic incubation at 26°C. Population levels were determined by cultural methods and metabolomic analysis was performed by Fourier Transform-Ion cyclotron-Mass Spectrometry on products, including a data treatment of 506 putative metabolites.

Results showed that the presence of *H. valbyensis* affected positively the growth of *B. bruxellensis* and *A. indonesiensis*, except for the trio with no obvious benefits. The two yeasts also had opposite effects on *A. indonesiensis* populations. Metabolomics analysis highlights metabolites exchanges as peptides and fatty acids involved in the yeast-yeast interaction. When *A. indonesiensis* was added, those metabolites were either consumed or not produced, echoing the disappearance of growth benefits. Moreover, *A. indonesiensis*' dehydroquinate production evokes an ecologically-driven strategy to attract nutrients providers, such as yeasts.

FP6/2 - Altering the probiotic epigenome, its potential application in fermentation

Presenting Author - *Yanzhuo Kong, Lincoln University, New Zealand*

Author/s - *Christopher Winefield, Philip Wescombe, Stephen On, Andrew Saunders, Venkata Chelikani*

Abstract Content

Epigenetics is the study of changes in gene expression and function that occur without a change in the underlying DNA sequence. These changes in humans, can be caused by a variety of factors, including dietary intake, environmental exposures, and lifestyle factors. Epigenetic modifications, such as DNA methylation and histone modifications, can alter regulation of genes impacting development and physiology. Consequently, manipulation of epigenetic processes has great potential for interdisciplinary applications, including in the fields of microbiology and fermentation science. This study aimed to investigate the impact of certain human dietary compounds on probiotic bacteria, with a specific focus on *Lactobacillus acidophilus*, widely regarded as a probiotic bacterium frequently present in the gut. The ultimate goal being to apply the acquired knowledge to enhance the effectiveness of probiotic strains used in fermentation processes. The study explored epigenetic impacts of six dietary compounds on the exemplar probiotic strains of *Lactobacillus*. The known DNA methyltransferase inhibitor, 5-aza-2'-deoxycytidine, was used as a positive control to compare activity of the chosen dietary compounds. Epigenetic changes were evaluated using whole genome bisulphite sequencing, RNA sequencing, and metabolomic profiling. The results show that each dietary compound has a unique epigenetic-based impact on regulation of gene expression in *L. acidophilus*. The study also investigates the application of epigenetically-modified probiotics in milk fermentation to develop a novel probiotic yogurt that could provide additional functional benefits in the gut and overall health benefits to the body.

FP6/3 - Fermentation of pea protein by mixed starter cultures of lactic acid bacteria and yeasts

Presenting Author - *Dor Zipori, University of Hohenheim, Germany*

Author/s - *Marina Rigling, Yanyan Zhang, Herbert Schmidt*

Abstract Content

Plant protein-based alternatives to meat and dairy products have grown in popularity in recent years. However, due to technological, nutritional, and sensory issues, the use of plant-based proteins introduces new challenges in the development of new products. Microbial fermentation has been proposed as a way to overcome these obstacles and produce more appealing products. Single and co-cultures of LAB and yeasts were used in fermentation experiments, which were assumed two have a synergistic effect on the reduction of off-flavor compounds in the pea protein-based product. First, a LAB strain capable of rapidly and strongly acidifying the pea protein matrix was selected. Then, mixed fermentations were performed with the LAB strain in combination with three yeast strains and compared to single strain fermentations in terms of pH and viable counts. Finally, the concentration of different flavor compounds was determined. A rapid acidification of the matrix was achieved by *Lactococcus lactis* strain. All three yeast strains tested could grow in the matrix as single and mixed culture along with *L. lactis* with comparable cell counts. Fermentation with a single strain culture of *L. lactis* led to an increase in the concentration of compounds associated with the “beany” off-flavors of peas, such as hexanal. A significant reduction in those compounds was achieved by fermentation with a *Yarrowia lipolytica* strain as single and in mixed culture together with *L. lactis*. Thus, the sensory attributes of a pea protein-based product can be enhanced through fermentation using a co-culture of both strains.

FP6/4 - Low N:P ratio and salinity in nano-filtered whey permeate induced over production of EPA by *Nannochloropsis oceanica*

Presenting Author - *Hossein Kiani, Instituto Ramón Y Cajal De Investigación Sanitaria (irycis), Ireland*

Author/s - *Yuchen Li, Qinge Ma, Brijesh Tiwari, Ronald Halim*

Abstract Content

Background: Whey is a byproduct of dairy factory and can be nano-filtered trapping proteins and lactose while a permeate is generated containing minerals and small organic molecules. Due to high mineral and phosphate content along with low carbon source, it is difficult to utilize or treat nano-filtered whey permeate (WP). Microalgae can capture CO2, Nitrogen and Phosphorus producing a biomass rich in omega-3 oil.

Objectives: Growth of oleaginous single-celled microalgae *Nannochloropsis oceanica* (NO), on WP was investigated by microscopy and flowcytometry.

Methods: Fluorescence microscopy was used to visualize cell wall and lipid structures. Fatty acid profile was analyzed by gas chromatography.

Results: Complex N source present in WP delayed the cell growth but finally the biomass concentration surpassed that of standard medium. High phosphate content and low N:P ration in WP contributed to increased cell size and EPA production. The presence of large cells and/or aggregates was observed because of low salinity while increasing number of small growing cells were also detected. BODIPY staining indicated that a population of medium and small sized cells tend to exhibit stronger fluorescence intensities suggesting that WP could induce fat accumulation in the cells. With 33% concentration, the major fatty acid was EPA accounting for 15% of biomass weight while DHA was also produced with a concentration of 5.6%. Total polyunsaturated fatty acid content was 55% accounting for 25% of the biomass. WP was shown to be a cost-effective medium for NO growth producing high concentrations of omega-3 fatty acids.

FP6/5 - Transmission routes of dairy propionic acid bacteria from the barn environment into raw milk

Presenting Author - *Carola Bücher, Austrian Competence Centre For Feed And Food Quality, Safety And Innovation (ffoqsi), Austria*

Author/s - *Tamara Rudavsky, Johanna Burtscher, Konrad J. Domig*

Abstract Content

Dairy propionic acid bacteria (dPAB) are frequently isolated from raw milk and have been found in biofilms from insufficiently cleaned milking systems and milking equipment. They are known for their potential to cause sensory defects and cheese blowing in raw milk cheeses, resulting in significant monetary losses for the producers, making strict milking hygiene essential. As data on the pathways of dPAB raw milk contamination are scarce, this study aims to elucidate the levels and transmission routes of dPAB contamination.

16 dairy farms were sampled in August 2022: air, feed, bedding, over 150 swab samples from the farm environment, cleaning-water residues of the milking system, and milk samples were collected. Milk samples were taken at different points along the milking system while only the first cow was being milked, in addition to bulk tank samples after all cows had been milked. Swabs and air samples were analyzed qualitatively, the remaining samples were enumerated on lithium glycerol agar with subsequent identification by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using the MBT (Bruker Daltonics) and an in-house database of additional dPAB spectra. dPAB were found in feed and bedding material. Contamination levels of raw milk samples varied from not detected in 1mL to log 4.3 CFU/mL, with levels increasing as the milk was transported through the milking system, with significant inter- and intra-farm variation in dPAB levels. This highlights the importance of farm management and milking hygiene, and may be useful in identifying critical points in the cleaning regimes of milking systems.

FP6/6 - Systematic evaluation of the suitability of phage products in the control of foodborne pathogens

Presenting Author - *Christoph Brieske, Max Rubner-Institut, Germany*

Author/s - *Hui Zhi Low, Christina Böhnlein, Charles M.A.P. Franz*

Abstract Content

Background: Foodborne bacterial pathogens can cause significant health and economic impacts worldwide. *Listeria (L.) monocytogenes* and *Salmonella* spp. frequently cause outbreaks and food recalls due to contamination. Bacteriophage (phage)-based products claiming to have broad host ranges against *Listeria* and *Salmonella* are commercially available. Although these products are already approved for use in Israel, Canada, China, Switzerland, Australia and New Zealand, they are still awaiting approval in the EU.

Objective: The aim of this study is to systematically investigate the efficacy of these phage products on clinical and food-associated *L. monocytogenes* and *Salmonella* spp. isolates currently circulating in Germany. Potential challenges that may be associated with this biocontrol method will also be evaluated.

Methods: Commercially available phage products targeting either *L. monocytogenes* or *Salmonella* were used. The susceptibility of clinical and food-associated *L. monocytogenes* and *Salmonella* spp. isolates was investigated by spot and plaque assays using incubation temperatures of 6°C, 20°C, and 37°C. In addition, we intend use flow cytometry to better characterize the infection and kinetics.

Results: Out of 20 clinical Salmonella isolates, 60% were susceptible to the phage product at 37°C. Results from other temperatures are pending. Of the clinical *L. monocytogenes* isolates (n=20), 65% were found to be susceptible to the product at 6°C. Food-associated *L. monocytogenes* isolates (n=18) showed 50% susceptibility at 6°C. Preliminary results demonstrate the effectiveness of phage products on current circulating strains of *L. monocytogenes* at temperatures relevant to food processing environments.

FP6/7 - How *Listeria monocytogenes* CC121 strains successfully adapt to the food processing environment.

Presenting Author - *Kathrin Kober-Rychli, University of Veterinary Medicine Vienna, Austria*

Author/s - *Eva Wagner, Stephan Schmitz-Esser, Martin Wagner*

Abstract Content - *L. monocytogenes* isolates have high variability regarding prevalence, pathogenicity and stress response. Strains belonging to CC121 are highly prevalent and are predominately isolated from food and food processing environments. There is evidence that CC121 strains are able to survive for months and even years in the food-producing environment.

We performed whole genome analysis of 70 CC121 strains from different origins and characterized several genetic feature essential for the survival of CC121 strains in the food-processing environment.

First, we identified the transposon Tn6188, which encodes QacH, a small multidrug resistance protein family transporter. QacH confers tolerance to quaternary ammonium compounds, commonly used as disinfectants in food processing facilities. Secondly, we characterized the stress survival islet 2 (SSI-2), consisting of a transcriptional regulator lin0464 and a PfPI protease lin0465. The PfpI protease is involved in alkaline and oxidative stress responses, but not in acidic, gastric, heat, cold, osmotic, and antibiotic stresses. In parallel, deletion of lin0464 decreased expression of the PfpI protease and subsequently survival under alkaline and oxidative stresses. Third, we discovered a 12.5 kbp insertion in CC121 genomes that encodes a protein with rearrangement hotspot (Rhs) repeats. We showed that expression of the Rhs C-terminus in Escherichia coli inhibited growth, indicating toxic activity of the Rhs protein. These data suggest that the Rhs insertion inhibits the growth of neighbouring cells, supporting the survival and growth of CC121 strains.

In conclusion, we demonstrated that CC121 harbors specific genetic features supporting adaption to the food processing environment and enabling persistence.

FP6/8 - Assessing the brewing potential of *Lachance thermotolerans* and *Lachance quebecensis* isolated from insects

Presenting Author - *Wendy Franco, Pontificia Universidad Catolica De Chile, Chile*

Author/s - *Wendy Franco, Valeria Galaz*

Abstract Content

Yeasts are ubiquitously present in different natural sources. Some of these yeasts have interesting characteristics for producing fermented food products. This study characterized *Lachancea thermotolerans* and *L. quebecensis* isolated from insects to determine their brewing potential. The yeasts were evaluated according to their fermentative potential in glucose and maltose-defined medium and their resistance to ethanol and hop. Finally, craft beer was elaborated at a laboratory scale (10 L). Both yeasts utilized glucose and maltose as the sole carbon source. The yeast utilized maltose as the only carbon source and produced ethanol, reaching values of 4.15 ± 0.25% (v/v). The yeast showed tolerance to alpha acids, up to 90 IBU, and 5% (v/v) ethanol. The craft beer produced with the yeast in monoculture experiments showed fruity flavors associated with ethyl acetate and isoamyl acetate. The ethanol content reached 3.20 ± 0.26% (v/v). The beer pH was 3.77 ± 0.1, with a lactic acid concentration of 1.85 ± 0.12 g/L. The sensory panel identified the beer as “fruity,” “floral,” “hoppy”, “sweet,” and “sour”. To our knowledge, this is the first time *L. quebecensis* was reported as a potential candidate for sour beer production with reduced ethanol content.

FP6/9 - Cooperation between food-associated microorganisms

Presenting Author - *Mareike Weber, Rheinische Friedrich-Wilhelms-Universitat Bonn, Germany*

Author/s - *Joana Esslen, André Lipski*

Abstract Content

Foodstuffs contain complex consortia of microorganisms interacting with each other in a competitive or cooperative way. Cooperative interactions of pathogens and/or spoilage microorganisms with commensal bacteria can threaten consumer's health or reduce shelf life due to accelerated deterioration. Signal molecules called autoinducers can be secreted to coordinate community-wide functions, e.g. bioluminescence, virulence, or biofilm formation. Autoinducer 2 (AI-2) molecules are produced by both Gram-negative and Gram-positive bacteria, and are thus assumed interspecies signals.

We assessed interspecies cooperation of isolates from a variety of foodstuffs. Isolates from meat, fish, raw milk cheese, and ready-to-eat food were identified via 16S rRNA gene sequencing, and tested for AI-2 production using bioluminescent *Vibrio campbellii* reporter strains. Depending on the foodstuff of origin, about 40-50 % of isolates produced AI-2. Independent of the foodstuff of origin, the genus *Pseudomonas* did not produce AI-2, while isolates of the genera *Kocuria*, *Enterococcus*, *Bacillus*, *Listeria*, *Brochothrix* and the order Enterobacterales were frequently tested positive for AI-2 production.

Combinations of two isolates displayed enhanced growth or biofilm formation compared to pure cultures, pointing to possible cooperation. We either observed enhanced growth in the planktonic phase, or enhanced biofilm formation, rather than a combination of both. In some cases, cell-free supernatants of bacterial strains exerted a growth or biofilm enhancing effect on other strains. Fluorescence-in-situ-hybridization (FISH) proved spatial proximity of the cooperating species within biofilms formed on stainless steel coupons, indicating beneficial effects of small distances between cooperating partners.

FP6/10 - The next-generation tools for risk assessment and precision food safety: investigation using metagenome-assembled genomes

Presenting Author - *Guerrino Macori, University College Dublin, Ireland*

Author/s - *Guerrino Macori, Leonard Koolman, Siobhán McCarthy, Séamus Fanning*

Abstract Content

Next Generation Sequencing (NGS) technologies and the application of bioinformatic approaches are redesigning microbiology and their applications, covering aspects of food quality and precision food safety. Use of NGS protocols includes, among others, a deep understanding of the genomes of microorganisms in pure culture using whole genome sequencing and importantly, metagenomics has allowed the extensive comprehension of the microbiota and microbiome of food. Microbial communities along food chains have increasingly been studied for describing the genetic diversity, functionality, and succession of spoilage microflora, and foodborne pathogens and for studying functional microorganisms used for producing food by fermentation. In this study, water kefir was used as a model for evaluating the performances of different sequencing approaches for the detailed description of complex microbial communities within foods. Several isolates were retrieved through culture-dependent techniques and were also identified as high-quality metagenome-assembled genomes (MAGs), including prominent probiotic species of the genus *Gluconobacter*, *Liquorilactobacillus*, *Lactiplantibacillus*, *Lentilactobacillus* and *Lacticaseibacillus*. Nanopore technology applications were used for describing the samples, through full-length 16S rRNA gene sequencing and shotgun metagenomic approach, coupled with software-controlled enrichment of the species isolated with culturing. Finally, the novel-identified species were sequenced from pure culture, providing a detailed characterisation of their genomes. These next generation of tools are going to change radically the risk assessment approaches and methods for better pinpointing the origins of contamination events, antimicrobial resistance spread and describing potential unknown pathogens, such as using metagenomics for resolving the pathogens present in food at the strain level using metagenome-assembled genomes.

FP6/11 - *In vitro* effects on gut metabolites of fermented brewers' spent grain containing dextran and oligosaccharides

Presenting Author - *Prabin Koirala, University of Helsinki, Finland*

Author/s - *Alice Costantini, Henry N. Maina, Carlo Giuseppe Rizzello, Michela Verni, Andrea Polo, Kati Katina, Raffaella Di Cagno, Rossana Coda*

Abstract Content

Background: Brewers' spent grain or BSG is a fiber and protein-rich food-grade side stream generated from breweries in large quantities globally. Its poor technological and sensory characteristics make it challenging to incorporate into food, and thus it remains largely underutilized. Lactic acid bacteria fermentation has been shown to improve the technological and sensory properties of BSG by producing in situ dextran and maltosyl-isomaltooligosaccharides with prebiotic properties.

Objective: To study the effects of in situ dextran and maltosyl-isomaltooligosaccharides on gut microbial metabolites during in vitro digestion simulation of BSG-bread.

Methods: BSG was fermented with Weissella confusa A16 in the presence of sucrose to induce the synthesis of dextran and maltosyl-isomaltooligosaccharides and was used as an ingredient in wheat bread. Digestion of BSG-breads was simulated in vitro in TWIN-SHIME gut model. Levels of fecal metabolites in the colon were analyzed at different stages of digestion.

Results: BSG-breads (with and without in situ dextran and oligosaccharides) positively influenced gut metabolites. Synthesis of short chain fatty acids increased with increased protein and fiber content of the bread. The bio-accessibility of amino acids increased, and the amount of ammonia decreased in presence of dextran and oligosaccharides simultaneously. Wheat-bread enriched with plant protein and fiber, and containing in situ synthesized polysaccharides upgraded BSG quality and usability as a food ingredient. Further investigations are ongoing for determining the preferential metabolism or carbon catabolite repression of different sugars during lactic acid bacteria fermentation in native BSG and the condition of dextran and oligosaccharides formation when enriched with sucrose.

FP6/12 - Resistome analysis on metagenomes from meat processing facilities.

Presenting Author - *José F. Cobo-Díaz, University of Lyon, Spain*

Author/s - *José F. Cobo-Díaz, Narciso M. Quijada, Vincenzo Valentino, Coral Barcenilla, Alba Puente, Francesca De Filippis, Danilo Ercolini, Avelino Alvarez-Ordóñez*

Abstract Content

Background: Meat processing environments (MPE) may act as a reservoir of antimicrobial-resistant microorganisms due to their high prevalence on farming animals. The spread of antimicrobial resistance genes (AMRG) through MPE in the meat industry along the food production process is an important concern that must be studied in depth.

Objectives: To describe the impact of different meat production processes on the spread of AMRGs along the meat production chain.

Methods: Microbiome composition and AMRG content were monitored on meat processing facilities through whole-metagenome sequencing (WMS). Raw materials, environmental samples and final products (both fresh meat and ripened products) were collected from 19 meat processing facilities. AMRGs were screened by assembly-free and -based approaches, using ResFinder1. Plasmids, lateral gene transfer events and integrons were detected on AMRG-carrying contigs using PlasmidFinder2/Platon3, Waafle4 and Integron_Finder5, respectively, while taxonomy was assigned using Kraken6.

Results: AMRGs were found in higher abundance on final products and facility surfaces than on raw materials. Tetracycline resistance genes were the most prevalent, followed by those related to beta-lactam and aminoglycoside resistance. A high percentage of aminoglycoside and tetracycline resistance genes were associated with plasmids, while beta-lactam resistance determinants were mainly located in chromosomes. Remarkably, certain critically important AMRGs were harbored by ESKAPEE bacteria (including *Acinetobacter baumanii* and *Staphylococcus aureus*).

FP6/13 - Whey-fruit smoothies: enrichment of phenolic compounds and improvement of protein digestibility through lactic acid fermentation

Presenting Author - *Elisabetta Trossolo, Free University of Bozen-Bolzano, Italy*

Author/s - *Elisabetta Trossolo, Ali Zein Alabiden Tlais, Stefano Tonini, Pasquale Filannino, Marco Gobbetti, Raffaella Di Cagno*

Abstract Content

Background: Scientific research is being increasingly directed toward developing innovative, safe and sustainable food for our people, our planet and our climate that will deliver fair food production and provide secure, affordable, and healthy food.

Objectives: Whey milk (protein source) was exploited as a novel ingredient of fruits smoothies (phenolic compounds source) through started-assisted fermentation.

Methods: Five starters belonging to different species were selected based on complementarity of pro-technological and functional performances to ferment whey fruit smoothie. After fermentation, bioactive compounds were quantified through high-performance liquid chromatography (HPLC) analyses. The effect of fermentation on functional attributes was evaluated in terms of antioxidant activity and protein digestibility.

Results: Compared to raw whey milk fruit smoothie (unstarted), the fermented counterpart exhibited distinct profiles of sugars, organic acids, ascorbic acid, phenolic compounds, and especially anthocyanins. The interaction between proteins and polyphenols enhanced anthocyanin release, especially under the activity of *Lactiplantibacillus plantarum* strains. The same bacterial strains outperformed other species in terms of protein digestibility and quality. With variations among starters culture, bio-converted metabolites were most likely responsible for the increase antioxidant scavenging capacity and the modifications in organoleptic properties.

FP7/1 - Population analysis of mixed bacterial communities by GroEL-Taxoproteomics

Presenting Author - *Simon Klaes, Technische Universität Berlin, Germany*

Author/s - *Shobhit Madan, Darja Deobald, Myriel Cooper, Lorenz Adrian*

Abstract Content

Bacterial communities are fundamental for many health-related, ecological, environmental, and biotechnological processes. Since proteins constitute a large share of the bacterial biomass and are responsible for metabolic activity, metaproteomics has become increasingly popular for characterizing bacterial communities. However, major challenges of metaproteomics are the high sample complexity impairing the detection of low-abundant proteins and the need for a sample-matched database for accurate protein identification. Here, we present a targeted metaproteomic approach to identify bacterial community compositions at the family level using the taxonomic marker protein GroEL, which is highly conserved and abundant in all bacteria. We call our method GroEL-Taxoproteomics. GroEL-Taxoproteomics comprises a Galaxy workflow for peptide identification coupled to a Python-based analysis script and can be performed with a sample-independent database. GroEL-Taxoproteomics was validated by applying it to raw metaproteome data from different microbial mock communities and real human gut samples. In addition, we experimentally determined the relative detection limit of GroEL-Taxoproteomics for detecting low-abundant bacterial taxa (at the family level). To reduce sample complexity and improve GroEL identification while simultaneously reducing the measurement time, proteins from crude extracts were separated by SDS-PAGE. The gel bands at GroEL's molecular weight size of approximately 60 kDa were cut out and analyzed by mass spectrometry followed by the GroEL-Taxoproteomics workflow established here. Our results show that GroEL-Taxoproteomics can overcome major challenges of metaproteomics and can be used to quantify biomass contributions of bacterial taxa in mixed communities.

FP7/2 - Nomenclature of prokaryotic taxa under the rules of the International Code of Nomenclature of Prokaryotes: updates from the 2022 revision

Presenting Author - *Aharon Oren, Faculty of Dental Medicine, Hebrew University of Jerusalem, Israel*

Abstract Content

Prokaryotic nomenclature is regulated by the International Code of Nomenclature of Prokaryotes (ICNP). The ICNP '2022 Revision', incorporating many changes proposed since the '2008 Revision', was approved by the ICSP and will soon be published in the International Journal of Systematic and Evolutionary Microbiology (IJSEM). One significant change is the inclusion of the rank of phylum. Phylum names are formed by addition of the suffix –ota to the stem of the name of the designated type genus. This has allowed the names of 43 phyla to be validly published as of December 2022. The cyanobacteria (phylum Cyanobacteriota) are now included in the ICNP. For cyanobacterial taxa named under the International Code of Nomenclature for algae, fungi, and plants, any of the names need satisfy only the requirements of that code for status equivalent to valid publication under the ICNP. A proposal to include the categories kingdom (Regnum) and domain (Dominium) is under consideration by the ICSP. The name of a kingdom is formed by the addition of the suffix –ati to the stem of the name of the designated type genus. The rules of the ICNP only deal with nomenclature of cultivated prokaryotes. Non-cultivated taxa can be described with the provisional status Candidatus. The list editors of the IJSEM have prepared five curated lists of Candidatus taxa up to the rank of class published before the end of 2022 and proposed numerous name corrections. A curated list of more than 180 Candidatus phylum names will soon be published.

Submitted on behalf of the Executive Board of the International Committee on Systematics of Prokaryotes

FP7/3 - A polyphasic characterization of *Clostridium* sp. Cl_52, a bacterium isolated from cheese with late blowing defect

Presenting Author - *Lucija Podržaj, University of Natural Resources and Life Sciences, Austria*

Author/s - *Lucija Podrzaj, Tina Krajnc, Johanna Burtscher, Konrad J. Domig*

Abstract Content

Clostridium is a wide heterogenous genus in the Bacillota phylum. The genus encompasses several species that may be responsible for food spoilage, including late blowing defect in cheese. Here we aimed to identify and characterize the strain Cl_52, isolated from cheese with late blowing defect. Morphological, cultural and physiological characteristics were investigated using cells cultivated anaerobically in Reinforced Clostridial Medium (RCM). Illumina reads were generated to assemble the draft genome. The genomic relatedness of Cl_52 to other *Clostridium* species was calculated by the BLAST-based Average Nucleotide Identity (ANI) score and digital DNA-DNA hybridization (dDDH), and analysis of 16S rRNA gene sequence. Cells were Gram-stain positive, rod-shaped, strictly anaerobic, catalase and oxidase negative. The strain forms creamy white colonies on RCM agar plates. Growth was observed at 25-37 °C (optimum, 30 °C), at pH values 4.0-8.0 (optimum, pH 5.5-6.5) and at the optimum NaCl concentration of 0 % (w/v). Based on the 16S rRNA gene analysis, Cl_52 was identified as a member of the *Clostridium* genus, sharing 95.4-98.9% sequence identity to *Clostridium tyrobutyricum* DSM 2637T as closest species. Draft genome yielded 3.08 Mbp with GC content of 30.49 mol%. ANI and dDDH values between Cl_52 strain and other *C. tyrobutyricum* strains ranged from 94.68 to 95.26%, and from 60.2 to 62.5%, respectively, and were below and/or on the borderline of the species delineation. The work presented here provides an initial characterization of Cl_52, which likely constitutes a separate and distinct genome species within the genus *Clostridium*.

FP7/4 - The diversity of *Bradyrhizobium* strains associated with indigenous South African Genisteae

Presenting Author - *Mabodiba Maake, University of Pretoria, South Africa*

Author/s - *Mabodiba Maake, Juanita Avontuur, Chrizelle Beukes, Tomasz Stępkowski, Emma Steenkamp, Stephanus Venter, Esther Muema*

Abstract Content - South Africa is the centre of origin for the legume tribe Genisteae as well as the centre of diversity for many of its species. Not much is known about the diversity of rhizobial symbionts of Genisteae indigenous to South Africa. Therefore, this study aimed to investigate the rhizobial diversity associated with indigenous Genisteae from South Africa. Eighteen *Bradyrhizobium* strains from *Argyrolobium* spp. were identified to the species level using phylogenetic analyses of the DNA sequences for five core genes (glnII, recA, dnaK, rpoB and gyrB). The resulting phylogenies separated the strains into five groups, three of which contained the type strains of *B. arachidis*, "*B. brasilense*", and *B. ivorense*. The other two groups appeared to be novel as they did not group with any known *Bradyrhizobium* species. Their uniqueness and novelty were supported by genomic and various phenotypic data. We accordingly propose the names *B. harveyanumense* sp. nov. (Arg816T) and B. robustumense sp. nov. (Arg237LT). However, only the strains of *B. harveyanumense* could nodulate the promiscuous host's cowpea and siratro. Furthermore, analysis of the common nodulation gene nodA, showed that all the *B. harveyanumense* strains and some of the *B. robustumense* strains formed part of nodA Clade XV, which is known to be unique to South African *bradyrhizobia*. The remaining *B. robustumense* strains formed part of Clade III, which is known to have a cosmopolitan, pantropical distribution. Taken together, our findings thus show that there are novel South African Bradyrhizobium lineages capable of interacting with Genisteae indigenous to South Africa.

FP7/5 - Towards understanding the role of microbial dark matter in Egypt's Solar Lake, Taba

Presenting Author - *Rehab Abdallah, The American University in Cairo, Egypt*

Author/s - *Ali HA Elbehery, Shimaa Farag, Amged Ouf, Mohamed Malash, Werner Liesack, Rania Siam*

Abstract Content

Modern-day extremophilic microbial mates are a window into the microbiology of early earth as they are considered metabolically analogous to those consortia that created Precambrian stromatolites. Microbial dark matter (MDM) is hypothesized to play a major role in the biogeochemical element cycling in extreme environments. Here we provide evidence for the contribution of MDM to the carbon, nitrogen, and sulfur cycling in Egypt's hypersaline heliothermal Solar-Lake.

Triplicate samples were collected from Solar-Lake sediments. DNA isolation followed by shotgun sequencing was performed using NovaSeq-6000. Functional annotation of both contigs and binned metagenome-assembled genomes (MAGs) was carried out by DRAM.

A major portion of archaeal (40%) and bacterial (17%) MAGs represented MDM. Lokiarchaeota and Hemidallarchaeota MAGs encoded the capacity for a mixotrophic lifestyle. The potential for methylated-amine-based methanogenesis and thiosulfate oxidation was detected in MAGs assigned to unclassified Bathyarchaeota and Methanofastidiosales, respectively, with the latter showing capacity for dinitrogen fixation. Among bacteria, MAGs affiliated with Zixibacteria and "RBG-13-66-14" showed the potential for carbon fixation and dissimilatory sulfate reduction. Marinisomatota MAGs exhibited the potential to degrade amorphous-cellulose, arabinan, mixed-linkage-glucans, xyloglucans and sulfated polysaccharides. Furthermore, MAGs of the KSB1 lineage possessed key genes for complex carbohydrate degradation, denitrification, and nitrogen fixation. Two novel Gemmatimonadetes and Myxococcota MAGs encoded photosystem-II, thereby suggesting that the bacteria represented by these MAGs participate in phototrophy along with Cyanobacteria. Collectively, our data show that MDM plays a major role in carbon fixation and degradation, methanogenesis, dissimilatory sulfate reduction, thiosulfate oxidation, denitrification, and nitrogen fixation in the Solar-Lake sediments.

FP7/6 - Prokaryotic and eukaryotic microbial diversity from three soda lakes in the East African Rift Valley determined by amplicon seq.

Presenting Author - *Oliyad Jeilu Oumer, Addis Ababa University, Ethiopia*

Author/s - *Erik Alexandersson, Eva Johansson, Addis Simachew, Amare Gessesse*

Abstract Content

Prokaryotic and eukaryotic microbial diversity in samples of the three soda lakes, Lake Abijata, Lake Chitu and Lake Shala, in the East African Rift Valley were determined using amplicon sequencing. Culture-independent analysis showed higher diversity of prokaryotic and eukaryotic microbial communities in all three soda lakes than previously reported. A total of 3,603 prokaryotic and 898 eukaryotic operational taxonomic units (OTUs) were found through culture-independent amplicon sequencing, whereas only 134 bacterial OTUs, which correspond to 3%, were obtained by enrichment cultures. This shows that only a fraction of the microorganisms from these habitats can be cultured under laboratory conditions. Of the three soda lakes, samples from Lake Chitu showed the highest prokaryotic diversity, while samples from Lake Shala showed the lowest diversity. Pseudomonadota (*Halomonas*), Bacillota (*Bacillus*, *Clostridia*), Bacteroidota (*Bacteroides*), Euryarchaeota (*Thermoplasmata*, *Thermococci*, *Methanomicrobia*, *Halobacter*), and Nanoarchaeota (*Woesearchaeia*) were the most common prokaryotic microbes in the three soda lakes. A high diversity of eukaryotic organisms were identified, primarily represented by Ascomycota and Basidiomycota. Compared to the other two lakes, a higher number of eukaryotic OTUs were found in Lake Abijata. The present study showed that these unique habitats harbour diverse microbial genetic resources with possible use in biotechnological applications, which should be further investigated by functional metagenomics.

FP7/7 - Surviving the skies: analysis of bioaerosol communities in Icelandic lava rocks

Presenting Author - *Aurélien Daussin, Matís, Iceland*

Abstract Content - Surface microorganisms are aerosolized into the atmosphere by wind and events such as volcano eruptions and dust storms. Before depositing, they experience stressful atmospheric conditions which preclude the successful dispersal of a large fraction of cells. Bacterial diversity and succession in different low-bacterial environmental surfaces are reasonably well characterized but have rarely been studied along with airborne atmospheric communities. In this study, we assessed and compared the bioaerosols communities of two Icelandic sites, the island Surtsey and the highland Fimmvörðuháls, by analysing in situ lithospheric microbial communities and after one year of settlement time. Additionally, we investigated the role of the atmosphere as a significant source for the microbial communities in soil and their strategies potentially employed to withstand atmospheric stresses. A combination of culture-dependent and culture-independent methods was used to describe and compare the microbiome communities. The source of the isolates was predictive by using a combination of literature review and bioinformatic analysis whereas the survival of airborne candidates was tested against simulated atmospheric stress factors. Most of the isolates were from both air and rocks samples and belonged to Proteobacteria, Actinobacteria and *Bacteroides*. These microbes derived from local sources, with a predominance of the seawater-related genus Pseudoalteromonas on the island. The airborne microbes that survived atmospheric stresses such as freeze-thaw and osmotic shocks play a significant role in shaping the soil communities. This study provides a better understanding of global microbial biogeographic patterns and improves our understanding of the role of bioaerosols in the subarctic environment.

FP7/8 - Mantel derived CO2 drives microbial life in the rare Eger Rift subsurface ecosystem

Presenting Author - *Daniel Lipus, GFZ German Research Centre for Geosciences, Germany*

Author/s - *Daniel Lipus, Zeyu Jia, Alexander Bartholomäus, Robert Bussert, Jens Kallmeyer*

Abstract Content - Frequent seismic activity and consistently high CO2 fluxes make the Eger Rift in Western Bohemia (CZ) a rare subsurface ecosystem and scientifically relevant location to study microbial behavior and assess how geologically derived compounds are utilized at depth. Explorations into microbial life in this unique ecosystem provide the opportunity to investigate how high CO2 levels and the associated mineralogy influence microbial community composition and metabolic activity. Furthermore, seismic activity in this region can cause abiogenic production of H2, potentially providing the basis for primary production through methanogenic archaea.

To gain insight into microbial processes associated with the high CO2 Eger Rift subsurface we investigated diversity and metabolic attributes of bacterial and archaeal communities in samples from drill cores and 100m deep groundwaters. We also assessed the ionic composition of the collected samples to gain insights on the geochemical conditions in this subsurface system.

Genomic analysis of core and water samples, covering depths between 17m and 230m, provided novel insights into a CO2-adapted microbial community. We detected strong Cyanobacteria and Proteobacteria signatures as well as unexpected archaeal diversity in sediments, and high abundances of acidophiles and sulfate reducers in water samples. Enrichment cultures from the recovered rock samples suggested active biological utilization of CO2 and H2, while reconstruction and annotation of MAGs provided insights into microbial processes driven by CO2.

Going forward our data will be used to further investigate cellular processes under high CO2 conditions and identify pathways and biomolecules which may be of industrial and biotechnological relevance.

FP8/1 - Learnings from FEMS Summer School for Microbiology Education: strategy, innovation, application and impact

Presenting Author - *Jonathan Tyrrell, Swansea University, United Kingdom*

Author/s - *Dr Jon Tyrrell*

Abstract Content

Background: As a Microbiology teaching academic, I am passionate about innovating how we communicate both in the context of public engagement and higher education. As such, in the summer of 2022 I was successful in my application to attend the FEMS Summer School for Microbiology Education (FSSME).

Objectives: This week-long congress aimed to develop skills of higher education teachers, whilst engender an environment to promote ideas around novel teaching strategies, and provide the opportunity for networking between teaching academics.

Method: The 2022 FSSME cohort were canvassed for feedback through questionnaires/conversation. Additionally, I applied many of the learnings from the Summer School in the development of a new module 'PMCM02 Clinical Microbiology & Infectious Disease', as part of the MSc Biomedical Sciences programme on which I teach, with evaluation collected from the cohort of 82 MSc students to provide a real life example of FSSME impact.

Results: It is clear that the FSSME was successful in it's aims- 100% of the 2022 cohort felt they were better, more enabled educators following their participation. All agreed there was a perfect balance between content, workshops and open discussion to promote brainstorming and collaboration. Applied to the context of PMCM02, feedback was overwhelmingly positive, with the course structure and interactive elements introduced as a result of FSSME ranking highly among the students preferred elements. Further thought is needed on how to maximise the collaborative community created by the FSSME.

FP8/2 - Playful Learning in Higher Education: MicroBEscape Room - Escape from the microbes!

Presenting Author - *Isabel Murillo, University of Bristol, United Kingdom*

Author/s - *Lydia Mason, Emma Stevenson, Laura Wright*

Abstract Content

Background: Games as pedagogical tools are becoming increasingly popular in higher education. New learning environments were created during the pandemic, and now is the perfect moment to use games such as escape rooms to produce innovative ways of delivering teaching.

Objectives: We have created an escape room game to enhance undergraduate students' experience when studying microbiology. The game is played by groups of students who must work together as a team to come up with answers that will unlock the solution to a microbiology challenge in a given time.

Methods and Results: The game has been designed around the specific microbiology curriculum and contains a series of props that create a suitable ambience. Clues and mysterious questions – amongst other stimuli - will guide the students through the game. The collaborative element of the activity enhances the group-building experience, and the theme of the game helps students revise the unit material. This game goes further than simply being the creation of a teacher. It has been created with the collaboration of first-year undergraduate students as co-creators, whose input has contributed ideas for clues and questions as well as the design of the game. This game is timeless and can be used year after year. A redesign can also be made if necessary to adapt the game to changes.

The game starts with an informative letter about an infectious bacterial strain, and in the game, many concepts explained in the unit are explored… Would you like to know more?

FP8/3 - The Bad Bugs Bookclub: an experiment in microbial literacy

Presenting Author - *Joanna Verran, Manchester Metropolitan University, United Kingdom*

Abstract Content

The Bad Bugs Bookclub was established in 2009. Its aim was to engage scientists and non-scientists in discussion about novels of fiction in which infectious disease forms part on the plot. Fourteen years and almost 100 books later (meeting reports and reading guides are posted on the bookclub website), the bookclub continues with its six meetings per year. How did the pandemic affect the bookclub's original aims?

The pandemic forced meetings online, but this resulted in increased membership (including international), enabled author participation and enhanced and widened discussion. Reflecting on achievements post-pandemic, it is apparent that learning has taken place for everyone: scientists have broadened their reading and engaged in rewarding and enlightening discussion, and non-scientists have been able to bring their knowledge, understanding and concerns to the meetings. The impact of the project has been significant to members, and the website provides a rich and accessible resource. The pandemic has enabled aims to be achieved and surpassed.

FP9/1 - Hypervirulent *Streptococcus pneumoniae* serotype 1 (ST217) displays high levels of shedding and transmission

Presenting Author - *Murielle Baltazar, University of Liverpool, United Kingdom*

Author/s - *Laura Catherine Jacques, Teerawit Audshasai, Marie Yang, Aras Kadioglu*

Abstract Content

Background: *Streptococcus pneumoniae* serotype 1 (ST217) is a major cause of invasive pneumococcal disease of high mortality. Despite exhibiting a low carriage prevalence within the population, ST217 has a high attack rate, which raises questions about the relationship between carriage and transmission of hypervirulent pneumococcal strains between individuals.

Objective: Using a novel model of transmission in adult mouse we studied the transmission dynamics of ST217 and serotype 2 strain D39 during both colonisation alone and co-infection with influenza A virus (IAV).

Methods: Donor "index" mice were intranasally infected with ST217, D39 or isogenic pneumolysin-deficient mutants and co-housed with recipient naïve "contact" mice. Three days later, all mice were infected with IAV. Pneumococcal transmission from index to contact mice was analysed by quantification of shedding and nasal colonisation. The host nasopharyngeal immune response and the role of the toxin pneumolysin in shedding and transmission were investigated.

Results: ST217 was shed in index mice at greater levels compared to D39. Upon viral co-infection, ST217 was shed and transmitted at a faster rate to contact mice and displayed higher transmission levels compared to D39. Interestingly, although the toxin pneumolysin did not play a role in shedding, upon pneumococcal acquisition, pneumolysin-dependant macrophage recruitment was observed in the nasopharynx of contact mice. In addition, ST217 expresses a thicker capsule compared to D39, a feature that may promote increased shedding and recolonisation following transmission, leading to a better transmissibility of serotype 1 (ST217) which could explain its success to disseminate within the population and cause outbreaks.

FP9/2 - Uncovering genetic markers of antimicrobial resistance in *Bacillus anthracis* using comparative genomic analysis

Presenting Author - *Tucker Maxson, United States Centers For Disease Control And Prevention, United States*

Author/s - *Will Overholt, Vasanta Chivukula, Victoria Caban-Figueroa, Thiphasone Kongphet-Tran, Blake Cherney, David Sue, Luz Medina, Lavanya Rishishwar, Andrew Conley, Julie Villan*

Abstract Content

Background: *Bacillus anthracis*, the causative agent of anthrax, is a highly infectious pathogen that is considered a potential bioweapon. Given the high mortality rate of inhalational anthrax, rapid administration of effective antimicrobial therapy is critical after exposure to spores. Sequencing can rapidly identify markers related to antimicrobial resistance (AMR) to inform treatment decisions. Expansive knowledge of genetic mutations associated with resistance is crucial to improving the accuracy of genetic-based AMR prediction; however, few DNA markers related to resistance to antimicrobials for B. anthracis have been reported.

Objectives: Identify and verify mutations associated with resistance to the second line antimicrobials, clindamycin and clarithromycin, in *B. anthracis* to catalog genetic features predictive of AMR.

Methods: Strains of avirulent (select agent-excluded) *B. anthracis* with elevated minimal inhibitory concentrations (MIC) against clindamycin and clarithromycin were isolated on media containing the respective antimicrobial. Mutations were identified through whole-genome sequencing (MinION, Illumina) and were evaluated in the context of broth microdilution results and comparative genomic analyses.

Results: Mutations involved in resistance to clindamycin were primarily found in genes encoding ribosomal proteins and rRNA. The accumulation of mutations tracked with increases in MIC and fit with the mechanism of action of clindamycin. Conversely, all strains generated with elevated clarithromycin MICs contained mutations in a single ribosomal protein gene, rplV. Mutations were not found in the drug-susceptible parent strain and were verified through Sanger and short-read whole-genome sequencing. The mutations could be identified within 1 hour through long-read sequencing and correlated to phenotypic resistance observed through broth microdilution.

FP9/3 - Analysis of microbial colonization during helmet molding therapy – a prospective study

Presenting Author - *Paul Jakob Schmid, Medical University Of Graz, Austria*

Author/s - *Jan Gaessler, Bernhard Remschmidt, Elisabeth Knaipp, Michael Schwaiger, Clemens Kittinger*

Abstract Content

Helmet molding therapy is a very effective treatment for positional plagiocephaly, a deformation of the skull in the first year of life. The helmet, which is worn 23 hours a day, brings the skull into a symmetrical shape. Adverse events can be pressure sores, erythema, skin erosions and infections [2], as well as malodor. Although skin erosions/infections, malodor and discoloration of the helmet are known, a contribution of the skin microbiota during helmet molding therapy has not been investigated yet. Therefore, this prospective study examined the microbial colonization of the helmet in infants undergoing helmet molding therapy. Samples were taken with a swab of the capillitium at the start of therapy, followed by swabs of the helmet at each check-up. The microorganisms were cultured on selective media and identified by MALDI-TOF. Pathogens were subsequently tested for antibiotic susceptibility. Additionally, molecular typing and phylogenetic classification was done for selected species. The helmet therapy was accompanied with an increased abundance of facultative pathogens on the inner helmet surface compared with the initial microbiota of the infants' skin. Most abundant pathogens were *Staphylococcus aureus*, *Bacillus cereus* and *Enterococcus faecalis*. Various antibiotic resistances were detected, however, no multidrug resistant strains, including MRSA or ESBL, were found. spa typing of *S. aureus* revealed a broad diversity of strains, but single cross-contamination events between patients were likely. Our results indicate that current hygiene measures during helmet molding therapy are not effective and the helmet can serve as a niche for facultative pathogens.

FP9/4 - Improved immune responses and tuberculosis protection by aerosol vaccination with rBCG expressing ESX-1 from *Mycobacterium marin*

Presenting Author - *Fadel SAYES, Institut Pasteur - Paris, France*

Author/s - *Fadel Sayes, Wafa Frigui, Alexandre Pawlik, Roland Brosch*

Abstract Content

Background: The current anti-tuberculosis (TB) vaccine, *Mycobacterium bovis* BCG, provides limited protection against pulmonary TB in adolescents and adults. To develop a virulence-neutral strain with enhanced immune signalling, we have previously integrated the extended esx-1 genomic region of *Mycobacterium marinum*, into BCG Pasteur. This recombinant strain named rBCG:ESX-1Mmar, is heterologously expressing ESX-1 functions of *M. marinum* and thereby modulates the host innate immune response via phagosomal rupture-associated induction of type I interferon (IFN) responses and enhanced inflammasome activity (1).

Objectives: Our objectives were to explore different vaccination routes for increasing the protection level by using rBCG:ESX-1Mmar in comparison to BCG.

Methods: We used different in vitro assays assays and murine models to characterize the *in vitro* and *in vivo* behavior of rBCG:ESX-1Mmar in comparison to a BCG wildtypestrain.

Results: We found that aerosol-vaccinated mice yielded higher frequencies of CD4+ and CD8+ T effector memory (TEM) cells in the lungs compared to subcutaneous immunized counterparts, while comparable poly-functional Th1 (IL-2, TNF-a and IFN-g) cytokine-producing subsets were observed in the spleen of the same vaccinated mice. Moreover, we detected significantly higher Th17 in the airways of aerosol-vaccinated mice compared to subcutaneous route without severe lung pathology despite local and transient inflammatory cytokine responses.

Finally, we show that vaccination of mice with BCG Pasteur or rBCG Pasteur:ESX-1Mmar via the aerosol route leads to significant improved TB protection and lower lung pathology compared to subcutaneous vaccination, as judged by mycobacterial loads and tissue score evaluations at one month post virulent *M. tuberculosis* infection.

FP9/5 - Investigating host factors underlying susceptibility to infection

Presenting Author - *Julia Sanchez-Garrido, Imperial College London, United Kingdom*

Author/s - *Yasaman Naemi Baghshomali, Jyoti S. Choudhary, Gad Frankel*

Abstract Content

Why do some people get severely ill when infected, whilst others do not? In order to attempt to address this question and mechanistically study host factors underlying disease severity we used *Citrobacter rodentium* (CR), a natural mouse-adapted pathogen that leads to different host-dependent infection outcomes. When infected with CR, C57Bl/6 mice develop a mild, self-limiting disease; conversely C3H/HeN mice succumb to infection and represent a severe model of colitis that resembles life-threatening human infection.

Given the early deterioration shown by C3H/HeN mice, we focussed in characterising innate immune responses in these hosts. Despite their inability to control the infection, we observed significantly higher neutrophil recruitment to the colon of C3H/HeN mice that coincided with tissue damage, heightened G-CSF levels and expansion of the neutrophil compartment in the bone marrow (BM). To investigate the intrinsic factors contributing to this response we performed proteomic profiling of BM and colonic neutrophils. While similar in homeostasis, BM neutrophils had distinct signatures in infected mice, where C3H/HeN neutrophils showed defects in terminal differentiation, including pathways such as chemotaxis. Colonic neutrophils from C3H/HeN mice also displayed differences, reflected in lower Ly6G expression, impaired migration to the infection site and increased pro-inflammatory cell death away from this site, thus contributing to the increased pathology and failure to control infection.

By providing insights into the regulation of neutrophil function and increased susceptibility to infection, we hope to pave the way to more targeted therapies that can reduce neutrophil-related tissue damage without impacting on their antimicrobial capabilities.

FP9/6 - Mucus-bacteria interactions: impact on Enterotoxigenic E*scherichia coli* virulence and interplay with the human gut microbiome

Presenting Author - *Lucie Etienne-Mesmin, Université Clermont, France*

Author/s - *Thomas Sauvaitre, Josefien Van Landuyt, Claude Durif, Charlène Roussel, Adeline Sivignon, Ophélie Uriot, Florence Van Herreweghen / Ghent University, Faculty of Bioscience Engineering, Center for Microbial Ecology and Technology (CMET) / Ghent / Belgium*

Tom Van de Wiele / Ghent University, Faculty of Bioscience Engineering, Center for Microbial Ecology and

Abstract Content

Background: The intestinal mucus layer has a dual role in human health constituting a well-known microbial niche that supports gut microbiota maintenance, but also acting as a physical barrier against enteric pathogens. Enterotoxigenic *Escherichia coli* (ETEC), the major agent responsible for traveler's diarrhea, is able to bind and degrade intestinal mucins, representing an important but understudied virulent trait of this pathogen.

Objectives: Our work aimed to describe how the mucus microenvironment could shape different aspects of the human ETEC pathophysiology.

Methods: Using a set of complementary *in vitro* approaches recapitulating the human digestive environment, we investigated the survival, adhesion, virulence gene expression, interleukin-8 induction and interactions with human gut microbiota of the human ETEC reference strain H10407.

Results: Using the TNO gastrointestinal model (TIM-1) simulating the physicochemical conditions of the human upper gastro-intestinal tract, we report that mucus secretion and physical surface sustained ETEC survival facing the upper gastrointestinal tract stresses. The integration of the host part through the Caco2/HT29-MTX co-culture model demonstrated that mucus secreting-cells favored ETEC adhesion and virulence gene expression, without impeding ETEC-induced inflammation. Furthermore, we show that the presence of a mucin-matrix tended to reduce ETEC colonization in a complex gut microbial background simulated by fecal batch experiments. Mucus-specific microbiota was also widely modified upon ETEC challenge suggesting a role in the pathogen infectious cycle. Using multi-targeted *in vitro* approaches, our work supports the major role played by mucus in ETEC pathophysiology, opening avenues in the design of new treatment strategies.

FP9/7 - Temporal changes in fecal microbiota of COVID-19 patients: a longitudinal cohort

Presenting Author - *Yangji CHOI, Lausanne University Hospital, Switzerland*

Author/s - *Yangji Choi, Tatiana Galperine, Jean-Luc Pagani, Claire Bertelli, Benoit Guery, Gilbert Greub, Antonios Kritikos (Institute of Microbiology, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland / Service of Infectious Diseases, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland)*

Matthaios Papadi

Abstract Content

SARS-CoV-2 infection, primarily characterized by respiratory manifestations, also causes gastrointestinal symptoms. Although triggering interest, the role of the gut microbiota along the gut-lung axis is still poorly understood in COVID-19. Thus, we aimed to study the impact of COVID-19 and its severity on the fecal microbiota in a longitudinal prospective cohort.

One hundred fecal samples were collected from 57 COVID-19 patients in ICU or internal medicine every 7 days, as well as from 19 non-COVID patients in ICU, among which 14 developed ventilator-associated pneumonia (Pneumonia group, antibiotics) and five remained without infection (Control group, non-antibiotics). The 16S rRNA amplicons (V3V4) sequenced on a MiSeq were processed by our in-house bioinformatic pipeline (https://github.com/metagenlab/zAmp). Statistical analyses were performed in R. SARS-CoV-2 viral loads in fecal samples were measured by qPCR.

Although similar at inclusion, Shannon alpha-diversity appeared significantly lower in COVID-19 and Pneumonia groups than in the Control group at day 7. Furthermore, the microbiota composition became distinct between COVID-19 and non-COVID-19 groups. The fecal microbiota of COVID-19 patients was characterized by increased *Bacteroides* and the Pneumonia group by *Prevotella*. In a distance-based redundancy analysis (db-RDA), only “COVID-19” and “patient” presented significant effects on the microbiota composition. Moreover, patients in ICU harbored increased *Campylobacter* and decreased short-chain fatty acids producing bacteria, such as Lachnospiraceae, *Roseburia* and *Faecalibacterium* as compared to patients in internal medicine. Both the stay in ICU and patient were significant factors affecting the microbiota composition. SARS-CoV-2 viral loads were higher in ICU than in non-ICU patients.

FP9/8 - Rescuer or enemy: the role of metformin in the antibiotic crisis

Presenting Author - *Lina Maarouf, University of East Anglia, United Kingdom*

Author/s - *Mark Webber, Benjamin Evans*

Abstract Content

Background: Metformin is a highly used antidiabetic drug prescribed to more than 120 million patients globally annually. However, it has shown some antibacterial activity against different bacterial genera. And it is known that the overuse of antibacterial agents can contribute to the emergence of antibiotic resistance.

Objectives: This study aimed to investigate the effect of the continuous exposure of different bacterial species to metformin on the emergence of less susceptible mutants to metformin and different antibiotics.

Methods: Six parallel replicates of *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* NCTC 6571 were exposed to metformin (1.25 mg/mL) for 18 passages. After exposure, the inhibitory activity of metformin was investigated on the evolved strains and controls using the broth microdilution method, and by determining growth curves in the presence of different concentrations of metformin (10, 5, 2.5, 1,25 and 0 mg/mL). Biofilm formation and protease production in different concentrations of metformin were also determined. All strains were whole genome sequenced using Illumina technology for detecting different mutations.

Results: The continuous exposure of bacteria to therapeutic concentrations of metformin did not select for any change in the MIC of metformin (10 and 40 mg/mL for *S. aureus* and *P. aeruginosa* respectively). However, there was a significant decrease in the ability of metformin to inhibit the growth of 2 *S. aureus* mutants compared to its inhibitory activity on the ancestor. Also, the biofilm and protease inhibition by metformin was reduced in 4 mutants of *P. aeruginosa* at the concentration of 10 mg/mL.

FP9/9 - Activity of human tear Lipocalin (LCN-1), produced in a plant-based system, against hypervirulent *Klebsiella pneumoniae* strains

Presenting Author - *Annarita Mazzariol, University Of Verona, Italy*

Author/s - *Denise Pivotto, Mattia Santoni, Anna Bertoncelli, Linda Avesani*

Abstract Content

Background: The spread of hypervirulent *K. pneumoniae* (hvKp) strains has become an urgent threat, due to their ability to cause infections in the community and the increasing failure of antimicrobial treatment. This study aimed to evaluate the activity of Human tear Lipocalin (TL), able of binding bacterial siderophores, in combination with antibiotics towards hvKp strains.

Material and methods: Production of recombinant TL was achieved in Nicotiana Benthamiana thanks to the magnICON™ technology. TL purification was carried out with chromatographic analyses.

Siderophore virulence factors, namely iroB, iucA, rmpA1, rmpA2 and peg-344 genes, in selected hvKp strains were confirmed through PCR.

Synergistic effect of TL with antibiotics was evaluated by growth curves of hvKp strains performed at the same time in different conditions: the strain alone, in presence of 4 µg/ml of TL, in presence of antibiotics (meropenem or ceftazidime) at concentration ranging from twice MIC to ¼ of MIC, with or without TL.

Results: TL does not affect the bacteria growth and we perform activity evaluation on presence of antibiotics using a TL concentration of 4 µg/ml.

When added twice of MIC value both, meropenem and ceftazidime, showed their activity on strong decresing of the growth curves. No additional effect was observed adding 4 µg/ml of TL.

Growth curve analysis when using lower antibiotics concentrations in particular ¼ MIC, showed that both, meropenen and ceftazidime, combined with TL register a decrease of almost 50% in comparison to the antibiotic alone, after 4 hours.

FP9/10 - The effect of chlorhexidine on the oral microbiota and the prevalence of antibiotic resistance genes

Presenting Author - *Sibylle Bartsch, University of Freiburg, Germany*

Author/s - *Eva Kohnert, Clemens Kreutz, Johan Peter Wölber, Ann-Sophie Burkhardt, Annette Anderson, Wolfgang Buchalla, Karl-Anton Hiller, Elmar Hellwig*

Abstract Content

Background and Objectives: Chlorhexidine (CHX) is an antiseptic and widely used in dentistry. It has been shown that biocides such as quaternary ammonium compounds can lead to cross-resistances to antibiotics, while CHX has only recently been considered in this regard [1]. The aim of the present study was to investigate the effects of intensive CHX application on the oral microbiota and the prevalence of antimicrobial resistance genes (ARGs).

Methods: Saliva and supragingival plaque samples were collected from patients who used CHX mouth rinses for four weeks following periodontal surgery. Three time points were studied: before and immediately after four weeks of CHX application and four weeks after discontinuation of CHX. DNA was extracted and a shotgun metagenome analysis was performed.

Results: Microbial diversity was reduced and oral streptococci dominated the microbiota directly after CHX application. Four weeks after discontinuation, the salivary microbiota recovered completely.

The prevalence of two ARGs, tetB(60) in the oral biofilm and tet(B) in saliva, tended to increase sharply during CHX use. Both genes encode efflux pumps and enable resistance to tetracycline. Additionally, tetB(60) confers resistance to the last resort antibiotic tigecycline. The tet(B) gene, which originates from Gram-negative species, was found in *Streptococcus suis* isolated from pigs in 2011 and *Streptococcus oralis* from the oral cavity in 2019, suggesting horizontal gene transfer [2, 3]. Correlations of these genes with CHX resistances and isolated streptococci from this work are the subject of further studies.

FP10/1 - Upgrading *Pseudomonas putida* by systems metabolic engineering for lignocellulose biotechnology

Presenting Author - *Pavel Dvorak, Masaryk University, Czech Republic*

Author/s - *Barbora Burýšková, Barbora Popelářová, Dalimil Bujdoš, Martin Benešík*

Abstract Content

Background: *Pseudomonas putida* KT2440 has drawn attention as a next-generation bacterial chassis for lignocellulose biotechnology. The bacterium became a host of choice for the valorization of lignin and was repeatedly employed to produce biochemicals from glucose. However, the lack of certain metabolic traits hindered its use for the utilization of (hemi)cellulosic xylose and oligosaccharides.

Objectives: This study presents our collaborative effort to empower *P. putida* with novel functions enabling co-utilization and valorization of lignocellulosic sugars.

Methods: Tools and approaches of systems metabolic engineering including multi-omics analyses, metabolic modelling, genome editing, or adaptive laboratory evolution (ALE) were adopted in this work.

Results: We initially introduced the upper xylose pathway from *Escherichia coli* and beta-glucosidase from *Thermobifida fusca* in *P. putida*. The strain grew on xylose and cellobiose and was able to co-utilize these sugars with glucose. The metabolism of xylose was then mapped by flux analyses and the growth was accelerated using knowledge-driven cuts and ALE. Omics analyses of obtained mutants highlighted the plasticity of *P. putida´*s metabolism. Growth of *P. putida* on glucose and cellobiose was improved by implanting sugar transporters from *Zymomonas mobilis* and *E. coli*. This intervention resulted in the accumulation of pyruvate or derived bioproducts. Secretion of the key metabolic intermediate was explained by an upgraded metabolic model, which pointed to the unregulated substrate uptake as a novel strategy for pyruvate overproduction in aerobic bacterial cultures.

The study provides a showcase of expanding the catalytic scope of a non-traditional bacterial host toward biotechnological applications.

FP10/2 - Anti-inflammatory effects of extracellular vesicles from rpsL K56N mutant of *Lacticaseibacillus rhamnosus* GG

Presenting Author - *Soyolmaa Jamiyanpurev, Shinshu University, Japan*

Author/s - *Fu Namai, Suguru Shigemori, Takeshi Shimosato*

Abstract Content

Background: Recently, probiotic lactic acid bacteria have been reported to offer numerous functional properties, but the effects vary greatly among individuals. We have previously applied ribosome engineering to *Lacticaseibacillus rhamnosus* GG to enhance the probiotic functions.

Objectives: One of the resulting mutants, MTK56N, suppressed expression of Tnfa compared to the wild-type strain (WT), suggesting an anti-inflammatory effect. This study focused on extracellular vesicles (EV) to identify and elucidate the mechanisms of anti-inflammatory response to MTK56N.

Methods: We examined the anti-inflammatory effects of supernatants from WT and MTK56N by incubating with them in the presence of RAW264.7 cells. Cytokine secretion was analyzed using ELISA. EV fractions were also collected, observed under TEM, and measured by TRPS (a single-particle measurement technique designed for measuring nanoparticles). We then analyzed the proteins from acquired EV, subjected samples from each strain to SDS-PAGE, and used the observed bands for proteomic analysis.

Result: ELISA indicated that the supernatant of MTK56N significantly enhanced TNF-α secretion in supernatant by RAW264.7 cells compared to WT. TRPS also showed that in MTK56N, the size of EV and the number of particles within UV were decreased compared to WT. SDS-PAGE revealed a band at approximately 31 kDa, later identified as glyceraldehyde-3-phosphate dehydrogenase in EV from MTK56N. These results suggest that ribosome engineering may alter EV size, secretion volume, and protein content. We plan to conduct further analyses of the anti-inflammatory effects of EV from MTK56N.

FP10/3 - Untapped biosynthetic potential of Antarctic soil bacteria

Presenting Author - *Stanislava Kralova, University of Vienna, Austria*

Author/s - *Mathias Flieder, Songcan Chen, Peter Spacek, Matej Bezdicek, Kristyna Dufkova, Martin Zehl, Sergey Zotchev, Thomas Rattei*

Abstract Content

Background: The growing problem of antimicrobial resistance motivated scientists to re-explore natural sources of bioactive secondary metabolites. Antarctica represents an extreme environment colonized by bacteria with unique adaptation mechanisms allowing them to thrive under harsh conditions. Such adaptations include production of secondary metabolites to inhibit competitors or sustain abiotic stresses, which predestines these microbes as source of natural products for biomedical use.

Objectives: The aim of this work was to recover novel bacterial taxa from Antarctic soils to access the biosynthetic potential hidden in yet uncultivated bacteria. The main objective is the activation of silent biosynthetic gene clusters enabling discovery of novel secondary metabolites, mainly through co-cultivation strategies.

Methods: Three isolation methods (pre-selection of spore-forming bacteria, low-nutrient and soil-extract based media) were applied to recover novel bacteria from Antarctic soils, predominantly targeting phyla with high biosynthetic potential such as Actinobacteriota, Proteobacteria and Firmicutes. Activation of silent biosynthetic gene clusters was attempted through targeted cultivation and co-cultivation. Screening for bioactive molecules and evaluation of their novelty was achieved by application of genomics, metabolomics, and bioactivity testing.

Results: A collection of 917 isolates was established. Recovered isolates were associated with four bacterial phyla including 77 isolates of novel species. Proteobacteria and Actinobacteriota represented the most abundant phyla. Specific media stimulated biosynthesis of several unknown natural products. Eight strains produced antimicrobial compounds against resistant and multidrug-resistant bacterial and fungal pathogens. Importantly, metabolomic profiling indicated that these strains produced several new secondary metabolites, which may be responsible for the observed antimicrobial activities.

FP10/4 - Marine bioprocess development: targeting the production of prodigiosin

Presenting Author - *Ricardo FS Pereira, Instituto Superior Tecnico, Portugal*

Author/s - *Carla CCR de Carvalho*

Abstract Content

The adaptation of marine microorganisms to continuously changing environmental conditions makes them promising sources for e.g. interesting compounds and enzymes. This is especially important for the health sector. The success in the development of marine bioprocesses depends on finding the conditions for bacterial growth and production in the laboratory. The objective of the study was to use high-throughput approaches to enable the determination of the best growth/reaction conditions for a marine *Serratia rubidaea* strain, and the development of the bioprocess to obtain high yields of prodigiosin and its purification within a week. To study the bacterium biochemical needs for high product production, medium engineering was used through the testing of different carbon and nitrogen sources, salt and metallic ions concentrations, and oxygen availability, at mL scale. The effect of environmental conditions was also studied, namely by varying temperature between 15 and 60 °C, pH between 5.5 and 9.0, and by changing light conditions. Using the mass transfer coefficient (kLa) as the scale-up criterion, the bioprocess was carried out in 2L bioreactors where several strategies were tested to enhance prodigiosin production. The obtained prodigiosion was extracted from both cells and media, using dichloromethane, and purified by the use of solid phase extraction. The purified sample was analysed by GC-MS to access its purity. This strategy resulted in the production in 24 h of ca. 980 mg/L of prodigiosin. This corresponds to a productivity of 40.8 mg/(L.h) which is, to our knowledge, higher than the best published results for *S. rubidaea*.

FP10/6 - Building computers and AI with synthetic-genetic engineered bacteria that can compute, solve mazes and add and subtract numbers

Presenting Author – *Sangram Bagh, Saha Institute of Nuclear Physics, HBNI, India*

Author/s - *Kathakali Sarkar, Deepro Bonnerjee, Rajkamal Srivastava*

Abstract Content

Background: The implementation of synthetic genetic logic circuits in living cells paved the way for human-designed computations to be performed by genetically engineered bacteria. Such cellular computations have enormous importance in biocomputer technology development at the micron scale, where microprocessor-based computers have limitations due to energy, cost and technological constraints.

Objective: Here, we designed and built artificial neural networks and biocomputers with synthetic-genetic engineered *E.coli*.

Methods: In this work, we have adapted the basic concept of artificial neural networks (ANNs) and designed, built and optimized synthetic genetic circuits in *E.coli*, where engineered bacteria worked as artificial 'neuro-synapses'. The engineered bacteria in a culture work as an artificial neural network.

Results: Experimentally demonstrated a broadly applicable single-layer ANN type architecture with molecular-engineered *E.coli* to perform complex irreversible computing like multiplexing, de-multiplexing, encoding, decoding, majority functions, and reversible computing like Feynman, double Feynman and Fredkin gates. Further, we expanded the capability of bacterial ANN and built bacterial computational devices, which can add and subtract binary numbers. Adapting the idea of distributed computing and applying it to synthetic microbiology, we build a biocomputer, that can solve simple maze problems. To our knowledge, this is the first artificial neural networks (ANNs) with synthetic-genetically engineered cells. This work represents a new approach to designing and building complex cellular computation and may have significance in establishing a new platform for cellular computing and in transforming bacterial cells into ANN-enabled hardware.

FP10/7 - Living therapeutic design for pernicious anemia by engineering probiotic yeast with synthetic biology approaches

Presenting Author - *Burcu Gündüz Ergün, Republic Of Turkey Ministry Of Agriculture And Forestry, Turkey*

Author/s - *Albayrak Şeker, Damla Albayrak*

Abstract Content - Pernicious anemia (PA) is a disease caused by vitamin B12 deficiency and results in symptoms such as weakness, pallor, fatigue, dyspnea, diarrhea, pale skin, psychosis. The disease is either autoimmune, where autoantibodies produced against the Intrinsic Factor (IF) inhibit its ability to absorb B12, or due to mutations in the IF gene. The current treatment involves taking pills or injections.

Living biotherapeutics are a new class of probiotics designed with synthetic biology tools for promoting health and well-being. *Saccharomyces boulardii*, the most widely used probiotic yeast for human and animal health, has anti-bacterial and anti-inflammatory effects, neutralizes toxins, and prevents acute diarrhea caused by antibiotics and infections. *S. boulardii* has qualified presumption of safety status and is a promising host for producing therapeutic and prophylactic protein-based biomolecules due to its eukaryotic post-translational modification capabilities.

This study aims to create a living therapeutic for pernicious anemia using *S. boulardii*. To avoid using antibiotic resistance markers, we used CRISPR/Cas9 to delete LEU2, TRP1, and URA3 genes, resulting in auxotrophic *S. boulardii* strains. IF expression cassette was constructed under the transcriptional control of the constitutive TEF1 promoter and cyc1 terminator, using the α-factor signal sequence for secretion. IF expression cassette was constructed with an auxotrophy replacement cassette as a bidirectional vector and integrated into the *S. boulardii* genome to generate a stable strain. The goal of the project is to combat IF-related B12 deficiency by using an engineered *S. boulardii* as a living biotherapeutic to secrete human IF in the intestine.

FP10/8 - Novel thermophilic carboxydotrophic microbes for syngas fermentation

Presenting Author - *Anastasia Galani, Wageningen University & Research, Netherlands*

Author/s - *Melissaviyanee Antony Venancius, Ben Tumulero, Detmer Sipkema, Diana Z Sousa*

Abstract Content

Carbon monoxide (CO) is a possible carbon and energy source for anaerobic microbes in hydrothermal environments. Thermophilic carboxydotrophs (i.e. microorganisms that can use CO) are promising bio-catalysts for syngas fermentation, which can be used for the conversion of waste-to-chemicals. To date, only a few thermophilic carboxydotrophs have been isolated from hydrothermal areas. Most of these environments remain unexplored for carboxydotrophic metabolism. In the present study, we investigated CO metabolism in several samples from hydrothermal sites at the island of São Miguel (Azores, Portugal). We obtained 19 thermophilic enrichment cultures that consistently consume CO and produce e.g. hydrogen (H2), methane and acetate. From these enrichments, several novel isolates were obtained that can use CO as sole energy and carbon source. Furthermore, the isolates can respire CO in the presence of several electron acceptors, such as thiosulfate. The results from this study contribute to fundamental understanding of the metabolic potential of the microbes inhabiting hydrothermal environments. Moreover, the obtained isolates expand the list of known thermophilic CO-utilizing microorganisms and can further be studied for their potential application for syngas fermentation.

FP10/9 - Rapid screening of antimicrobial compounds targeting transcription through an in vitro molecular beacon-based assay

Presenting Author - *Thuy Duong Pham, University Of Camerino, Italy*

Author/s - *Thuy Duong Pham, Lucia Cimarelli, Attilio Fabbretti, Roberto Spurio, Anna Maria Giuliodori*

Abstract Content

An extensive screening has been carried out in our laboratory, looking for bioactive metabolites produced by environmental microorganisms of our Culture Collection of Microorganisms (CCM). To this aim, we have developed an assay using a molecular beacon to rapidly track bacterial transcription in the presence of newly isolated secondary metabolites. The system relies on the fluorescence generated from the specific hybridization between the molecular beacon and an RNA target synthesized in vitro by the *Escherichia coli* RNA polymerase. The absence of fluorescent signals depicts the inhibition of RNA synthesis, possibly because of the RNA polymerase stalling. The antibacterial metabolites extracted from a subset of selected environmental microorganisms were assayed in vitro for their ability to inhibit transcription. As result of this screening procedure, two metabolite extracts, obtained from bacteria labeled as MES784 and MES1160, were found to display significant ability to block transcription. This result was confirmed by denaturing urea polyacrylamide gel electrophoresis, which demonstrated the absence of the gel band corresponding to the transcribed RNA. Following High-performance liquid chromatography purification, fractions from MES784 retained their activity against the transcription reaction and produced a significant growth inhibition of multi- and extreme-resistant clinical strains of *Staphylococcus aureus*. The producer microorganism, whose draft genome assembly and annotation was generated based on Illumina sequencing, is a *Streptomyces* strain close to *Streptomyces vinaceus*, predicted to contain a large set of biosynthetic gene clusters. These findings demonstrate the efficiency of the assay in the fast identification of potential new antibiotic molecules targeting bacterial transcription.

FP10/10 - Leaderless bacteriocin producing phage against enterohemorrhagic *Escherichia coli*

Presenting Author - *Yoshimitsu Masuda, Kyushu University, Japan*

Author/s - *Tatsuya Uedoi, Tahir Noor Mohammadi, Ryota Daimon, Ken-ichi Honjoh, Takahisa Miyamoto*

Abstract Content

Background: Lytic bacteriophages are effective tools for controlling bacterial infections in humans and foods. Leaderless bacteriocins (LLBs) are simple bacteriocins produced by Gram-positive bacteria, which do not possess an N-terminal leader peptide in the precursor, implying that they are active immediately after translation.

Objectives: This study aimed to construct novel LLB-producing phages (LLB-phages) against enterohemorrhagic Escherichia coli (EHEC) by simply introducing LLB structure genes into phage genome.

Methods: Using CRISPR-Cas9 editing system, the structural gene (lnqQ) of lacticin Q (LnqQ) was introduced into the genome of the EHEC lytic phage ECP52. To enhance LLB expression during the phage lytic cycle, additional LLB gene (aucA) and promoters were introduced, resulted in five different types of LLB-ECP52s. The properties of the LLB-ECP52s, such as LLB productivity, propagation activity, and lytic activity, were evaluated.

Results: The constructed LLB-ECP52s, Phol-LA and Pend-LA, in which both lnqQ and aucA were introduced under the promoters of holin or endolysin genes of ECP52, formed clear haloes in agar plates containing both E. coli and B. coagulans, indicating significant production of LLBs. Compared with parental ECP52, the infection and propagation activities of all LLB-ECP52s were not disadvantaged by LLB expression. In fact, their burst sizes were significantly larger than that of ECP52. Finally, the lytic activities of LLB-ECP52s against EHEC host strains were significantly enhanced. These results indicate the possibility of freely designing the strength or timing of LLB productions from LLB-phages in future.

FP10/11 - Unusual terpenoids via bacterial methyltransferases – Investigation of enzymes and products and potential applications

Presenting Author - *Markus Buchhaupt, Dechema-forschungsinstitut, Germany*

Author/s - *Laura Drummond, Max Kschowak, Hendrik Schewe, Svenja Sommer, Holger Zorn, Jeroen Dickschat*

Abstract Content

The natural substance class of terpenoids includes many important pharma compounds, aroma molecules and agrochemicals. Several different microbial terpenoid-producing platforms have been developed and are applied in industrial processes. Although terpenoids show an extremely wide range of different structures, their building block repertoire is limited to the C5 compounds DMAPP and IPP and their condensation products in most organisms. However, several bacteria developed a structure expansion strategy to increase the precursor diversity by the introduction of additional methyl groups. Methyltransferases acting on IPP, DMAPP, GPP (C10) or FPP (C15) have been identified in respective bacterial secondary metabolism pathways, which finally lead to unusual terpenoids with additional methyl groups.

The objectives of our work are the investigation of different aspects of the metabolic pathways towards the unusual terpenoids and the promotion of biotechnological application. Within this presentation, we will give an overview about the field and show some highlights from our recent research. We will demonstrate the identification and characterization of suitable methyltransferases and show examples for their successful implementation in production pathways. Analytical strategies to specifically detect the unusual terpenoids and to investigate mechanisms will be shown. Finally, examples for the potential applicability of novel terpenoid compounds in the aroma field will be presented.

FP10/12 - Engineering *Rhodotorula toruloides* for improved lipid production

Presenting Author - *Zongbao Zhao, Dalian Institute of Chemical Physics, China*

Abstract Content

The basidiomycetous yeast *Rhodotorula toruloides*, one of so-called red yeasts, has been recognized as a powerful host for the production of lipids, carotenoids and related biomolecules, because it can use diverse compounds including some phenolic ones derived from lignocellulosic biomass. Over the past 10 years or so, we and others have been streamlining this yeast to be a more user-friendly host. We first completed high-quality genome sequencing of *R. toruloides* NP11 and annotated over 8100 protein-coding genes, suggesting the uniqueness of this yeast over many other ascomycetous ones. Further multi-omic analysis of cells cultured under diverse growth conditions provided rich information regarding the molecular bases of lipid accumulation and cellular responses to nutrient limitation. To develop genetic tools, we identified promoters and terminators, established methods for DNA transformation, RNA interference, homologous gene targeting, and efficient co-expression of multiple genes. More importantly, the CRISPR-Cas system has been successfully adopted to promote gene knockout and homologous recombination. Thus, various platform strains of *R. toruloides* have been generated. Specifically, we have been devising *R. toruloides* strains for better phenotypic features of industrial concerns and improved production of valuable lipids such as diacylglycerols and plant terpenoids. In this presentation, research progresses will be summarized and future directions be discussed.

FP10/13 - Pangenome-Scale mathematical modelling of ANAMMOX bacteria metabolism

Presenting Author - *Roman Bielski, Loughborough University, United Kingdom*

Author/s - *Roman G. Bielski, M. Ahsanul Islam*

Abstract Content

Removal of fixed nitrogen compounds such as ammonium and nitrite from wastewater is of critical importance for balancing the nitrogen cycle and protecting aquatic environments from eutrophication. Anaerobic AMMonium Oxidising (ANAMMOX) bacteria have recently been employed for fixed nitrogen removal purposes in wastewater treatment processes. These specialised bacteria convert ammonium and nitrite into nitrogen gas anaerobically, which significantly reduces the amount of energy required for aeration in the conventional aerobic wastewater treatment processes. However, slow growth rates of ANAMMOX remain a major obstacle towards their widespread use in industrial wastewater treatment processes. Thus, a pangenome-scale, constraint-based mathematical model of ANAMMOX bacteria metabolism has been developed to accelerate their growth on a range of substrates by identifying metabolic bottlenecks. The main metabolic limitation was identified in the energy metabolism of these bacteria concerning the production of ATP. The extremely low efficiency of the electron transport chain combined with very high growth-associated maintenance energy is mainly responsible for the slow growth of ANAMMOX. Moreover, different ANAMMOX species were found to conserve energy using a variety of different redox couples, and the modelling simulations revealed their comparative advantages under different growth conditions. The pangenome-scale model also helped identify the dispensable catabolic reactions that have demonstrable beneficial effects on enhancing the growth rates of ANAMMOX bacteria. Thus, the detailed, systems-level metabolic model of ANAMMOX will be instrumental in designing ANAMMOX-assisted efficient and effective industrial wastewater treatment processes for sustainable aquatic environments and the balanced nitrogen cycle.

FP10/14 - Multiplexed retron-based genome editing in prokaryotic and eukaryotic organisms.

Presenting Author - *Alejandro Gonzalez, Gladstone Institutes, United States*

Author/s - *Santiago Lopez, Alfonso Matias Rojas Montero, Seth Shipman*

Abstract Content

In the last years a potential tool to produce template DNA inside cells is a bacterial system called retron that recently has been shown to be involved in phage defense. Retrons are tripartite systems composed by a reverse transcriptase (RT) a contiguous non-coding RNA (ncRNA) with two region msr and msd, and an additional protein or RT-fused domain with diverse enzymatic functions. Targeted reverse-transcription activity of retrons is being used to produce a specific single-stranded DNA (ssDNA) donor in vivo via engineering msd region4,5. Combined with single-stranded annealing proteins (SSAPs) in bacteria and with Cas9 in yeast and human cells, retron-derived donors has been tested to efficiently edit genomes across kingdoms of life. For many biotechnological and therapeutic applications, editing of multiple DNA loci in a single genome is required. Therefore, in this work we develop multiplexed retron-based genome editing for the first time. To reach this aim we designed new retron architectures that encoded several ssDNA donors showing that this group of prokaryotic RTs represent a versatile bioengineering tool for editing up to 5 loci simultaneously in both prokaryotic and eukaryotic cells with efficiencies >90%. Finally, we use this technology to engineer the lycopene metabolic path in *E. coli* and *S. cerevisiae* showing that retron-based genome editing could be used to increase the production of compounds of interest.

FP10/15 - Engineering *P. aeruginosa* phages with reduced genomes and improved functionalities towards bacterial detection and control

Presenting Author - *Diana P. Pires, University of Minho, Portugal*

Author/s - *Luciana Meneses, Rodrigo Monteiro*

Abstract Content

Background: *P. aeruginosa* is a bacterial pathogen responsible for several infections, urgently requiring the development of new treatments. Bacteriophages have emerged as a promising therapeutic approach and their properties can be enhanced by phage-engineering.

Objectives: Design and assemble chimeric phages with improved functionalities that could be used for bacterial detection (by cloning the NanoLuc® luciferase) and for bacterial control (by cloning genes interfering with quorum-sensing (QS) pathways).

Methods: Since the cloning of extra genes into the phage genomes require additional space, we first deleted up to 48% of genes with unknown functions from phage vB_PaeP_PE3 using the yeast-based phage engineering platform. The fitness of these phages was studied both in vitro as well as in vivo (Galleria mellonella). Then, the reduced phage was used for the introduction of the NanoLuc® and the QS-interfering genes aiiA and pvdQ. Detection assays were performed with the reporter phage and infection assays were performed with anti-QS phages.

Results: Our data demonstrated that the knockout of genes with unknown functions did not impair the phages' antibacterial properties. The assembled reporter phage was capable of reliably detect 500 CFU/mL within 7h or an average 1 CFU/mL after 24h, and no false positives were observed. The *P. aeruginosa* infection experiments revealed that the OD of cultures infected with anti-QS phages were significantly lower between 6 and 48h of infection comparatively to wildtype phage. Overall, this work demonstrated that chimeric phages hold a great potential to be used in the future for both diagnostic and therapeutics.

OTS1/3 - *In situ* incubations reveal key players in chitin degradation in Arctic hydrothermal deep-sea sediments

Presenting Author - *Katharina Sass, University of Bergen, Norway*

Author/s - *Hasan Arsin, Anita-Elin Fedøy, Ida Helene Steen, Runar Stokke*

Abstract Content

Deep-sea hydrothermal vents are among the most extreme habitats on Earth and represent targets for marine bioprospecting and biodiscovery. However, the full extent of the heterotrophic capabilities in these environments is still being explored. These habitats already constitute important environments to find new microbial solutions that are urgently needed in the bioprocessing industry, where the degradation of complex organic materials is often a major challenge. These materials include chitin, a biopolymer that has a direct impact on carbon and nitrogen cycles in the ocean. Therefore, understanding its impact on the composition and function of the diverse heterotrophic microbial community is crucial.

Here, we report on data of long-term in situ enrichments on chitin, indicating responsible microorganisms involved in chitin degradation. In addition to typical heterotrophic microorganisms, we identified potential novel chitin degraders within the uncultivated candidate phylum KSB1. Thus far, KSB1's role in carbon metabolism has not been described. Metagenomic and phylogenetic investigations indicate a relationship of candidate KSB1 MAGs with MAGs assembled from the Guaymas Basin hydrothermal sediments, for which a significant role in hydrocarbon degradation was already described. By using different bioinformatical approaches we identified various chitin degrading enzymes of the KSB1 phylum. After heterologous expression and purification, the respective enzymes showed high chitin degrading activity and hence support a significant role in the carbon cycle for KSB1 bacteria. Furthermore, the identified novel lineages are likely adapted to dealing with the industrial substrates in the incubation chambers, and thus provide novel sources for enzyme mining.

OTS1/4 - Unraveling the presence and function of non-methylotrophic methanogenic communities in hypersaline microbial mats

Presenting Author - *Jose Q. García-Maldonado, Centro De Investigación Y De Estudios Avanzados Del Instituto Politécnico Nacional, Mexico*

Author/s - *Alejandro López-Cortés, Santiago Cadena, Hever Latisnere-Barragán, Patricia J. Ramírez-Arenas, Ricardo Vázquez-Juárez, Maurilia Rojas-Contreras,*

Abstract Content

Methanogenic archaea lacking the Wood-Ljungdahl pathway with a hydrogen-dependent methylotrophic pathway to reduce carbon dioxide, have been detected in a wide variety of habitats, however, the occurrence of this metabolism in hypersaline microbial mats has been little explored. The aim of this study was to stimulate the hydrogenotrophic and hydrogen-dependent methylotrophic methanogenic pathways under microcosms experiments of hypersaline microbial mats to elucidate the changes in the methanogenic communities when these conditions are present in the system. Microbial mats were collected in June 2019 and 2021 from two evaporation ponds. Microcosms incubations were designed to enrich all the methanogenic pathways, with special interest on the hydrogen-dependent methylotrophic route. The methanogenic community was assessed by amplicon sequencing of the mcrA gene as well as by metagenomic shotgun sequencing. Biogas production was observed for all the treatments in the experiment, being trimethylamine the preferred substrate. Changes in the methanogenic communities were observed depending on the supplemented methanogenic substrate. In addition to Methanosarcinales, in this study members of the Methanobacteriales, Methanomicrobiales, Methanomassiliicoccales, Candidatus Methanofastidiosales, Methanocellales, Methanococcales and Methanopyrales orders, were detected. Moreover, several mcrA environmental sequences were significantly different from those previously reported and did not match with any known methanogenic archaea, suggesting the presence of specific environmental clusters of methanogenic archaea in the studied site.

OTS1/5 - Unusual coexistence of microbial biodiversity in Deception Island, Antarctica

Presenting Author - *Jenny M. Blamey, Universidad de Santiago de Chile y Fundacion Biociencia, Chile*

Author/s - *Jenny M. Blamey*

Abstract Content

Within Antarctica, Deception Island constitutes a uniquely rare ecosystem that comprises an unusual diversity of extreme environments that coexist in a very small geographical area confined in this active stratovolcano. This includes frozen environments such as permafrost, glaciers, and frozen lakes, but also, geothermal sites, fumarole emissions and marine hydrothermal vents. This makes it a hotspot for the discovery of extremophilic microorganisms with multiple stress resistance and interesting biomolecules. Because of its challenging nature, the microbial structure and dynamics of this ecosystem is severely understudied. The presence of radically different environmental conditions coexisting in close proximity permits us to expect an unusually diverse microbial community, mostly composed of extremophilic microorganisms capable of surviving various sources of stress factors.

The intrinsic fragility of this pristine ecosystem further highlights the value of getting a snapshot of its microbial inhabitants and some of their biomolecules. Here we will present some of the microorganisms isolated from Deception Island and the remarkable biochemical properties of macromolecules as enzymes derived from them. This includes substrate specificity, optimum temperature, pH and thermostability.

OTS1/6 - Hiding in plain sight: unveiling extremophilic microorganisms living in medieval manuscripts

Presenting Author - *Cecilia Flocco, Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Germany*

Author/s - *Anika Methner, Franziska Burkart, Alicia Geppert, Jörg Overmann*

Abstract Content

Background: Current cultural heritage conservation approaches largely catalogue microbes as detrimental agents to be eliminated. Our interdisciplinary research across life sciences and humanities challenges the prevailing conservation paradigm by perceiving heritage objects as an archive of untapped microbial diversity and cultural-historical clues.

Objectives: This work aims to unveil the microbial communities inhabiting written heritage objects and assess their specific physiological adaptations, biotechnological potential and value as biographical probe to trace the history of the object.

Methods: our interdisciplinary approach combined cultural heritage history with microbiological (classic cultivation, microbial physiology assays) and biomolecular analyses (high-throughput sequencing, genome mining and comparative genomics) to dissect the microbiome of a medieval parchment Bible of the XIV century and assess its specific adaptations and biotechnological potential. Representative, novel taxa were selected for polyphasic identification and in-depth genomic analyses.

Results: Approximately 400 microbial isolates were obtained using a diversity of cultivation conditions (~55) covering a wide range of nutritional requirements. Endospore-forming microorganisms adapted to low water availability and/or alkaline conditions (representing several Bacillaceae genera) were predominant, followed by human and animal skin associated microorganisms (*Staphylococcus* spp.). Their adaptation reflects the physicochemical habitat intrinsic to parchment and the techniques used to produce a writable surface out of animal hives. Concurringly, genome mining of two representative novel isolates revealed the occurrence of genes associated to salt tolerance mechanisms, such as the complete ectoine biosynthesis pathway, a central component of the compatible solute salt tolerance strategy, as well as various stress tolerance mechanisms (such as arsenic resistance).

OTS2/2 - Vaginal lactobacilli exert prebiotic effects towards gut bifidobacteria

Presenting Author - *Carola Parolin, Dipartimento di Farmacia e Biotecnologie; Università di Bologna, Italy*

Author/s - *Barbara Giordani, Angela Abruzzo, Claudio Foschi, Luca Laghi, Antonella Marangoni, Barbara Luppi, Beatrice Vitali*

Abstract Content

Background: Colonization of infants' gut by *Bifidobacterium* spp. is crucial for the prevention of gastrointestinal diseases and the correct maturation of immune system. Infants born by C-section generally display low bifidobacteria amount and high risk of developing gastrointestinal disorders, suggesting that vaginal partum and bifidobacteria-enriched gut microbiota in newborns are interconnected. Healthy vaginal microbiota is generally dominated by *Lactobacillus* spp. that, in virtue of their well-known physiological benefits to vaginal ecosystem, are widely used as probiotics. Recently, *Lactobacillus* postbiotics have also gained interest.

Objectives: Lactobacillus strains of vaginal origin were investigated for their potential to promote the growth of Bifidobacterium spp. widely represented in the gut tract, in the perspective of unravelling possible contribution of lactobacilli to gut microbiota development and of designing novel strategies to improve infants' health.

Methods: Heat-inactivated cells and culture supernatants recovered from vaginal L*actobacillus crispatus*, *Lactobacillus gasseri*, *Limosilactobacillus vaginalis*, and *Lactiplantibacillus plantarum* strains were evaluated for bifidogenic activity. Such activity was measured towards planktonic cultures and biofilms of *Bifidobacterium* spp.

Results: Vaginal lactobacilli significantly stimulate *Bifidobacterium* spp. grown in free-floating and biofilm forms; such effect is dependent on *Lactobacillus* species and growth phase. Importantly, no stimulating effect on an intestinal strain of *Escherichia coli* was observed.

Besides already known probiotic effects, we demonstrated that vaginal lactobacilli inactivated cells and supernatants can exert a strong prebiotic activity on bifidobacteria, thus suggesting a novel approach based on *Lactobacillus* postbiotics to trigger a favorable bifidogenic shift in the infant gut.

OTS2/3 - Diverse *Bacteroides* species and strains of the intestinal microbiota positively and negatively interact with each other

Presenting Author - *Daniel Unterweger, Christian-albrechts-universität Zu Kiel, Germany*

Author/s - *Hanna Fokt, Gabija Sakalyte, Rahul Unni, Daniel Unterweger*

Abstract Content

The mammalian intestine is a unique ecosystem for thousands of bacterial species and strains. How naturally co-existing bacteria of the microbiota interact with each other is not yet fully understood. Here, we systematically studied over 100 interactions between bacteria of the genus Bacteroides that were isolated from the intestine of healthy mice. We find a vast diversity of interactions ranging from positive to negative. Intraspecific interactions are dominated by mutualistic and parasitic interactions. Interspecific interactions are subject to intraspecific diversity and differ between hosts. These findings on obligate host-associated bacteria (i) identify novel molecular mechanisms by which bacteria affect each other and (ii) demonstrate high strain-level variation of bacteria-bacteria interactions. The results have implications for our basic understanding of the microbiota and for the design of synthetic microbial communities.

OTS2/4 - Mycobiota and diet-derived fungal xenosiderophores promote *Salmonella* gastrointestinal colonization

Presenting Author - *Judith Behnsen, University of Illinois at Chicago, United States*

Author/s - *William Santus, Amisha P Rana, Jason R Devlin, Kaitlyn A Kiernan, Carol C Jacob, David M Underhill, Joshua Tjokrosurjo, University of California Irvine, Irvine, United States*

Abstract Content

The fungal gut microbiota (mycobiota) has been implicated in diseases that disturb gut homeostasis, such as inflammatory bowel disease. However, little is known about functional relationships between bacteria and fungi in the gut during infectious colitis. Here we investigated the role of fungal metabolites during infection with the intestinal pathogen *Salmonella enterica* serovar Typhimurium, a major cause of gastroenteritis worldwide. We specifically focused on siderophores, small molecules that allow efficient iron uptake in iron-limited environments such as the inflamed gut. We analyzed the role of fungal siderophores during *Salmonella* infection using conventionally raised and gnotobiotic mice, different mouse diets, mycobiota sequencing, and in vitro approaches. We found that, in the gut lumen, both the mycobiota and fungi present in the diet can be a source of fungal siderophores. The ability to use fungal siderophores, such as ferrichrome and coprogen, conferred a competitive growth advantage to *Salmonella* strains expressing the fungal siderophore receptors FhuA or FhuE in vitro and in a mouse model. Our study highlights the role of inter-kingdom cross-feeding between fungi and *Salmonella* and elucidates an additional function of the gut mycobiota, revealing the importance of these understudied members of the gut ecosystem during bacterial infection.

OTS2/5 - A microbiome-derived metabolite intercepts the communication between Enteropathogenic *E. coli* and *Vibrio cholerae*

Presenting Author - *Neta Sal-Man, Ben-Gurion University, Israel*

Author/s - *Orna Gorelik, Lara Holoidovsky, Michael M. Meijler, Neta Sal-Man*

Abstract Content

Reported numbers of diarrheal samples exhibiting co-infections or multiple infections, with two or more infectious agents, are rising, likely due to advances in bacterial diagnostic techniques. Bacterial species detected in these samples include *Vibrio cholerae* (V. cholerae) and enteropathogenic *Escherichia coli* (EPEC), which infect the small intestine and are associated with high mortality rates. It has previously been reported that EPEC exhibit enhanced virulence in the presence of *V. cholerae* owing to their ability to sense and respond to elevated concentrations of cholera autoinducer 1 (CAI-1), which is the primary quorum-sensing (QS) molecule produced by *V. cholerae*. In this study, we examined this interspecies bacterial communication in the presence of indole, a major microbiome-derived metabolite found at high concentrations in the human gut. Interestingly, we discovered that although indole did not affect bacterial growth or CAI-1 production, it impaired the ability of EPEC to enhance its virulence activity in response to the presence of *V. cholerae*. Furthermore, the co-culture of EPEC and V. cholerae in the presence of B. thetaiotaomicron, an indole-producing commensal bacteria, ablated the enhancement of EPEC virulence. Together, these results suggest that microbiome compositions or diets that influence indole gut concentrations may differentially impact the virulence of pathogens and their ability to sense and respond to competing bacteria.

OTS2/6 - Hfq RIL-seq in *Clostridioides difficile* reveals a network of sRNAs regulating sporulation initiation

Presenting Author - *Franziska Faber, Julius-Maximilians-Universität of Würzburg, Germany*

Author/s - *Manuela Fuchs, Franziska Faber*

Abstract Content

Background: The enteric pathogen *Clostridioides difficile* persists in the intestinal tract by forming antibiotic resistant endospores that contribute to relapsing and recurrent infections. Despite the importance of sporulation for *C. difficile* pathogenesis, molecular mechanisms regulating sporulation initiation remain ill defined. Recently, posttranscriptional regulation mediated by the RNA binding protein Hfq and small regulatory RNAs (sRNAs) has been implicated in modulating sporulation in *C. difficile*.

Objective: We wanted to elucidate the role of sRNAs and Hfq in the posttranscriptional gene regulation in C. difficile with a focus on the sporulation pathway.

Method: We applied RIL-seq (RNA interaction by ligation and sequencing) that relies on ligation of Hfq-bound RNA pairs and thereby directly captures and identifies interaction partners.

Results: Using RIL-seq, we identify Hfq as a global RNA matchmaker and reveal a rich post-transcriptional regulatory network. We discover two novel sRNAs, which we name SpoX and SpoY, that target the master regulator of sporulation Spo0A thereby fine-tuning the formation of endospores in this important human pathogen. We show that SpoX and SpoY base-pair with the 5‘ UTR and early coding sequence of Spo0A, respectively, to modulate Spo0A protein levels with opposite effects. Whereas SpoX increases spore frequency, the activity of SpoY leads to decreased/delayed sporulation. Our work reveals an elaborate RNA-RNA interactome controlling the physiology and virulence of *C. difficile* and identifies a post-transcriptional regulatory mechanism in the production of *C. difficile* endospores which are critical for the pathogen’s resistance to traditional antibiotic therapies and for its transmission to new hosts.

OTS3/2 - Morphological and biochemical insights into the biofilm formation of *Syntrophobacter fumaroxidans* and methanogens

Presenting Author - *Anna Doloman, Wageningen University & Research, Netherlands*

Author/s - *Diana Sousa*

Abstract Content

Background: Microorganisms are often found in organized assemblages, biofilms, where they interact and exchange biochemical molecules. However, no knowledge exists on the underlying factors of biofilm formation in the anaerobic methane-producing cocultures of fatty-acid oxidizing acetogenic bacteria and hydrogenotrophic methanogenic archaea. Mechanistic insights into their aggregation abilities are crucial for understanding of ecological relationships among these environmentally- and application-relevant anaerobic microorganisms.

Objectives: Examine morphological and biochemical changes associated with cell aggregation in cocultures of syntrophic propionate-oxidizing *Syntrophobacter fumaroxidans* and hydrogenotrophic methanogens, *Methanospirillum hungatei* or *Methanobacterium formicicum*.

Methods: Bi-cultures of *S. fumaroxidans* and methanogens, 10% v/v each, were grown at 37°C in a bicarbonate-buffered mineral salt medium containing propionate (20 mM) and 1.5 bar N2/CO2 (80/20 (v/v)). Morphology of cocultures was monitored with fluorescent and scanning electron microscopy. Substrate/product turn-over rates were monitored with high-performance liquid/gas chromatography. Changes in the gene expression in dispersed and aggregated cocultures were investigated through RNA sequencing and differential expression analysis (DEseq).

Results: We observed formation of aggregates in bi-cultures with either of the methanogens within 5 months of cultivation. Localization of *S. fumaroxidans* within aggregates differed depending on the partner methanogen. Biofilm aggregates had 2x faster substrate/product turnover rates, compared to the dispersed cocultures. DEseq results point to statistically significantly higher expressed genes for signal transduction, polysaccharide secretion, iron transporters and chemotaxis in the aggregated cocultures, compared to the dispersed ones. Future studies will investigate cocultures' time-resolved genes expression profiles and production of signaling molecules to fully understand the mechanisms of biofilm formation.

OTS3/3 - Untangling the metabolism of a dichloromethane degrading *Dehalobacter* strain for bioremediation

Presenting Author - *Olivia Bulka, University of Toronto, Canada*

Author/s - *Radhakrishnan Mahadevan, Elizabeth Edwards*

Abstract Content

Background: Dichloromethane (DCM) degradation is a critical step in chloroform (CF) bioremediation at many contaminated groundwater sites across the globe, more than 717 of which are on the EPA's National Priority List for remediation in the United States alone. Several *Dehalobacter restrictus* strains can respire CF to DCM through reductive dechlorination, but remediation generally stops there, with DCM accumulating in the system until it inhibits further CF biodegradation. Furthermore, though some DCM-degrading microbes have been identified, most are very sensitive to CF, rendering their use for bioremediation in complex contaminated sites impractical.

Objective: Here we present a novel *Dehalobacter* strain—the first microbe found to degrade CF and DCM concurrently—and use genome-scale metabolic modelling and experimental validation to elucidate the pathways that allow this tandem degradation.

Methods & Results: The genome of this *Dehalobacter* strain was assembled from metagenomic sequencing of a mixed microbial bioaugmentation culture, and was then used to adapt and curate a draft metabolic model. We performed additional experimentally informed curation, thermodynamic analysis, and dynamic Flux Balance Analysis to propose a mechanism of cyclical electron shuttling for respiration of CF to DCM, circumventing the requirement of an added electron donor to the system. Additionally, we demonstrate the possibility of carbon assimilation from DCM through the Wood-Ljungdahl Pathway and pyruvate oxidoreductase.

Significance: Modeling the metabolism this *Dehalobacter* strain improves understanding of CF and DCM dechlorination energetics, which informs strategies for culture maintenance and scale-up, and benefits contaminated sites where the culture is employed for remediation worldwide.

OTS3/4 - Expression of flagellar and type III secretion systems is under stochastic and deterministic regulation in *Pseudomonas syringae*

Presenting Author - *Nieves Lopez-Pagan, IHSM-UMA-CSIC, Spain*

Author/s - *José S. Rufián, María-Antonia Sánchez-Romero, Laurent Aussel, Josep Casadesus, Fernando Govantes, Javier Ruiz-Albert, Carmen R. Beuzon*

Abstract Content

We previously described bistability and phenotypic heterogeneity of the type III secretion system (T3SS) of *Pseudomonas syringae* (Rufián et al., 2016). First example of such phenomenon in a plant pathogen. Here, we describe heterogenous flagellar expression leads to phenotypic heterogeneity within *P. syringae* populations. We find that although as reported flagellin is downregulated inside the plant, it is still expressed by a part of the bacterial population that maintains high expression levels during colonization of the plant apoplast. We demonstrate that expression of the T3SS and flagellar systems undergo counter regulation that is displayed at a single-cell level as T3SSON/FlagellaOFF and T3SSOFF/FlagellaON subpopulations. Despite this counter regulation, T3SSON/FlagellaON and T3SSOFF/FlagellaOFF bacteria can also be found within the apoplast at significant levels. Genetic analysis of the elements involved shows that counter-regulation is reciprocal: altered levels of T3SS transcriptional activator HrpL affect flagellar expression and altered levels of flagellar master regulator FleQ affect T3SS gene expression. But it also shows that the heterogeneity of each of these systems arises through independent mechanisms and display different dynamics. The regulatory loops involved in establishing T3SS and flagellar heterogeneity in *P. syringae* are different to those described for these systems in animal pathogen, suggesting convergent evolution of heterogeneity. Finally, we analyze the biological implications of heterogeneity and propose that, through a division of labor strategy, heterogeneity may provide adaptive value to this pathogen. This is one of the few examples where phenotypic heterogeneity is analyzed in natural conditions within the context of host colonization.

OTS3/5 - Genotype-Phenotype-Fitness mapping in *E. coli*

Presenting Author - *Anthi-Maria Kouvatsou, University of Manchester, United Kingdom*

Abstract Content

Central to understanding and predicting evolution is the ability to predict the effects of mutations. Mutations can alter the immediate phenotype of the gene or regulatory element they appear in, and those changes can impact organismal fitness. I will present our attempts to experimentally and computationally connect mutations in bacterial promoters (genotype) to their effects on gene expression levels (phenotype), and then link changes in phenotypes to organismal fitness.

In a synthetic plasmid system, we have generated promoters with different architectures – meaning a different number and relative position of transcription factor binding sites. We are exploring how promoter architecture impacts the regulatory logic and expression of the associated gene. We are introducing random mutations into these promoters to understand the relationship between promoter architecture and mutational effects.

Mutations in promoters alter gene expression levels. To study the consequences of such changes, we are using a metabolic model and Flux Balance Analysis of *E. coli*. We explored how changes in expression levels of every gene involved in *E. coli* metabolism alter organismal fitness and identified general trends of this relationship. We are also investigating potential factors that determine why changes in gene expression affect fitness the way they do.

The result of these investigations will be forming a genotype-phenotype-fitness map. This will have important benefits for future research, as from a single sequence (with no other information) one will be able to use the patterns observed to predict its phenotype and fitness without conducting lab experiments.

OTS3/6 - Understanding the Role of *Escherichia coli* Hydrogenase-2 subunits in proton flux under different glucose concentrations

Presenting Author - *Karen Trchounian, Department of Biochemistry, Microbiology, and Biotechnology, Yerevan State University, Armenia*

Author/s - *Liana Vanyan, Anait Vassilian, Anna Poladyan, Karen Trchounian*

Abstract Content

E. coli Hydrogenase-2 (Hyd-2) encoded by hybOABCDEFG operon has an important role in cell bioenergetics. This thesis examines the effect of specific subunits of Hyd-2 on proton flux in Escherichia coli in response to different glucose concentrations.

E. coli BW25113 wild-type and single-deletion mutants of Hyd-2 were grown in the presence of 2 g L−1 glucose and proton flux was determined using pH-selective electrode.

When 2 g L-1 glucose was supplemented total proton flux was 1.55 mmol min-1, and significant changes were not observed in mutants lacking HybA, HybB, HybD, HybE, and HybO subunits. In hybG and hybF mutants, proton flux increased by ~22% and ~38%, respectively, and in hybC mutant, it decreased by ~23%.

DCCD-sensitive H+ flux in wild type was 0.466 mmol min-1. Compared to the wild type, FOF1-ATPase contribution was reduced ~3-fold in hybC and hybG mutants, and ~1.5-fold in hybO.

When 8 g L-1 glucose was supplemented wild type and mutants show similar total fluxes. The contribution of FOF1-ATPase was ~2.3 higher compared to 2 g L-1. In hybA, hybO mutants DCCD-sensitive fluxes were 0.4 mmol min-1: ~60% lower compared to wild type. Meanwhile in hybB and hybC mutants decreased by ~40% compared to wild type.

The findings of this study provide new information on the functional aspects of Hyd-2. At low glucose concentration from HybO-HybC-system, HybC acts as the catalytic subunit, while at high concentrations, HybO is active. Moreover function of different subunits depends on glucose concentration.

OTS3/7 - Regulation and effects of the RpoS-RssB interplay in *Vibrio cholerae*'s life style

Presenting Author - *Martina Wölflingseder, Universität Graz, Austria*

Abstract Content

The severe diarrheal disease cholera is caused by the Gram-negative bacterium *Vibrio cholerae*. The bacteria exist not only in their natural aquatic environment but also infect the human host. The transition between these two different habitats has to be strongly regulated by different mechanisms, such as by the alternative sigma factor RpoS. RpoS is known as a general stress response regulator in many different bacteria, however its output regulatory response is varying in many ways. In *V. cholerae* RpoS can alter the transcription of genes involved in signaling pathways such as motility, biofilm formation, and pathogenesis. For example, it was shown that the motility of *V. cholerae* is activated by RpoS in the end phase of the infection allowing the bacteria to return to the aquatic environment. By focusing mainly on nutrient poor conditions, we investigated the interplay of RpoS and its anti-sigma factor RssB. RpoS positively regulates the transcription of rssB, in its active state RssB induces the proteolysis of RpoS, and in the end RssB itself gets degraded. This interplay is important not only for motility and chemotaxis phenotypes under in vitro conditions, but also for the colonization fitness in our in vivo model and postinfectious survival. Furthermore, we could identify the FexB/ArcB kinase as possible activator of the RssB protein.

OTS3/8 - Genome-wide analysis of *Haemophilus influenzae* genes reveals epigenetic regulation of the FNR regulon

Presenting Author - *Celia Gil-Campillo, Spanish Research Council (csic), Institute Of Agrobiotechnology (idab-csic), Spain*

Author/s - *Celia Gil-Campillo, Gabriel Gutiérrez, Irene Rodríguez-Arce, Jeroen D. Langereis, María Antonia Sánchez-Romero, Junkal Garmendia, Begoña Euba. Institute Of Agrobiotechnology, Spanish Research Council (Idab-CSIC)-Government of Navarra, Mutilva, Spain*

Abstract Content

Background: *Haemophilus influenzae* is a human host-adapted pathogen causing chronic lower airway infections and recurrent exacerbations in chronic obstructive pulmonary disease patients. By using transposon insertion sequencing (Tnseq), we screened bacterial genes required for infection in a murine model of airway infection, identified and validated the methyltransferase Dam.

Objectives: To study the role of Dam GATC methylation in the regulation of *H. influenzae* gene expression, and the contribution of such epigenetic regulation to this host-pathogen interplay.

Methods: We followed two complementary approaches:

(i) RNA sequencing (RNA-seq) to profile differential gene expression when comparing WT and dam mutant strains.

(ii) methylome analysis in a panel of *H. influenzae* PacBio genomes to screen hypo/hemi-methylated GATC sites in non-coding regions.

Results: The oxygen sensitive FNR regulator and the FNR-regulated genes ytfE, dmsA and cydD, were overexpressed upon dam inactivation. Further analysis identified GATC motifs in the fnr, dmsA and cydD promoter regions, and Dam methylation of these sites was confirmed. Conversely, methylome analyses recurrently showed GATC hypo/hemi-methylation in a region containing two GATC motifs upstream of htpG gene, the proximal one overlapping with a putative FNR binding site. Analysis of such GATC sites revealed possible phenotypic heterogeneity in the above mentioned proximal motif, further tested by ad-hoc generation of fluorescent reporter strains for single-cell analyses. Together, our results shed light on Dam methyltransferase contribution to *H. influenzae* pulmonary infection, highlight epigenetic regulation of the *H. influenzae* FNR regulon and its likely involvement in airway infection and bacterial response to environment stress conditions.

OTS3/9 - Convergent within-host evolution of a *Klebsiella pneumoniae* clone during a large hospital outbreak

Presenting Author - *Greta Zaborskytė, Uppsala University, Sweden*

Author/s - *Karin Hjort, Birgitta Lytsy, Linus Sandegren*

Abstract Content

Background: During colonization and infection of the human host, bacteria can acquire mutations that make them better adapted to the host niche. The critically important pathogen *Klebsiella pneumoniae* often causes nosocomial outbreaks that present a great opportunity to study within-host evolution, but the resolution of evolutionary trajectories is often complicated by the co-existence of multiple clones.

Objectives: We explored the evolution of a single *K. pneumoniae* clone in individual patients during a 5-year hospital outbreak.

Methods: We have analyzed the isolates of an outbreak clone from 110 patients with asymptomatic colonization and infections (UTI, wound, blood, and lung). Whole-genome sequencing was combined with phenotypic characterization of systemic virulence properties in the *Galleria mellonella* infection model, survival in human serum, mucoviscosity, growth under limited iron conditions, biofilm formation on silicone surfaces, and gastrointestinal colonization in mice.

Results: Phylogenetic analysis supported the emergence of short-lived host adaptations rather than the spread of particular lineages. Signatures of a strong positive selection were present in numerous genes and intergenic regions, especially relating to siderophores and iron metabolism, cell surface structures (capsule, LPS, outer membrane porins), and signaling (diguanylate cyclases, two-component systems). Phenotypically, the isolates showed frequent attenuation in virulence, altered regulation of capsule synthesis, and iron uptake and utilization. A highly increased biofilm formation capacity was associated with an advantage during GI colonization in mice. Overall, our study illustrates the power of combining large-scale genomic and phenotypic analysis to understand the evolutionary within-host trajectories and the infection-related phenotypes of *K. pneumoniae*.

OTS4/4 - Isogenic heterogeneity in lifespan follows an evolutionary trade-off in response to amino acid identity

Presenting Author - *Kiyan Shabestary, Imperial College London, United Kingdom*

Abstract Content

In the wild, microorganisms mainly operate at suboptimal growth conditions with fluctuations in nutrient abundance . Constrained by finite resource allocation principles and subject to fitness pressure, adaptation to suboptimal conditions often takes the form of a strategic choice between two conflicting tasks: growth or survivability maximisation. Here, we systematically study the impact of single amino acid on cellular metabolism and report isogenic macro-heterogeneity in response to a change in nitrogen quantity and quality conserved in *Saccharomyces cerevisiae* strains. Cells exposed to a nitrogen down-shift differentiate into subpopulations of different sizes, chronological lifespans and growth resumption capabilities. We monitor the metabolic response of the subpopulations using a protein-tagged GFP library coupled to high-throughput microscopy and single-cell tracking. We show that depending on the nitrogen source and quantity available, cells can choose to either maintain or abort this differentiation process. We propose that this macro-heterogeneity is a case of bet-edging where subpopulations operate at distinct spaces of the growth-survivability trade-off depending on the amino acid present. These results establish amino acids as important signalling molecules for chronological lifespan and growth rate determination.

OTS4/5 - The Hrk1 kinase as a modulator of ion homeostasis and acetic acid stress tolerance in yeast

Presenting Author - *Miguel Antunes, iBB - Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Portugal*

Author/s - *Deepika Kale, Olga Zimmermannová, Marie Kodedová, Hana Sychrová, Isabel Sá-Correia*

Abstract Content

Background: Acetic acid-induced stress is frequently encountered in industrial processes, having a negative impact on the growth and metabolism of *Saccharomyces cerevisiae*. The Hrk1 kinase is an important acetic acid tolerance determinant and a potential ion homeostasis modulator. Hrk1 belongs to a family of kinases involved in the regulation of nutrient uptake and metabolism. To adapt and tolerate acetic acid-induced-stress, *S. cerevisiae* employs various mechanisms to restore intracellular pH (pHi) relying on the activity of *alkali* cation/H+ exchangers and of plasma membrane H+-ATPase. Potassium ions are essential for the regulation of pHi, cell volume, and membrane potential. Potassium ion homeostasis regulation mechanisms include the activation of the Trk1/2 transporters and the Na+,K+/H+ antiporter Nha1.

Objective: This study aimed to investigate the acetic acid stress tolerance mechanisms and ion homeostasis regulation in *S. cerevisiae*, specifically focusing on the roles of the Hrk1 kinase and potassium transporters.

Methods: A combination of phenotypic characterization and evaluation of physiological parameters was employed in cells deleted for HRK1 alone or in combination with potassium transporter encoding genes, which were cultivated under various stresses.

Results: Hrk1 plays a significant role in the modulation of potassium homeostasis in the absence and presence of acetic acid stress based on growth phenotypes, and membrane potential and pHi measurements. These findings provide new insights into the mechanisms of tolerance to acetic acid stress and ion homeostasis regulation and may have important implications for improving the performance of yeast in industrial processes.

OTS4/6 - Comparative analysis of transcriptomic responses in wine yeast cells triggered by yeast extracellular vesicles or whole cells

Presenting Author - *Miguel Mejias Ortiz, Institute of Grapewine and Wine Sciences (ICVV-CSIC-UR-GR), Spain*

Author/s - *Ana Mencher, Pilar Morales, Jordi Tronchoni, Ramón González*

Abstract Content

Background: The use of non-Saccharomyces yeast species in the wine industry has been increasing in recent years. These alternative yeasts are often used in conjunction with *Saccharomyces cerevisiae* to ensure complete fermentation. However, this can lead to potential interactions between the different yeast species, which can impact the outcome of wine fermentation. Our previous research has demonstrated that when *S. cerevisiae* comes into contact with other wine yeast species, there are transcriptional responses with both common and species-specific features (Curiel et al., 2017).

Objectives: In this study, we test the hypothesis that extracellular vesicles (EVs) play a role in the biological interactions.

Methods: *S. cerevisiae* cultures were exposed to purified EVs from Metschnikowia pulcherrima. We then evaluated the impact of these EVs on the physiology of *S. cerevisiae* using transcriptomic analysis and compared the results to the response of *S. cerevisiae* to whole M. pulcherrima cells under the same experimental conditions.

Results: Our analysis reveals an important overlap in the transcriptional responses in S. cerevisiae induced by either *M. pulcherrima* cells or EVs. Both appear to induce transcription of genes related to glycolysis and ribosomal activity and repress vacuolar transport. These results confirm that *S. cerevisiae* directly responds to competing species under wine-like conditions and provide the first experimental support for the hypothesis that recognition mechanisms involve, at least in part, EVs. Based on these findings, we proceeded to evaluate the reaction of *S. cerevisiae* against EVs from other yeast species of oenological interest.

OTS4/7 - The role of polyamines in the pathobiology of the emergent fungal pathogen *Emergomyces africanus*

Presenting Author - *Elizaveta Koroleva, Stellenbosch University, South Africa*

Author/s - *Alfred Botha, Barbra Toplis, Sybren de Hoog*

Abstract Content

Emergomyces africanus is an emerging opportunistic pathogen causing severe morbidity and mortality. Although little is known about this fungus, its morphological transition to a pathogenic yeast-like phase in the human host is a notable virulence mechanism. Research suggests that polyamines may play a pivotal role in dimorphism, however the functions of these biogenic amines in fungi remain enigmatic. The aim of this work was, therefore, to characterise polyamine metabolism of *E. africanus* and obtain an indication of its role in the thermally induced dimorphic switch.

We evaluated polyamine production of two strains of *E. africanus* in mycelial and yeast-like phases using ultraperformance liquid chromatography tandem mass spectrometry analysis, and by determining activities of key polyamine synthesis enzymes. Additionally, we quantified growth in the presence of exogenous polyamines and investigated the effect of polyamine biosynthesis inhibitors cyclohexylamine and difluoromethylornithine on growth and dimorphism.

In this first report of the polyamine profile of *E. africanus*, we reveal spermidine as the major intracellular polyamine, and detect a variety of secreted polyamines, with spermidine and agmatine constituting the major proportion. Activity of several polyamine biosynthesis enzymes was found, with significant differences in ornithine decarboxylase, arginase and agmatinase expression between morphological phases. Agmatinase upregulation in the yeast-like phase was in accordance with enhanced growth in the presence of agmatine. Both inhibitors attenuated dimorphic switching, with strain-specific differences in inhibitor potency, but only cyclohexylamine affected fungal growth. Taken together, our study provides compelling evidence for the role of polyamines in the pathobiology of *E. africanus*.

OTS4/8 - The mycoparasitic behavior of the fungus *Trichoderma atroviride* is shaped by a hidden chemical crosstalk

Presenting Author - *Susanne Zeilinger, University of Innsbruck, Austria*

Author/s - *Dubraska Moreno-Ruiz, Alexander Lichius, Kristina Mißbach, Rainer Schuhmacher, Stefan Leibetseder, Martina Marchetti-Deschmann,*

Abstract Content

Mycoparasitism is a characteristic trait of *Trichoderma* fungi. The perception of host fungus-derived signals is essential for successful mycoparasitism and small secreted metabolites are supposed to play a major role in these interactions.

We used *Trichoderma atroviride* to study details of the mycoparasitic interaction. Chemosensing testing revealed that conidial germlings and microcolonies preferentially respond to self-signaling cues and compounds secreted by plant roots, while the perception of host fungi had no major relevance at this early developmental stage. Host fungus recognition emerged later in fully differentiated mycelium which showed directional growth towards certain host-derived culture supernatant fractions. Early interaction between *T. atroviride* and living host fungi was characterized by repeated switching between positive and negative chemotropism, evidencing a stress response probably triggered by host-derived substances. Accordingly, various low molecular weight metabolites released by both fungi could be localised in the interaction zone of a *T. atroviride – B. cinerea* co-culture underpinning a chemical cross-talk between *Trichoderma* and host fungus. Metabolome analyses revealed that the vast majority of the detected substances is located ahead of the hyphal growth front and that some are produced in a light-dependent manner.

T. atroviride mycoparasitism hence is governed by environmental and endogenous cues being as well strongly dependent on the developmental stage. The mycoparasitic interaction is accompanied by the release of a plethora of low molecular weight metabolites by both interaction partners. Mature *T. atroviride* hyphae are capable of a differential response to the presence of host-derived signals, which finally culminates in the mycoparasitic attack.

OTS5/2 - The emerging pathogen *Stenotrophomonas maltophilia* exhibits antibacterial activity

Presenting Author - *Cristian Crisan, Emory University, United States*

Author/s - *Daria Van Tyne, Joanna Goldberg*

Abstract Content

Background: *Stenotrophomonas maltophilia* is a Gram-negative bacterial pathogen that colonizes many organs including the heart, brain, urinary tract, lungs, eyes, bones, and kidneys. Septicemia caused by the bacterium has mortality rates as high as 65%, and many isolates are multidrug-resistant. *S. maltophilia* is found in diverse environments like water, soil, and plants, but can also contaminate hospital equipment. During infections and in external environments, *S. maltophilia* lives in polymicrobial communities where it must compete with other bacteria.

Objective: Mechanisms used by *S. maltophilia* to engage in competitive behaviors are largely unknown. The objective of this study was to explore how a *S. maltophilia* sputum isolate eliminates competitor bacteria.

Methods: We performed co-cultures experiments of *S. maltophilia* with competitor cells. We used confocal microscopy to visualize spatial structures of fluorescently labeled bacterial co-cultures.

Results: We discovered that a clinical sputum *S. maltophilia* isolate can efficiently eliminate Escherichia coli and Burkholderia cenocepacia cells. We identified a gene responsible for this phenotype and engineered an *S. maltophilia* mutant with a deletion in this gene. The *S. maltophilia* mutant has a significantly reduced ability to kill *E. coli* and *B. cenocepacia*. Furthermore, the mutant is impaired at competing against a *Pseudomonas aeruginosa* isolate from the same patient. Finally, we demonstrated that the *S. maltophilia* mutant forms well-mixed structures with *P. aeruginosa*, while the wild-type *S. maltophilia* forms distinct clusters when co-cultured with *P. aeruginosa*. Findings described here could lead to the identification of novel antibacterial proteins and new therapies.

OTS5/3 - Intraspecific diversity of type VI secretion system effector sets in *Pseudomonas aeruginosa*

Presenting Author - *Antonia Habich, Christian-albrechts-universität Zu Kiel, Germany*

Author/s - *Antonia Habich, Daniel Unterweger*

Abstract Content

Bacteria use type VI secretions systems (T6SSs) to deliver effector proteins into neighboring cells and the extracellular space. How a bacterium benefits from its T6SSs depends on the effectors, which enable bacteria to kill other microbes, manipulate eukaryotic cells or obtain nutrients. Most of what we know about effectors, and thus about the function of T6SSs in *Pseudomonas aeruginosa*, is based on few reference strains. Little is known about effector diversity within the species. Here, we use comparative genomics to systematically test for the intraspecific diversity of T6SS effectors in roughly 2000 phylogenetically distinct *P. aeruginosa* strains.

We found prevalent effectors, which are omnipresent and conserved across the species, and variable effectors, which vary between strains. The combination of prevalent with variable effectors results in an effector set. We observed a tremendous diversity of effector sets within the species, which can partly be explained by horizontal gene transfer between strains of different phylogroups.

All effectors with nutrient-acquiring activity are prevalent, suggesting T6SS-mediated nutrient uptake to be a wide-spread trait in *P. aeruginosa*. We further show that the prevalent effector TseT contributes to virulence in vivo, which indicates a role of the T6SS in pathogenicity across strains, whereas variable effectors with known anti-eukaryotic activity contribute to strain-level variation of virulence. Variable effectors with anti-prokaryotic activity provide the genetic basis for intraspecific killing and could provide a competitive advantage in mixed microbial communities.

These findings show the distinct contribution of T6SS effectors to the intraspecific diversity of a bacterial pathogen.

OTS5/4 - Molecular mechanism of ixotrophy behavior in a predatory marine bacterium

Presenting Author - *Yun-Wei Lien, ETH Zurich, Switzerland*

Author/s - *Gregor Weiss, Kang Soo Lee, Go Furusawa, Martin Pilhofer*

Abstract Content

To ensure survival in the environment, bacteria evolved diverse strategies to compete with other organisms. Several members of the family *Saprospiraceae* are known to have a specialized and very efficient antibacterial behavior called ixotrophy. It is characterized by catching prey bacteria by an unknown mechanism followed by lysis of the prey cells. The underlying molecular mechanism of ixotrophy is poorly understood. Genome analysis revealed contractile injection system (CIS) gene clusters in ixotrophy-active bacteria. Bacterial CISs resemble a bacteriophage-tail and play a major role in bacterial cell-cell interactions. Hence, we hypothesized a potential role of CISs in the ixotrophy of *Saprospiraceae*.

Here we identify the macromolecular machinery involved in ixotrohpy of the filamentous multicellular gliding bacterium *Aureispira* sp. CCB-QB1. Cryo-electron tomography revealed that every *Aureispira* cell harbors multiple contractile injection systems (CISs), working in a type VI secretion system-like mode of action. These novel CISs were attached to the cytoplasmic membrane and connected to an extracellular antenna-shaped structure. Light microscopy and functional assays further showed its involvement in bacterial killing and revealed a so far unknown mode of regulation. We further show that grappling hook-like structures on the cell surface of *Aureispira* might be involved in catching flagellated prey bacteria.

In summary, we comprehensively characterized a new mechanism of a bacterial predator to shape its ecological niche by using a novel CIS.

OTS5/5 - Optimized recombinant production of the bacteriocin garvicin Q by *Corynebacterium glutamicum*

Presenting Author - *Christian Desiderato, Institute of Microbiology and Biotechnology, Germany*

Author/s - *Carolin Müller, Alexander Schretzmeier, Katharina Hasenauer, Alexander Reiter, Valentin Steier, Bruno Gnannt, Marco Oldiges, Bernhard Eikmanns, Christian Riedel*

Abstract Content

Background: Bacteriocins are ribosomally synthesized antimicrobial peptides, that kill target bacteria or inhibit their growth. They are used in food preservation and are discussed as potential alternatives to conventional antibiotics. Basic research and potential applications are limited by availability of bacteriocins in sufficient amount and purity. To adress this problem, we recently established Corynebacterium glutamicum as production host for bacteriocins.

Objective: The aim of the study was to optimize recombinant production of the bacteriocin garvicin Q (GarQ) using C. glutamicum.

Methods: Automated strain construction was used to generate a library of genes for GarQ fused with different signal peptides. GarQ production was detected and quantified using live fluorescent biosensor cells. Chromatographic methods and mass spectrometry were applied to verify secretion and correct processing of GarQ.

Results: Secretion of GarQ via the Sec-system of C. glutamicum was established by screening different signal peptide – GarQ combinations. We observed that acidic pH and supplementation with CaCl2 and tween 80 reduced adsorption of GarQ to biomass of producing cells, improved stability of the product and accelerated recombinant GarQ production. Cultivation at limited oxygen supply led to increased GarQ production due to prevention of oxidation of the peptide. In addition, disruption of the secretion stress-related protease HtrA increased the recombinant production of GarQ. We produced about 100 µg GarQ ml-1 with our optimized set up, which is the highest amount of recombinant bacteriocin produced with C. glutamicum so far.

OTS5/6 - Membrane protein effectors are secreted by the *Legionella pneumophila* type IV secretion system via an inner membrane intermediat

Presenting Author - *Samuel Wagner, Interfaculty Institute for Microbiology and Infection-medicine Tübingen (IMIT), Germany*

Author/s - *Silke Malmsheimer, Iwan Grin*

Abstract Content

Type IV protein secretion systems are versatile molecular machines that facilitate the secretion of bacterial effectors into eukaryotic host cells. Besides secreting soluble effector proteins from the bacterial cytoplasm or the periplasm into host cells, T4SS were also shown to transport hydrophobic membrane proteins that find their final destination inside host cell membranes. While we could show previously that a substantial number of membrane effectors feature a reduced hydrophobicity of their transmembrane segments to avoid targeting to and insertion into the bacterial inner membrane, many membrane effectors are highly hydrophobic and the question arises whether these follow a two-step secretion pathway with an inner membrane intermediate.

Here we show by cell fractionation and proteomics analysis that sufficiently hydrophobic membrane effectors are integrated into the inner membrane of *Legionella pneumophila*.

Further data provide evidence that these proteins are extracted from the membrane towards the cytoplasm, facilitated by a cytoplasmically localized C-terminus and small periplasmic loops. The secretion of these membrane effectors is largely dependent on the T4SS chaperones IcmS and IcmW as well as a C-terminal signal but the secretion requirements are distinct from their soluble counterparts. Our work highlights once more the amazing versatility of T4SS in secreting effector proteins from different bacterial compartments and provides the first insight into the mechanism of membrane protein secretion by these secretion systems.

OTS5/7 - The inner membrane TonB protein as a novel antibiotic target

Presenting Author - *Isabelle SCHALK, University of Strasbourg, France*

Author/s - *Carsten Peukert, Véronique Gasser, Till Orth, Sarah Fritsch, Mark Brönstrup*

Abstract Content

Background: In Gram negative bacteria, most nutrients, like iron complexes, heme, carbohydrates, nickel complexes and vitamin B12 are transported by TonB-dependent transporters (TBDTs) across the outer membrane. The energy necessary for the uptake of molecules by these transporters is provided by the inner membrane protein TonB. TonB is in complex with two other proteins ExbB and ExbD in the inner membrane and forms a molecular motor that uses the proton gradient of the inner membrane to convey energy to TBDTs, allowing the active transport of nutrients into the periplasmic space.

Objectives: Our goal was to block the protein interaction between TonB and TBDTs, hoping that this blocks the import of nutrients and therefore bacterial growth.

Methods: 6 peptides inhibiting the interaction between TonB and TBDTs have been designed. The peptides designed were too large for a passive permeation through porins or the lipid bilayer of the outer membrane, and thus required a vectorization of the peptides to two synthetic siderophores (iron chelating compounds). The ability of these compounds to enter *Pseudomonas aeruginosa* cells and block bacterial growth was investigated.

Results: A few conjugates repressed bacterial growth in *P. aeruginosa* strains with minimal inhibitory concentrations of 0.5, 4 and 0.1 µM.

OTS5/8 - Virulence factors of the bacterial fish pathogen *Tenacibaculum maritimum*

Presenting Author - *Sophanit Mekasha, University of Oslo, Norway*

Author/s - *Dirk Linke*

Abstract Content

Background: A member of phylum Bacteroidetes *T. maritimum* is the causative agent of tenacibaculosis, a severe and economically important fish disease in the aquaculture industry worldwide. The genome of the gram-negative fish pathogen consists circular chromosome with 2866 predicted protein-coding genes. As the molecular virulence factors involved in tenacibaculosis outbreaks are not fully understood, existing prevention and treatment strategies of the disease are limited.

Background: The objective of the current study is to identify and characterize virulence factors involved in tenacibaculosis in the quest to develop reliable vaccines and treatments.

Methods: Five *T. maritimum* species, two commercial and three field isolates, are included in comparative studies of virulence mechanisms using molecular, proteomic and biochemical methods. In brief, combination of the complete genomic data of the *T. maritimum* type strain NCIMB 2154T and LC-MS proteomic analysis of the secretome of the five *T. maritimum* strains is used to identify the protein profiles of the strains. In addition, surface exposed virulence proteins are explored using surfaceome proteomic of the type strain.

Results: Both secretome and surfaceome proteomic data show the presence of varying degree of potential virulence proteins in all strains such as nucleases, adhesins, metallo-proteases, type IX secretion system, iron uptake systems and hemolysins.

Two multi-domain collagenases are among the abundantly secreted proteins in the secretome of the type strain, which confirms our earlier observation on the presence of gelatinase/collagenase activity in the secretome on gelatin from fish skin. Data from characterization of the two collagenases will be shown.

OTS6/2 - Membrane channel engineering for microbial cell factories

Presenting Author - *Liam Richard Jenkins Sánchez, Ghent University - Marine Biology Department, Belgium*

Author/s - *Lobke Sips, Inge Van Bogaert*

Abstract Content

Background: Microbial membranes are naturally impermeable to most compounds, meaning that specialized transporter proteins are needed to enable the import and export of biomolecules. This makes transporter a key, yet understudied area of engineering for cell factories like Escherichia coli. In particular, aquaporins (AQPs) are of great interest as they enable small polar molecule transport, many of which are of industrial concern, and they do not consume energy to function. Since their structure is well-described, they are also promising targets for engineering.

Objectives: Transmembrane channels specific for small polar molecules will be engineered to facilitate the transport of compounds of interest, while retaining intermediates. Focus is placed on polyols of industrial concern (1,3-propanediol) and C1 carbon substrates (CO2, methanol and others). These improved channels will be deployed in production strains to demonstrate their potential for cell factories.

Methods: Two strategies are used, the small scale testing of natural channel variants, and a large scale method involving semi-rational mutagenesis of channel residues involved in substrate selectivity. To screen these libraries, biosensors are used to measure intracellular substrate concentrations during transport, resulting in a scalable fluorescent output that can be analyzed in plate readers but also using Fluorescence Activated Cell Sorting (FACS).

Results: Biosensors for the detection of 1,3-propanediol and C1 substrates have been tested and developed, and AQPs are being screened for improved transport. The impact of AQPs in 1,3-PDO transport was confirmed experimentally with this methodology combined with channel deletion and reconstitution.

OTS6/3 - *Candida boidinii*, an oleaginous methylotrophic yeast species attractive as a platform for methanol-based biomanufacturing

Presenting Author - *Marta Neves Mota, iBB - Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Portugal*

Author/s - *Margarida Palma, Isabel Sá-Correia*

Abstract Content

Background: Methanol is an attractive non-food alternative feedstock for biobased manufacturing. However, methanol efficient use as carbon and energy sources is essential and methanol toxicity(1) can limit bioprocess productivity. Yeast diversity can be explored to search for robust native methylotrophic strains to be used as platforms for biomanufacturing, in particular to produce microbial oils, similar to vegetable oils for sustainable biodiesel production(2,3).

Objectives: Search for yeasts that combine methylotrophy and oleaginousness, isolated from different habitats. Molecular identification of *Candida boidinii* isolates and characterization of their methanol-based-growth and lipid(triacylglycerols) production.

Methods: Yeasts were isolated using YPD agar plates supplemented with chloramphenicol(4). For molecular identification, genomic DNA was extracted, using the phenol:chloroform:isoamyl alcohol method, and used as template for amplification of D1/D2 domain of 26S ribosomal DNA(rDNA) and the internal transcribed spacer (ITS) region of rDNA. Yeast growth(OD600nm), methanol consumption(HPLC), and lipid production(Nile Red staining and GC analysis) were assessed.

Results: Four *C. boidinii* strains were isolated from traditional (Mértola region, Portugal) curation of olives soaking waters, collected over 3 years. Three others were isolated from contaminated soil superficial layer from fuel stations. Growth medium optimization led to the selection of 2% (v/v) methanol for further studies. The 7 strains fully consumed methanol after, at the most, 40 h of cultivation in shake flasks, and accumulate lipids, both with different efficiencies. Adaptive laboratory evolution experiments are ongoing for robustness increase and improvement of native methylotrophy of *C. boidinii* as a promising platform for methanol-based biomanufacturing, in particular of microbial oils.

OTS6/4 - Construction of a genome reduced *Paenibacillus polymyxa* chassis

Presenting Author - *Giulia Ravagnan, University of Münster, Germany*

Author/s - *Meliawati Meliawati, Stephan Noak, Johannes Kabisch, Jochen Schmid*

Abstract Content

The demand for highly robust and versatile microbes is increasing in biotechnology. *Paenibacillus polymyxa* DSM 365 has great potential as a cell factory: it is non-pathogenic, various tools for genetic and genomic engineering are efficiently estabilshed and it presents a broad metabolic capability (production of antimicrobials and commercially relevant compounds, e.g. exopolysaccharide and 2,3-butanediol). Yet, optimisation of yields and rates are still required in order to reach industrial productions.

In this study, we initiated a top-down construction of a chassis strain of *P. polymyxa* aiming at enhancing its genomic stability and improving both its growth parameters and the production of 2,3-butanediol. Mobile genetic elements, phage-related genes and secondary metabolites clusters were removed through the utilization of a CRISPR-Cas9 system. Each generated knock out was tested for changes in biological fitness during growth both on rich and minimal medium. Additionally, the knockouts lacking the production of different antimicrobial peptides were further characterised for 2,3-butanediol production. Future work will sequentially combine the deletions of the best performing genome-reduced strains resulting in an improved *P. polymyxa* chassis.

OTS6/5 - Biosynthetic activity of *Streptomyces netropsis* IMV AC-5025 under that action of exogenous compounds

Presenting Author - *Mariia Loboda, Zabolotny Institute Of Microbiology And Virology, Ukraine*

Author/s - *Mariia Loboda, Liudmyla Biliavska*

Abstract Content

Background: Streptomycetes are known to be distributed in soil microbiocenosis. They produce a wide range of metabolites that improve the soil's phytosanitary conditions and provide a priming effect on plants. Streptomyces netropsis IMV Ac-5025 produces antibiotics, including polyenes, phytohormones (auxins, cytokinins, gibberellins, and abscisic acid), lipids, amino acids, peptides, etc. Polyene antibiotics are believed to be important to research because many aspects of their structure and biosynthesis haven't been studied. The aim of the work was to research the effect of the exogenous isopentenyl adenosine, indole-3-carbinol, β-sitosterol and their complex action on the polyene antibiotics and other biologically active substances biosynthesis by the strain.

Methods: Gas Chromatography, High-performance liquid chromatography, general microbiological and biochemical methods. Statistical analysis of the results was carried out by using ANOVA program. The correlation between the biosynthesis of polyene antibiotics and phytohormones and sterols by the producer was clarified and confirmed experimentally, which makes it possible to use factor analysis as a method of theoretical prediction. Exogenous compounds had different effects on the accumulation of polyenes and growth regulatory metabolites (auxins, cytokinins, sterols) in the producer biomass. Exogenous β-sitosterol caused super synthesis of polyenes by streptomycete and their excretion from the cells. The complex action of 12.5μg/mL of indole-3-carbinol and 5 μg/mL of β-sitosterol increased the polyene antibiotics biosynthesis 2.4-fold.

Results: These results are important for understanding the complex relationships of metabolic pathways in streptomycetes and provide an opportunity to create in one biotechnological process bioproducts with specified properties.

OTS6/6 - Bioengineering Sustainable Structural Colours in Flavobacteria

Presenting Author - *Laura Catón, University of Cambridge, United Kingdom*

Author/s - *Laura Catón, Alexander Campos, Daniel Dominguez-Pérez, Aldo Barreiro, Constantinos Patinios, Raymond Staals, Silvia Vignolini, Cambridge University, Cambridge, United Kingdoom*

Colin Ingham, Hoekmine BV, Utrecht, Netherlands

Abstract Content

Background: Colour is nearly ubiquitous within the living world and may provide sustainable colourants for industry. Structural colour (SC) is a consequence of light interacting with ordered nanostructures, and it is responsible for the most vivid and brightest colours in the natural world. Despite a plethora of work describing the optical physics and diversity of SC in living organisms, little is known about the underlying genes, biochemical pathways, and evolution of SC.

Objective: We used a model organism to understand SC, *Flavobacterium* IR1, which rapidly organises into an ordered nano-scale 2D periodic lattice crystalline colony, giving angle-dependent coloured patterning when illuminated with white light. We identified biologically relevant proteins and pathways involved in SC.

Methods: IR1 is genetically amenable, allowing exploration of the genes that specify colony organization and to create SC. We generated a transposon insertion mutant library, conducted comparative proteomic analyses on selected IR1 SC mutants and used these data to target a CRISPR-Cas12 gene editing system to modify key genes.

Results: A detailed picture of the relationship between genes and biochemical pathways is being developed and will be presented. For example, the figure shows a key transcriptional regulator (hypA16) in the turning of SC in IR1, the consequences of a KO on colour and cell morphology, a demonstration of genetic complementation and the consequences of this KO on the proteome. We work to understand SC and bioengineering new enhanced SC bacterial optical structures as new materials.

OTS6/7 - Metabolic burden and resource allocation in engineered *Pseudomonas putida* strains: the fate of amino acids

Presenting Author - *Marleen Beentjes, Technical University Munic, Germany*

Author/s - *Andreas Kremling, Katharina Pflüger-Grau*

Abstract Content

The biotechnological production of heterologous proteins is often limited by metabolic burden as it interferes with the host's cellular capacity to allocate resources. When cellular resources are drained towards heterologous gene expression, additional stress is created, which often results in low host productivity and a decrease in the growth rate. In this study, the goal was to investigate whether resupplying cellular resources (in our case amino acids) could help to alleviate metabolic burden and boost heterologous protein production in engineered *Pseudomonas putida*. To this intend, four different synthetic fusion-proteins were designed, consisting of eGFP fused to proteins with varying size and amino acid composition. The resulting strains were grown in minimal medium, with and without amino acid supply and bacterial growth, protein production rates and amino acid uptake were analysed. Amino acids can have different faiths once taken up; they can be used as building blocks for protein synthesis, as energy source, or as nitrogen source, and/or as carbon source. Interestingly, we could show that the size of the heterologous protein does not have a direct influence on the metabolic burden arising in *P. putida*. The supplementation of amino acids to metabolically burdened strains reduced the duration of the lag phase and promoted growth, while, surprisingly, the protein production rate decreased. This decrease in protein production rate became less apparent when the translation rate of the fusion protein was increased. This hints towards a non-equal distribution of the additional resources between growth-related processes and processes involved in heterologous protein production.

OTS6/8 - Effects of dynamic substrate supply on methanogenesis

Presenting Author - *Wenyu Gu, Swiss Federal Institute of Technology Lausanne, Switzerland*

Author/s - *Jörg Deutzmann, Nicole Matis, Alfred Spormann*

Abstract Content - Bioelectrochemical power-to-gas presents a promising technology for CO2 reduction and long-term storage of excess renewable energy in the form of methane. The core of this technology- biocatalytic conversion of CO2 to methane was carried out by (mainly hydrogenotrophic) methanogen (Deutzmann et al., 2015; Kracke et al., 2020). The successful application of the technology can be promoted by better understanding of the physiology of methanogen, especially methanogenesis activities under different substrate condition and intermittent supplies.

In batch cultures, we determined Km for CO2 in a mesophilic methanogen *Methanococcus maripaludis* and a thermophilic methanogen *Methanothermobacter marburgensis*, showing their capability to reduce CO2 at rates close to umax when CO2 is low. We then cultivated *M. maripaludis* in chemostat reactors and showed that H2 and CO2 limitation did not affect the yield or methanogenesis activity. To explore the effect of intermittent substrate on methanogen, we investigated *M. maripaludis*' recovery after being starved for H2 vs. CO2 using batch cultures. We found that *M. maripaludis* is more robust for H2 starvation as compared to CO2. Yet, the difference is only evident when cells are starved for more than 3 days: longer lag phases and lower initial methanogenesis rates were observed when *M. maripaludis* was starved for CO2 vs. H2 for 3 days and 5 days, respectively. These characterisations show methanogenesis is a robust platform for power-to-gas as the intermittent power supplies from solar or wind.

OTS6/9 - Development of efficient bioprocesses using isolated marine bacteria

Presenting Author - *Carla C. C. R. de Carvalho, Instituto Superior Tecnico, Portugal*

Author/s - *Carlos JC Rodrigues, Ricardo FS Pereira*

Abstract Content

The oceans cover over 70% of the surface of the Earth, but their biological resources are largely underexplored. Marine bioprospecting allows the targeted and systematic quest for enzymes and bioactive compounds required for e.g. the health, food and pharmaceutical industries. Using proper culturomics techniques, we isolated hundreds of bacterial strains from marine samples collected biodiversity hot-spots, including extreme environments. We showed that these techniques allow the isolation of ca. 45% of the bacterial cells present in a marine sample, thus overcoming the dogma that most marine bacteria do not grow under laboratory conditions. The isolation allowed the assessment of the biocatalytic properties of the cultivated bacteria and the search for commercially interesting compounds. Among the enzymes detected were lipases/esterases, transaminases, inulinases, hyaluronidases, and cellulases. High throughput screening methods were developed to accelerate the discovery of novel biocatalysts, whilst bioreactor design improved production yields and downstream processing. A *Bacillus subtilis* strain exhibiting significant inulinase activity allowed the production of 2.2 g sugars/(g protein.h) in a stirred bioreactor, while cells of bacterium *Glutamicibacter arilaitensis* immobilized in a continuous packed bed reactor produced 1.16 g of benzyl alcohol/(gCDW.L.h). A *Serratia rubidaea* strain, isolated from a shallow water thermal vent, was used to produce prodigiosin, reaching a productivity of 40.8 mg/(L.h). The results clearly demonstrate the benefit of using marine resources for biotechnological applications.

OTS7/2 - Antibiotic-resistant bacteria in the air of Poznań, Poland

Presenting Author - *Ryszard Koczura, Adam Mickiewicz University Poznań, Poland*

Author/s - *Joanna Mokracka*

Abstract Content

Background: Antibiotic resistance concerns not only clinical isolates, but also strains from various environments. Little is known, however, about antibiotic-resistant bacteria in the air, an unfavorable environment for microorganisms, in which they usually cannot grow.

Objectives: The aim of the study was to evaluate the presence of bacteria resistant to selected antimicrobials in the outdoor air in Poznań, Poland, and to determine the presence and quantity of genes conferring that resistance as well as integrons' integrase genes.

Methods: Air samples were taken by using a portable air sampler and gelatin membrane filters. Antibiotic-resistant bacteria were isolated on BHI agar supplemented with tetracycline, cefotaxime or ciprofloxacin. Integron-integrase genes and antibiotic resistance genes (ARGs) were detected by conventional PCR. Quantitative analysis of ARGs was carried out with the use of digital PCR.

Results: The number of bacteria resistant to tetracycline was equal to 5.0×10^2 cfu/m3, to cefotaxime – 0.9×10^2 cfu/m3, to ciprofloxacin – 1.1×10^2 cfu/m3, whereas the number of bacteria harbouring class 1 integrons amounted to 7.6×10^2 cfu/m3.

Resistance to tetracyclines was conferred by tet(A), tet(B), tet(C), tet(E), and tet(M) genes. Cefotaxime-resistant isolates carried blaCTX, blaSHV, blaTEM, blaOXA, blaGES, and blaVEB genes. Among fluoroquinolone resistance genes, qnrA, qnrB, qnrD, and qepA were detected. The variable region of class 1 integrons contained gene cassettes responsible for resistance to aminoglycosides (aadA) and trimethoprim (dfrA). The concentration of selected ARGs in the air metagenome varied from 2.5×10^3 to 4.5×10^4 copies/m3.

OTS7/3 - Priority antibiotic-resistant pathogens get a ride with microplastics in rivers

Presenting Author - *Isabel Silva, University of Coimbra, Portugal*

Author/s - *Marta Tacão, Isabel Henriques*

Abstract Content

This study aimed to characterize priority antibiotic-resistant pathogens colonizing microplastics in rivers.

For this, sterilized particles (0.5 to 1 mm) of polypropylene (PP), polyethylene (PE) and a mixture of PP, PE and polyethylene terephthalate (PET) were submerged in a polluted Portuguese river. After 30 days, bacteria colonizing microplastics were cultivated in m-FC supplemented with cefotaxime, ciprofloxacin or meropenem. Isolates were typed by BOX-PCR, identified and Enterobacteriaceae isolates were chosen for further characterization using conventional molecular methods and/or genome sequencing.

Isolates belonged to Escherichia (n=38), Shigella (n=1), Klebsiella (n=9), Raoultella (n=2), Citrobacter (n=11) and Enterobacter (n=5) and 86% were multi-drug resistant. Of 11 carbapenem-resistant Enterobacteriaceae, 10 harbored carbapenemases encoding genes [blaKPC (n=8) and blaGES (n=6)]. Two KPC producers were able to transfer blaKPC to a receptor strain through conjugation. Cefotaxime-resistant strains (n=23) harbored blaCTX-M (70%), blaTEM (61%) and blaSHV (39%). All CTX-M producers harbored the intI1 gene and 63% were able to transfer blaCTX-M to a receptor strain. The sequence type (ST) for *E. coli* and *K. pneumoniae* isolates harboring blaCTX-M or blaKPC included clonal lineages recognized as high-risk clones (i.e. ST10 or ST131, and ST15 or ST17, respectively). Enterobacteriaceae-resistant to ciprofloxacin (n=53) harbored plasmid-mediated quinolone resistance genes [qnrS (30%), qnrVC (8%), qnrB (25%) and aac(6')-Ib-cr (40%)] and intI1 (64%). Mutations in gyrA (70%) and parC (72%) genes were also frequently detected.

Our results showed that microplastics may serve as a vector for the proliferation and dissemination of priority antibiotic-resistant pathogens harboring antibiotic-resistance genes of clinical concern.

OTS7/4 - Ecology of hospital sinks inter-kingdom microbiome and its role as antimicrobial resistance reservoirs

Presenting Author - *Ana Elena Pérez-Cobas, Instituto Ramón Y Cajal De Investigación Sanitaria (irycis), Spain*

Abstract Content

Background: Water sinks of hospital wards harbour microbial communities that could carry antibiotic-resistant commensal opportunistic pathogens (COPs) and act as antimicrobial resistance (AMR) reservoirs. Those communities persist in abiotic surfaces through mechanisms such as biofilms, where complex microbial interactions occur. However, the “sink microbiome” ecology is primarily unknown.

Objectives: This work aims (1) to characterise the composition and dynamics of bacterial, fungal and protozoa populations inhabiting hospital sinks and (2) to decipher the interactions of transient COPs with resident microbes.

Methods: Sinks from 14 rooms in a hospital ICU ward were sampled every three months for two years (2021-2023). The characterisation of the bacterial, protozoa and fungal communities was based on 16SrRNA, 18SrRNA and ITS Illumina sequencing. The bioinformatics and statistics were performed with QIIME2 and R software to detect putative microbial interactions. In addition, >1000 isolates from COPs were identified (MALDI-TOF) and screened for blaESBL/blaMBL genes (Multiplex-PCR). Protozoa were isolated on 2%-TSA plates to characterise bacteria-protozoa interactions.

Results: The inter-kingdom microbial diversity varied significantly over time (Kruskal-Wallis p-values<0.05) but not between sinks (p-values>0.05). However, significant differences in composition occurred between sinks and over time (PERMANOVA, p-values<0.05). In contrast, ~20 bacterial species were consistently recovered, highlighting the stability of specific taxa in the sink environment, including COPs. The culturomics detected carbapenemases (219)/ESBLs (335) in ~20 species comprising pathogens. In addition, amoeba-bacteria interactions were experimentally identified. Inter-kingdom microbial interactions could contribute to ecosystem stability and should be considered in preventive strategies to control nosocomial outbreaks and AMR transmission in hospitals.

OTS7/5 - Exploring the in-situ evolution of Nitrofurantoin resistance in clinically derived Uropathogenic *Escherichia coli* isolates.

Presenting Author - *Phillip Aldridge, Newcastle University, United Kingdom*

Author/s - *Maxime Vallée, Chris Harding, Judith Hall, Aaron Tan*

Abstract Content - Nitrofurantoin has been re-introduced as a first-choice antibiotic to treat uncomplicated acute urinary tract infections in England and Wales. Its mode of action involves initial reduction by nitroreductases, to generate electrophilic intermediates that inhibit protein and nucleic acid synthesis. Highly effective against common uropathogens such as *Escherichia coli*, its use is accompanied by a low incidence (<10%) of antimicrobial resistance. Resistance to Nitrofurantoin is predominantly via the acquisition of loss-of-function, step-wise mutations in the nitroreductase genes nfsA and nfsB.

To explore the in situ evolution of NitR, longitudinal uropathogenic *E. coli* isolates recovered from two rUTI patients, were exploited. Growth rate analysis identified a 2-10% slower doubling time for Nitrofurantoin resistant strains, but statistically, these data suggested there was no fitness advantage of evolved strains over their sensitive predecessor (ANOVA P-value = 0.13). Genetic manipulation of E. coli to mimic nitrofurantoin resistance evolution, again confirmed no fitness advantages (ANOVA P-value = 0.22). Rather, further analysis argued that a first-step mutant gained a selective advantage, at sub-MIC (4-8 mg/L) nitrofurantoin concentrations.

Correlation of these findings to Nitrofurantoin pharmacokinetic data suggests that the low incidence of *E. coli* NitR, within the community, is driven by urine-based nitrofurantoin concentrations that selectively inhibit the growth of *E. coli* strains carrying the key first-step loss-of-function mutation.

OTS7/6 - Characterization and genome analysis of a novel phage ϕEF212 infecting vancomycin-resistant *Enterococcus faecalis*

Presenting Author - *Aylin USKUDAR GUCLU, Baskent University, Turkey*

Author/s - *Suleyman Yalcin*

Abstract Content

This study aimed to isolate and characterize a lytic *E. faecalis* bacteriophage to provide the basis for the development of new therapeutic agents against vancomycin-resistant *Enterococcus faecalis* infection. The microbiological, physicochemical and genomic characterization of isolated phage was demonstrated by host range analysis, one-step growth curve, MOI, adsorption assay, thermal and pH stabilities, transmission electron microscopy (TEM). Whole-genome-sequencing of was performed by nanopore-sequencing. The complete genome sequence was assembled using *Shasta*, and then annotated using RASTtk. *VIRIDIC* was used to calculate the intergenomic similarities. The phylogenetic trees were constructed (MegaX) based on aminoacid sequences of major capsid protein and of large terminase subunits. The presence of tRNA, 16S rRNA, antibiotic resistance, pathogenicity, and virulence genes were investigated by using tRNAscan-SE 2.0, BLASTn against 16S ribosomal RNA sequences, VirulenceFinder 2.0, ResFinder 4.1, and PathogenFinder respectively. Genome of the phage was mapped by CGview. TEM analysis demonstrated that belong to *Siphoviridae*. ϕEF212 has a genome 40.690 kbp with 35.7% G+C content, and contains 67 functional genes. ϕEF212 shared the closest relationship with phage EFACPT1 (88.35%) with the query-coverage 89%. There were no tRNAs, 16S rRNA and virulence or resistance genes in phage's genome. ϕEF212 has lytic activity against some vancomycin-resistant, -susceptible clinical isolates and *E. faecalis* ATCC29212. The burst size was 4200 PFU/ml and latent period was 20 min, and the optimal MOI was 0.01. ϕEF212 had the lysis-related-genes, N-acetylmuramoyl-L-alanine amidase and holin. The microbiological, physicochemical and genomic characteristics of this phage suggest that it may represent candidate therapeutic agents against *E. faecalis* infection.

OTS7/7 - Mechanistic evaluation of the treatment sequence on phage-antibiotic synergistic combinations against multidrug-resistant AB2

Presenting Author - *Subhankar Mukhopadhyay, The Chinese University of Hong Kong, Hong Kong*

Author/s - *Pengfei Zhang, Kenneth K.W. To, Yannan Liu, Changqing Bai, Sharon S.Y. Leung*

Abstract Content

Background: Antimicrobial resistance (AMR) is a complicated global issue with potentially disastrous health and economic implications. Phage-antibiotic synergy (PAS) is one of the promising treatment strategies to counter the AMR-crisis.

Objectives: This study aims to mechanistically evaluate the treatment sequences on PAS combinations against a multidrug resistant (MDR) strain of *Acinetobacter baumannii* (MDR-AB2).

Methods: Phage vB_AbaM-IME-AB2 (IME-AB2 in short) and colistin were used as the model phage and antibiotic to screen for optimal combination ratio demonstrating PAS against MDR-AB2 using a modified checkerboard assay. Three treatment sequences were studied: (1) phage-first, (2) simultaneous and (3) antibiotic-first. Studies investigating the bacterial morphologies, colistin binding and outer membrane perturbation and phage-resistance development under different treatment sequences were conducted to correlate with the overall antibacterial performance.

Results: Treatment order plays crucial roles on killing planktonic bacteria and removing biofilms (phage first > simultaneous > antibiotic first) of the optimized PAS combinations. Pre-treating bacteria with phage prior to colistin addition decapsulated the bacteria to improve colistin binding, promoting the colistin-mediated killing. For simultaneous treatment, the concomitant addition of phage could also promote the colistin binding and facilitate the outer membrane disruption for better bacterial killing. Surprisingly, pretreating bacteria with colistin only slightly improve the antibacterial efficiency. Among the three studied treatment sequences, the suppression of phage-resistance development via simultaneous administration of phage and antibiotic while maintaining an acceptable antibacterial efficiency can address a key need for phage therapy.

OTS8/2 - Bacteriocins as microbiome-editing tools in a simplified human intestinal microbiota

Presenting Author - *Natalia Soledad Rios Colombo, APC Microbiome Ireland, Ireland*

Author/s - *Jessey-Joy Siebert, Mariana Perez-Ibarreche, Lorraine Draper, Des Field, Paul Ross, Colin Hill,*

Abstract Content

Background: Bacteriocins are antimicrobial peptides produced by bacteria of many genera that have been studied for decades as food bio-preservatives or as alternatives to antibiotics. They also have potential as precise modulators of the gut microbiome, which is linked to human health. However, rigorous evidence is required to determine if bacteriocins can act as microbiome-editing tools to shape communities in a desirable direction.

Objectives: We assess the effect of different bacteriocins on a Simplified Human Intestinal Microbiota (SIHUMI), using a set of bacteriocin-producing strains (Bac+) and their corresponding isogenic non-producers (Bac-). Bacteriocins from different classes and spectrum of activity were selected, including lantibiotics and pediocin-like peptides.

Methods: SIHUMI is a bacterial consortium of seven diverse human gut species that can be individually tracked in a complex media using qPCR. The Bac+ and Bac- strains were superimposed on the SIHUMI system, and samples were taken at intervals up to 48 h for genomic DNA extraction. The genome copy number of each SIHUMI member was evaluated using specific primers.

Results: We were able to determine the behavior of the consortium over time and evaluate how the system is impacted by different bacteriocin producers. Our results show that it is possible to shape the composition of the community in a predictable way by targeting multiple members or specific members with either broad or narrow spectrum bacteriocins. While we recognize that SIHUMI is a simplified model, it provides useful insights into the possible mechanisms by which the microbiome could be shaped by bacteriocins.

OTS8/3 - Regulation of microbiota-derived GABA within the human gut ecosystem

Presenting Author - *Benoit Pugin, ETH Zurich, Switzerland*

Author/s - *Kun Ye, Nize Otaru, Anouk Wandeler, Anna Greppi, Markus Arnoldini, Christophe Lacroix, Kun Ye, Nize Otaru, Anouk Wandeler, Serafina Plüss, Denisa Mujezinovic, Anna Greppi, Markus Arnoldini, Christophe Lacroix, Benoit Pugin*

Abstract Content - The neurotransmitter GABA is produced by certain gut commensals through the glutamate decarboxylase (GAD)-system and may play a role in maintaining mental health. However, the primary taxa and factors contributing to microbiota-derived GABA production remain unexplored. This study fill this gap by using comparative genomics, transcriptomic and metabolic assays, and in vitro intestinal and gnotobiotic models.

Results showed that GABA production is a prevalent trait in the highly abundant intestinal genus Bacteroides, with >94% harboring the GAD-system and producing GABA. To understand GABA regulation, we examined the function of the GAD-system in B. thetaiotaomicron. We generated GAD-system mutants (ΔgadB; ΔglsA) and found that GABA protect cells against acid stress. Further, the expression of gadB and glsA was upregulated (>2-fold) in response to acidic and osmotic stress. Importantly, Bacteroides-derived GABA was produced at pH and osmolality of the proximal colon (pH5.8; 340mmol/kg).

We confirmed our findings using in vitro intestinal models with fecal-derived microbiota (n=5), with pH and osmotic variations altering GABA levels. Higher GABA was associated with Bacteroides and Bifidobacterium. In gnotobiotic mice (minimal communities), the pH of the cecum was inversely correlated with cecum GABA levels (r=-0.82, p=0.01), with more GABA observed in gnotobiotic vs germ-free mice (468.0±57.7 vs 76.6±4.5µmol/g feces; p=0.0007).

Together, gut bacteria contribute to GABA levels, with pH and osmolality representing key regulating factors. The intestinal physicochemical conditions thus play a key role in regulating microbiota-derived biomolecules. Future studies will explore the precise interactions of microbiota-derived GABA with intestinal/peripheral tissues and mental health.

OTS8/4 - Deconstructing synthetic communities to understand the plant microbiome

Presenting Author - *Omri Finkel, The Hebrew University, Israel*

Abstract Content

How plants evolved to shape their microbiota is a long-standing question in ecology and evolution. The microbiome forms a crucial part of how plants adapt to changing environments and that this microbiota optimization should be manifested in a strong genomic and phenotypic signature of adaptation. To study plant adaptation to cooperation with their microbiota, we must develop methods to carefully manipulate the soil microbiota which serves as the primary source of microbes for the plant. To understand how interactions within the microbiome affect plant health, my colleagues and I have been designing and constructing plant-microbe microcosms encompassing synthetic communities composed of hundreds of microbial isolates. By editing these communities, we are able to identify keystone elements in the community with strong effects on plant health.

OTS8/5 - Analysis of the fungal plastisphere and its biodegradation potential

Presenting Author - *Aurélie Philippe, LUBEM, Univ. Brest, France*

Author/s - *Cyril NOËL, Camille LACROIX, Anne-Laure CASSONE, Marie SALAUN, Jean-François BRIAND, Jean-François GHIGLIONE, Emmanuel COTON and Gaëtan BURGAUD, Univ Brest, INRAE, Laboratoire Universitaire de Biodiversité et Écologie Microbienne, F-29280 Plouzané, France*

Abstract Content

Background: Environmental contamination by plastic is a worldwide problem. Recent research have highlighted the plastic degradation potential of marine microorganisms, including marine fungi. This raises questions about the diversity, distribution and activity of fungal communities associated with marine plastic waste, as well as their potential to degrade plastic polymers.

Methods: In the framework of the French ANR project MycoPLAST, different types of plastics, voluntarily immersed in various coastal marine habitats, were collected and analyzed using culture-independent and -dependent approaches.

Objectives: The aim was to explore the plastic-associated mycobiome, via metabarcoding analyses, targeting the V4-V5 18S rRNA gene and ITS2 region, and culturomics (i.e. high-throughput culturing).

Results: Using metabarcoding, our results show that fungal communities associated with plastic polymers differed from those found in the surrounding waters. They also significantly differed between sampling sites and polymer types (biodegradable/conventional). Temporal changes of colonization patterns were also observed after about 30 days, which may be related to the switch of the original microbial biofilm into biofouling communities. Overall, this work validates the view that the fungal kingdom is an integral part of the "plastisphere". Using culturomics, over 2000 fungal isolates were obtained from marine plastic waste. A laser nephelometry approach, dedicated to high-throughput screening of fungal isolates, was applied to assess their ability to utilize different kind of plastics (PE/PHBV/PS/PCL/PET/PVC) as a single carbon source and highlighted 16 promising fungal isolates (out of 500 tested to date) with a limited or broad spectrum of use (use of a single or multiple polymers).

Acknowledgements/References - This project was funded by the French National Research Agency in the framework of the ANR-19-CE04-0001 Mycoplast project.

OTS8/6 - Benchmarking shallow metagenomics using synthetic communities

Presenting Author - *Nicole Treichel, RWTH Aachen University Hospital, Germany*

Author/s - *Charlie Pauvert, Thomas Hitch, Thomas Clavel*

Abstract Content

Next-generation sequencing techniques are essential for microbiome research. However, their widespread use contrasts the low number of studies testing their output. Shallow metagenomics (sMG) is an emerging technique to overcome the main limitations of the two most common approaches, 16S rRNA gene amplicon and deep shotgun sequencing.

Here we systematically assessed the effects of shotgun sequencing depth on taxonomic and functional readouts using comprehensive datasets from synthetic microbial communities of multiple complexities.

Six synthetic communities of varying complexity (10-70 strains) and two abundance profiles (equal vs. staggered) were sequenced at up to nine depths (0.1-10 Gb/sample). Background effects are tested by spiking DNA extracted from the intestinal content of germfree mice. The genomes of all strains are used as references during analysis, enabling both de-novo and targeted analysis.

Main readouts include: (i) number and abundance of strains; (ii) occurrence of spurious taxa; (iii) number and diversity of genes and functional pathways; (iv) strain-level resolution analysis; (v) metagenome-assembled genome (MAG) reconstruction and their quality. Our analyses show that sequencing depth can be chosen according to abundance variance and expected diversity of the microbial environment, as well as by the read-outs to be generated. Analyses so far indicate that less than 1 Gb/sample can be sufficient for most analysis, except accurate MAG reconstruction.

This work will provide guidelines for researchers to optimize costs without sacrificing accuracy and maximise the benefit of metagenomic data. The high-complexity communities generated in this work are available as ready-to-use DNA stocks.

OTS8/7 - Metaproteomics: an effective tool in the meta-omics toolbox

Presenting Author - *Tim Van Den Bossche, Belgium*

Abstract Content

Background: Microbial communities are major drivers of biogeochemical cycles, vital components of the biosphere including plants and animals, and key factors in human health. High-throughput genome sequencing technologies have revealed the immense diversity of microbes and the complexity of the ecological interactions within microbiomes. In the past, deciphering the functioning of these complex microbial systems has been a daunting challenge, but is now becoming more approachable due to recent technological advances that allow diving much deeper into the functional biomolecular machinery. Metaproteomics is one such emerging approach that is beginning to dramatically advance our understanding of microbiome functioning through analyzing the spatio-temporal expression of microbial genes and dynamics within a microbial consortium. It therefore perfectly complements the genetic potential and gene expression information obtained through metagenomics and metatranscriptomics, respectively. In addition, metaproteomics is also highly complementary to metabolomics in explaining the phenotype of biological systems. Importantly, due to sequence variation among metabolic enzymes, metaproteomics data provides insight into "which organism is doing what" in a microbiome ecosystem and enables identification of specific members of the microbiome that are responsible for a change in abundance of a given metabolite.

Objectives and results: During this presentation, an overview of the possibilities of metaproteomics will be given, the robustness of the field will be demonstrated, and a short explanation about the Metaproteomics Initiative will be provided.

OTS8/8 - Capturing the human gut volatilome: non-destructive, continuous VOCs detection during colonic in-vitro fermentation

Presenting Author - *Andrea Dell'Olio, Wageningen University & Research, Netherlands*

Author/s - *Franco Biasioli, Vincenzo Fogliano, Josep Rubert*

Abstract Content

Background: Current human gut metabolomic research focuses on microbial non-volatile metabolites, leaving volatile organic compounds (VOCs) as a missing component.

Objective: This research effort intends to provide more in-depth information on how gut microbiota process food components during fermentation by merging non-invasive, continuous analytical approaches with an in-vitro gut simulator to track the time evolution of small molecules released by gut microbial consortium into the headspace.

Methods: Due to their complementary analytical capacities, two techniques are presented for efficient separation and identification of analytes and rapid quantitative analysis without sample preparation. The proposed approaches in this investigation are automated Head-space Solid Phase Micro Extraction coupled with Gas Chromatography-Mass Spectrometry (HS-SPME-GC-MS) and Proton Transfer Reaction Time of Flight Mass Spectrometry (PTR-ToF-MS). The goal is to link GC-MS profiling with PTR-ToF-MS untargeted detection to be able to track a subset of masses of interest to reveal concentration change during gut fermentation.

Results: The anaerobic in-vitro gut fermentation performed directly in 20mL vials followed over 24 hours by HS-SPME-GC-MS and PTR-ToF-MS was able to detect the release in time of several short chain fatty acids (SCFAs) and medium chain fatty acids (MCFAs) derived from 24h oat bran fermentation. The detected acids were co-released after 4 hours of fermentation and their relative abundance increased in time as showed for butanoic acid. The information collected can be used to analyse the dynamics of bacterial foraging on complex undigestible food substrates to gain new mechanistic insights on the gut bacterial ecosystem.

OTS9/2 - *Candida glabrata*'s genomic plasticity shapes the clinical evolution of biofilm formation

Presenting Author - *Inês Vieira Costa, iBB - Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Portugal*

Author/s - *Maria Zolotareva, Mafalda Cavalheiro, Mónica Galocha, Miguel Cacho Teixeira*

Abstract Content

Candida species are the most common cause of fungal infections worldwide. *Candida glabrata* is increasing in prevalence, which is particularly concerning due to its intrinsic resistance to azoles, and emerging resistance to other antifungals. Its virulence is also attributed to the ability to adhere to host mammalian cells, other microbes, and abiotic surfaces, like catheters, to form biofilms. Although biofilms are of great advantage to pathogenic yeasts, there appears to be little information on how the ability to make biofilms evolves within clinical isolates. To shed some light on the issue, we analysed the phenotypic variability of biofilm formation of a collection of *C. glabrata* clinical isolates. Next, we paired a comparative genomics approach with experimental microevolution of clinical isolates towards a stronger biofilm phenotype, to identify key regulators or effectors of biofilm formation in *C. glabrata*. We found that specialization towards increased biofilm formation occurs rapidly, adhesin remodelling and polymorphism accumulation in effectors of different regulatory levels: from epigenetic to post-translational. We also characterized the clinical and evolved isolates through virulence and adhesion assays. Some evolved isolates show a drastic increase in adherence to epithelial cells, but there is no clear correlation between biofilm formation and virulence/adhesion between all clinical isolates. Nevertheless, we were able to identify genes with a predicted role in biofilm formation, from uncharacterized adhesins from the EPA and PWP gene families, to transcription factors and telomeric silencing proteins. Those genes appear as promising targets for biofilm disruption and markers of biofilm evolution.

OTS9/3 - How did the modern western lifestyle influence the evolution of *Candida albicans*?

Presenting Author - *Mathias Jansen, Leibniz Institute for Natural Product Research and Infection Biology, Germany*

Author/s - *Mathias Jansen, Sascha Brunke, Bernhard Hube*

Abstract Content

In modern Western societies, the yeast *Candida albicans* is frequently found as a commensal member of the gut microbiota, which serves as a reservoir for severe infections. The human gut microbiota has, however, changed significantly during industrialization. Interestingly, colonization with *C. albicans* was found to be rare in human populations which remained mostly isolated from Western influences [1]. Furthermore, environmental *C. albicans* strains are very rarely described and investigated. Here, we examined over 30 non-western-lifestyle-adapted ("non-adapted") strains from the environment, isolated human populations, and animals to elucidate the changes *C. albicans* has undergone during its co-evolution with humans.

We found that the human-derived strains damaged epithelial cells more than the environmental isolates. Some of the commensal human isolates caused even more damage than important highly virulent clinical isolates. Furthermore, when testing for in vitro growth with different dietary sugars, most of the environmental strains showed reduced growth as compared to the standard clinical reference strain. Importantly, several non-adapted strains showed resistance against typical antifungals indicating a non-clinical source of antifungal resistance.

To understand how *C. albicans* has adapted to the conditions of the modern western gut, we performed laboratory evolution experiments. Non-adapted strains were passaged for several months in media containing different types of dietary sugars, simulating important aspects of the Western diet. A direct comparison showed that after the adaption, the strains grew better with their selected for dietary sugar but showed reduced growth with other dietary sugars. Interactions with host cells and possible antifungal resistances will be investigated.

OTS9/4 - Predatory microeukaryotes in wastewater treatment plants: Diversity & Function

Presenting Author - *Kenneth Dumack, University of Cologne, Germany*

Author/s - *Kenneth Dumack, Jule Freudenthal, Nils Heck, Nina Pohl, Marcel Dominik Solbach*

Abstract Content

In general, microeukaryotes (protists) are poorly investigated as molecular tools underlie numerous biases that do not apply to research with prokaryotes. Accordingly, wastewater has a long history of microbiologists' interest, but so far almost the entirety of studies focussed on prokaryotes.

I will present a summary of four recent studies where we explored the whole wastewater microbiome from 13 wastewater treatment plants (Freudenthal et al. 2022, Pohl et al 2021, Solbach et al. 2021, Heck et al. (unpublished)). Via a combination of various meta-omics techniques, we were finally able to assess the whole wastewater microbiome, including prokaryotes, fungi, protists, and microscopic metazoa.

We focussed our analyses on the biotic interactions in wastewater. Our findings comprise several novel insights: 1. Microeukaryotic predators shape the community composition of prokaryotes and thus are integral for well-performed wastewater treatment. 2. Microeukaryotic gut parasites are highly active in wastewater but are effectively reduced during denitrification, likely also partly due to microbial predation. 3. The so-far overlooked, but most abundant wastewater protist worldwide, Rhogostoma minus, hosts Legionellales and potentially functions as a vector for Legionnaire's disease in humans.

I will illustrate that with the now available tools, it is finally time to study microbial eukaryotes in wastewater.

OTS9/5 - A systematic evaluation of the potential anti-parasitic activity of low molecular weight heparins against human malaria parasite

Presenting Author - *Muqdad Hmoud, American University of Iraq-Sulaimani, Iraq*

Author/s - *Muqdad Hmoud, Paul Horrocks*

Abstract Content - Malaria disease still poses a global burden and challenge, through the spread of antimalarial drug and insecticide resistance. Despite the efforts in malaria eradication and elimination, malaria is still responsible for 627 000 deaths and 241 million infection cases world wides according to the World Health Organization report 2020.

There is an urgent need to develop a new approach for malarial treatment to aid the current advances in antimalarial drug development. Anticoagulants of unfractionated and low molecular weight heparins have been used in the treatment and prevention of thrombotic events such as deep vein thrombosis and pulmonary embolism. Unfractionated heparin as an antithrombin agent has been reported to pose an inhibitory effect against intra-erythrocytic stage infection of *P. falciparum in vitro*, the most virulent among other malaria parasites. The inhibitory activity of heparin seems to be mediated via targeting initial events of merozoite invasion to erythrocytes leading to the parasite's erythrocytic life cycle inhibition. Here we report the anti-plasmodial activity of different commercially available low molecular-weight heparins using a novel approach to measuring cell viability**.**

OTS9/6 - An in vivo mouse model of Prototheca infection

Presenting Author - *Tomasz Jagielski, University Of Warsaw, Poland*

Author/s - *Angelika Proskurnicka, Mateusz Iskra, Agnieszka Kwiatek*

Abstract Content

Prototheca spp. are unicellular, achlorophyllous algae, which are the only known plants capable of causing opportunistic infections (protothecosis) in animals and humans.

The aim of the study was to explore the virulence of the Prototheca algae in vivo using a murine model of infection.

Three pathogenic (*P. wickerhamii, P. bovis, and P. ciferrii*) and one saprophytic (*P. stagnora*) species were used to experimentally inoculate mice of both immunocompetent and immunodeficient phenotype. The study was carried out on 54 groups of 10-week-old, female mice (6 individuals per each), depending on the inoculum (algae or PBS as a control), challenging dose (i.e. 5x106 or 5x107 CFU/mL), and inoculation route (subcutaneous, intramammary, and intraperitoneal). Six weeks post-infection, the animals were euthanized, and their organs were explanted, weighted, homogenized, plated on Sabouraud agar, and incubated for 72 h at 30°C.

A fifth (19.4%) of wild-type mice and almost twice that (37.5%) of immunodeficient animals showed signs of infection. *P. ciferrii* accounted for the majority of infections (45.1%), followed by *P. bovis* (34.2%), and *P. wickerhamii* (20.7%). The intramammary route was the most efficient resulting in over a third of all infections. Among healthy mice, the bulk of infections were due to *P. ciferrii* (71.4%), mostly as a result of subcutaneous inoculation (46.4%). Whereas among immunosuppressed animals, most of infections were induced intraperitoneally (37%) with *P. bovis* as the major pathogen (37%). In both groups of animals, the most commonly affected organs were mammary glands. No infection was produced upon inoculation with *P. stagnora*.

OTS9/7 - The role of the nitrogen limitation in the pathobiology of *Cryptococcus*.

Presenting Author - *Alfred Botha, Stellenbosch University, South Africa*

Author/s - *Caylin Bosch, Heinrich Volschenk, Zoë Bhana*

Abstract Content

Environmental stress often causes phenotypic changes among pathogenic cryptococci such as the basidiomycetous yeast *Cryptococcus neoformans*. Nitrogen limitation is a common stressor occurring in many ecosystems and under such a condition a shunt has been observed within the metabolism of basidiomycetous yeasts towards carbohydrate and lipid accumulation. Thus, we contended that nitrogen limitation might increase the size of the carbohydrate-rich capsule, and result in the accumulation of the membrane lipid, ergosterol, in *C. neoformans*. Therefore, since capsule production is a known cryptococcal virulence factor and ergosterol a drug target, we hypothesised that nitrogen limitation impacts cryptococcal pathobiology. To test this hypothesis, we cultured *C. neoformans* at 37 °C in media with high (0.53 g/l), and low (0.21 g/l) NH4Cl concentrations, and then determined capsule sizes microscopically, total lipid content gravimetrically, ergosterol content spectrophotometrically, and antifungal drug tolerance using standard culture-based tests.

We found that the lower nitrogen condition decreased total lipid content, but enhanced capsule size and ergosterol content, and increased tolerance towards antifungal drugs amphotericin B and fluconazole. We subsequently used RNA-sequencing to determine the transcriptomic response to the above-mentioned low nitrogen condition and found that the expression of numerous virulence genes, including CTR4 and CGP1, was being modulated. Also observed, was the upregulation of antifungal tolerance-related genes, including genes involved in ergosterol biosynthetic processes and cell wall integrity. Overall, our findings provide insight into the survival of *C. neoformans* in nitrogen-poor ecological niches and suggest that pre-adaptation to these conditions may increase the virulence of this yeast.

OTS10/2 - Microbiome-mediated approach for designing synthetic microbial community for stress mitigation in agriculture

Presenting Author - *Rashi Tyagi, Indian Institute Of Technology Delhi, India*

Abstract Content

The vulnerability of agricultural system to various biotic stresses is responsible for decline in global crop productivity by nearly 20-40%. The commonly used chemical pesticides possess serious ecological implications while bioinoculants have reduced persistence and survivability under in vivo conditions. Microbiome-assisted rhizosphere engineering aims to re-structure the rhizospheric microbiome that benefits the plant by stress mitigation and growth promotion. The present study aims to devise a strategy based on rhizosphere engineering to combat biotic stress *Fusarium udum*, in Cajanus cajan by generating a synthetic microbial community (SMC). This is done by differentiation and categorization of bacterial taxa in *Fusarium*-infested and pathogen-free soil samples using Illumina MiSeq sequencing. Next, bacterial strains with antagonistic potential against *Fusarium* were isolated from rhizospheric soil samples of Cajanus cajan. The isolates were catalogued based on their plant-growth-promoting (PGP) and biocontrol properties thus, generating a culture bank of antagonistic strains exhibiting functional redundancy. Further, various possible combinations of compatible strains generated from the catalogue was assessed through a novel approach of iterative deconvolution technique to establish the best performing SMC. The efficacy of best possible SMCs was tested by seed germination assays followed by in planta assays. Thus, a robust, synthetic microbial community with indigenous multi-trait PGP strains for sustainable mitigation of biotic stress (*Fusarium*) with proven efficacy in host (Cajanus cajan) was generated. The study offers a new approach to strategic designing of SMCs which can be extended for other applications as well.

OTS10/3 - Nitrate-dependent anaerobic methane oxidation (N-DAMO) as a bioremediation strategy for agriculture-affected waters

Presenting Author - *Martyna Glodowska, Radboud University Nijmegen, Netherlands*

Abstract Content

Agricultural drainage ditches are subjected to high anthropogenic nitrogen input leading to eutrophication and greenhouse gas emissions. Nitrate-dependent anaerobic methane oxidation (N-DAMO) could be a promising remediation strategy to decrease methane (CH4) emissions and nitrate (NO3-) concentration simultaneously. Therefore, we aimed to evaluate the potential of N-DAMO to remove excess NO3- and decrease CH4 release from agricultural drainage ditches. Microcosm experiments were conducted using sediment and surface water collected from three different sites: a sandy-clay ditch (SCD), a freshwater-fed peatland ditch (FPD), and a brackish peatland ditch (BPD). The microcosms were inoculated with an N-DAMO enrichment culture dominated by *Candidatus Methanoperedens* and *Candidatus Methylomirabilis* and supplemented with 13CH4 and 15NO3-. A significant decrease in CH4 and NO3- concentration was only observed in the BPD sediment. In freshwater sediments (FPD and SCD) the effect of N-DAMO inoculation on CH4 and NO3- removal was negligible, likely because N-DAMO microorganisms were outcompeted by heterotrophic denitrifiers consuming NO3- much faster. Overall, our results suggest that bioaugmentation with N-DAMO might be a potential strategy for decreasing NO3- concentrations and CH4 emission in brackish ecosystems with increasing agricultural activities where the native microbial community is incapable of efficient denitrification.

OTS10/4 - Effect of feeding milk fermented by probiotics on the occurrence of diarrhea and diarrhea-associated zoonotic-bacterial-pathogen

Presenting Author - *Belen Fresno, Technical University of Denmark, Denmark*

Author/s - *Anna Luiza Farias Alencar, Henrik Læssøe Martin, Hanne Skovsgaard Pedersen, John Elmerdahl Olsen, Annette Nygaard Jensen*

Abstract Content

Background: Calf-diarrhea is a major cause of death in calves yielding high economic losses. Besides virus and parasites, it is caused by the zoonotic-bacterial-pathogens Salmonella Dublin (SD), Enterotoxigenic Escherichia coli (ETEC) and Clostridium perfringens (Cp). Probiotic-based feeding might contribute to prevent disease and zoonotic-transmission, therefore supporting a sustainable cattle-industry

Objective: We aimed to investigate the effect of milk fermented by a 4-probiotic-strain-combination (FMP)1 on occurrence of diarrhea and diarrhea-associated bacterial-pathogens

Methods: 143 newborn calves (from 3 Danish dairy-farms) were allocated into treatment- (n=72, fed FMP for the first 8-weeks-of-life) or control-group (n=71, fed regular farm-milk). Feces were scored weekly for diarrhea and collected at 3 time points (n=355). Diarrheic samples (n=118) were tested for the presence of SD, ETEC, and Cp by qPCR.

Results: Occurrence of diarrhea was 18.6% (farm1), 22.4% (farm2) and 15.7% (farm3). Incidence was higher during the first 3-weeks-of-life than from 4-to-8-weeks. The effect of FMP-treatment seemed to be farm-dependent, suggesting a link to farm-management. Thus, while no effect was observed for farm2, for farm1 diarrhea incidence was significantly reduced by the treatment in calves over-3-weeks-old and for farm3, the treatment-group showed significantly higher occurrence than the control-group in calves up-to-3-weeks-old (Fig 1). Limited detection of ETEC and SD did not allow for assessment of FMP-treatment effect, while occurrence of Cp (118 samples) was significantly reduced by the FMP-treatment except for farm3 (Fig 1). Overall, FMP-treatment may help decrease pathogen and diarrhea occurrence, thereby supporting agricultural sustainability, however the expected effect might be affected by farm-practices

OTS10/5 - Unlocking the potential of microbiome-based solutions as green biofertilizer for sustainable agriculture

Presenting Author - *Annamaria Bevivino, ENEA - National Agency for New Technologies, Energy and Sustainable Economic Development, Italy*

Author/s - *Jonas Hett, Lisa Cangioli, Alessia Fiore, Marina Caldara, Nelson Marmiroli, Daniel Neuhoff, M. Costanzo, A. Mengoni, E. Maestri, G. Aprea, A. Ambrico, R. Magarelli, M. Trupo, M. Gullì, S. Graziano, E. Ercole, F. Sev, S. Tabacchioni, A. Brunori, A. Pihlanto*

Abstract Content

Background: Many efforts in the recent years are being made in developing new inoculants microbial consortia not only for growing crops sustainably but also for preserving soil health; in this context, the question of whether natural soil microbiomes are negatively or positively affected by adding foreign microorganisms needs to be addressed.

Objectives: The main objectives of the present work are to exploit the potential of specifically selected microbial consortia (MC)1,2 for attaining sustainable agricultural production systems and to assess the impact of their application in field on indigenous rhizosphere microbial diversity.

Methods: MC were applied alone or in association with Arbuscular Mycorrhizal Fungi (AMF) and Biochar in open field under conventional and organic management and compared with commercial microbial products. The plant growth and diversity, composition, and relative abundance of bacterial community in maize rhizosphere soil were investigated at different maize growth stages and with different fertilization levels.

Results: The application of MC exerted a positive effect on plant growth at lower fertilization levels3, while did not significantly affect species diversity and richness of native rhizosphere microbial communities. A great impact of biochar on rhizosphere soil microbiome was found suggesting that functionalization of biochar with MC seems a promising approach for microbiome modulation and enhancing plant growth. Overall, our results suggested that multifunctional MC may be effectively exploited as biofertilizer in sustainable maize cultivation without altering the biodiversity or the resident microbiota, thus removing risks of long-term impacts on natural biodiversity.

OTS10/6 - Levan oligosaccharides and levan-metabolizing bacteria as novel biocontrol agents for strawberry

Presenting Author - *Triinu Visnapuu, Unversity of Tartu, Estonia*

Author/s - *Craig Ehrenreich, Evelin Loit, Barbara De Coninck, Wim Van den Ende*

Abstract Content

Strawberry is one of the most popular fruit crops with a significant market share in Europe. Fungal pathogens, e.g. *Botrytis cinerea*, are causing rot on strawberry plants which results in significant economic loss. Instead of intensive use of agrochemicals to control fungal pathogens, more sustainable and environmentally friendly solutions, such as bio-control organisms (BCOs) or natural protective compounds, should be implemented.

A β-2,6-linked polyfructan, levan, and levan-type oligosaccharides (LOS) are candidate prebiotics and potential immune system stimulators for various organisms. Recently it was revealed that a LOS mixture acts as an elicitor of the plant immune response against *B. cinerea*.

The aim of the study is to isolate and select potential BCOs for strawberries to boost plant immunity or have protective effect against *B. cinerea*. Levan-degrading and LOS-producing capabilities of BCOs might be highly relevant to their protective effect.

Culture-based and bioinformatic methods were applied to isolate and select potential BCOs applicable in strawberry context. For strain isolation tryptic soy and R2A medium was used and levan degradation was evaluated on minimal medium. Levan and LOS were produced enzymatically using levansucrase and endo-levanase, respectively.

Several strains with the potential to act as BCOs were isolated and identified from Estonian wild and domesticated strawberries and their activity towards levan and LOS was revealed. In addition, several bacterial strains from culture collections were investigated as potential BCOs. In planta experiments regarding the synergism between BCOs and LOS are ongoing.

OTS10/7 - Secondary metabolite-rich endophytic *Bacillus halotolerans* strains Cal.l.30 and Cal.f.4 as a compatible consortium

Presenting Author - *Polina Tsalgatidou, University Of Peloponnese, Greece*

Author/s - *Eirini-Evangelia Thomloudi, Eirini Baira, Costas Delis, Vasilios Demopoulos, Anastasia Venieraki, Panagiotis Katinakis,*

Abstract Content

An effective dual-strain consortium of plant growth promoting endophytic bacteria can be developed considering their compatible interaction in biofilm formation under in vitro and ex vivo conditions. In the present study, the novel endophytic *Bacillus halotolerans* strains Cal.l.30 and Cal.f.4 presented an efficient biofilm formation when co-inoculated in vitro and in planta. Bacterial root colonization of *Arabidopsis thaliana* seedlings was increased as a consortium compared to single-strain formulation, suggesting a synergistic interaction. A preventive formulation of the compatible consortium on detached olive fruit and grape berries significantly reduced incidence and severity of anthracnose (*Colletotrichum acutatum*) and gray mold (*Botrytis cinerea*) disease, respectively, compared to single-strain treatment. Both co-inoculated strains successfully colonized the plant tissues at the point of formulation, presenting a synergistic suppression of the fungal pathogens. Genome mining revealed that both *B. halotolerans* Cal.l.30 and Cal.f.4 are supported with a wide arsenal of secondary metabolite biosynthetic gene clusters (SM-BGCs) encoding for metabolites with diverse antimicrobial properties and/or involvement in biofilm formation and colonization, such as fengycin, surfactin, mojavensin A, bacilysin, bacillaene, bacillibactin, subtilosin A and a FAS-PKS (Fatty Acid Synthases- Polyketide Synthases) biosynthetic gene cluster. Further UHPLC-HRMS chemical analysis detected the secretion of the above secondary metabolites and other antimicrobial compounds like azelaic acid, 15-hydroxypentadecanoid acid and 2-hydroxyphenylacetic acid. The combination of the compatible B. halotolerans strains Cal.l.30 and Cal.f.4 exhibiting multiple genomic and metabolic features may provide new aspects to the creation of a successful consortium for sustainable agriculture.

OTS10/8 - Effect of plant beneficial microorganism application on maize in two contrasting growing size in two contrasting growing seasons

Presenting Author - *Loreen Sommermann, Anhalt University of Applied Sciences (AUAS), Germany*

Author/s - *Jan H. Behr, Doreen Babin, Narges Moradtalab, Soumitra Paul Chowdhury, Ioannis Kampouris, Davide Francioli, Theresa Kuhl-Nagel - Leibniz Institute of Vegetable and Ornamental Crops (IGZ), Plant-Microbe Systems, Großbeeren, Germany;*

Michael Rothballer - Institute of Network Biology, Helmholtz Zentrum München, German Research Center for Environmental Health, Ger

Abstract Content

Intensive farming strategies can in the long run compromise soil health and plant performance due to the accumulation of plant pathogens and loss of soil biodiversity. The research question was to determine to what extent the use of beneficial microorganisms (BM) affects the indigenous rhizosphere microbiome as well as plant health under different farming practices. A long-term field experiment served as the study site comparing two tillage practices (mould-board plough vs. cultivator tillage) and two nitrogen (N) fertilization intensities (intensive N-fertilization including fungicides/plant growth regulators vs. reduced extensive N-fertilization without fungicides/plant growth regulators) in 2020 and 2021, respectively. Maize plants were drench-inoculated twice with a BM-consortium (Bacillus sp., Pseudomonas sp., Trichoderma sp.) at early stages of plant development. In both growing seasons, successful root and soil colonization of the three strains was detected in all variants. In addition, a significant influence of the BM on the indigenous bacterial/archaeal and fungal community composition analyzed by high-throughput sequencing of 16S rRNA gene and ITS2 amplicons was observed. However, a significant increase in biomass and nutrient content of the inoculated plants could only be detected in the 2020 growing season characterized by severe spring drought. A significant decrease in selected stress-related genes and reduced levels of physiological stress-indicators was observed in inoculated plants compared to control plants. This multidisciplinary study provides insights into the influence of applied beneficial microorganisms on plant-microbial interactions under field conditions in two contrasting growing seasons, which could be useful to develop future microbe-assisted strategies for sustainable agriculture.

OTS11/2 - Essential gene-wide susceptibility testing of antisense oligomers in *E. coli*

Presenting Author - *Linda Popella, University of Wurzburg, Germany*

Author/s - *Jakob Jung, Lars Barquist, Jörg Vogel*

Abstract Content

The growing emergence of multidrug-resistant bacterial pathogens urgently demands the development of alternative antibiotics. Antisense oligomers (ASOs), such as based on peptide nucleic acid (PNA), can exert potent bactericidal effects when designed to sequester the ribosome binding site of an mRNA of an essential gene. Over the years, various essential genes have been investigated for their vulnerability to PNA targeting in Enterobacterales, with the acpP gene encoding acyl carrier protein being considered the most susceptible PNA target. However, it remains unknown what makes a potent PNA or PNA target. Here, we present a systematic investigation of the growth inhibitory efficacy of PNAs targeting 293 selected essential genes in *E. coli* BW25113 and uropathogenic *E. coli* (UPEC). We reveal strain-independent versus strain-specific antibacterial PNA activities. Our screen has produced 27 and 57 potent antibacterial PNAs, corresponding to 15 and 51 essential target genes, in BW25113 and UPEC, respectively. Bioinformatic analyses will enable us to decipher common traits of the identified most efficient PNA sequences and their highly susceptible gene targets. Further, characterization and testing of UPEC-specific PNAs on a broader panel of Enterobacterales may help to generate species-specific antibacterials. Overall, this study gives new insights into sequence- and target gene-dependent differences in PNA susceptibility and provides information on putative universal predictors of PNA efficacy.

OTS11/3 - Seaweed-associated microbes as a source of cell wall-degrading enzymes for biocontrol of *Phytophthora* oospores

Presenting Author - *Josie Mainwaring, Victoria University Of Wellington, New Zealand, New Zealand*

Author/s - *Chelsea Vickers, Monica Gerth*

Abstract Content

Phytophthora species cause devastating dieback and root-rot diseases in thousands of plants worldwide. A key part of the *Phytophthora* disease cycle is the sexual production of 'oospores': metabolically-dormant infection propagules which allow the pathogen to survive across seasons and beyond. Oospores are by far the most formidable of the pathogen targets; common chemical treatments have limited efficacy against them. The resilience of oospores is attributed to their thick, multi-layered cell walls, the major components of which are highly branched β-1,3-(1,6)-glucans cross-linked with cellulose and β-mannans. I am exploring the potential of carbohydrate-active enzymes that target these components as biocontrol tools against *Phytophthora* oospores.

Seaweeds (macroalgae) also have cell walls constructed from complex, cross-linked polysaccharides, sometimes bearing notable similarities to those of *Phytophthora* species. Seaweed-associated microorganisms therefore represent an untapped source of carbohydrate-active enzymes with potential relevance to oospore cell walls. Indeed, bacteria isolated from a New Zealand green seaweed (*Codium fragile*) encode a wide range of putative enzymes of interest. One such isolate – the newly described *Rhizobium* sp. nov. C1 (1) – and its cell wall-degrading enzymes, will be the focus of this presentation. I used bioinformatic analyses to identify enzymes of interest, which I then heterologously expressed, purified, and studied. I show that these enzymes are highly active on oospore cell wall-related substrates and significantly decrease oospore viability and germination. These results, plus ongoing work on the potential of *Rhizobium* sp. nov. C1 and its companions to be used directly as biocontrol agents, will be presented.

OTS11/4 - Anti-biofilm activity of fluorocytosine (5-FC) in *Escherichia coli* requires its conversion to a nucleotide and involves PBP1B

Presenting Author - *Srikanth Ravishankar, University Of Milano, Italy*

Author/s - *Karen Leth Nielsen, Paolo Landini, Elio Rossi*

Abstract Content

Background: Anti-virulence agents, i.e. drugs that selectively "disarm" pathogenic bacteria are considered as a promising strategy to combat the alarming issue of antibiotics resistance in acute and chronic infection causing-opportunistic pathogens. Several antimetabolites, like fluorocytosine (5-FC) have been shown to inhibit virulence factors' production in Gram negative bacteria, yet their mechanism of action is not understood.

Objectives: The aim of our work is to elucidate the molecular mechanism behind 5-FC inhibition of biofilm determinants in *E. coli* MG1655.

Methods: Phenotypic assays, β-glucuronidase reporter assay, RT-qPCR, and generation and screening of E. coli overexpression library were performed.

Results: At subinhibitory concentrations for growth, 5-FC impairs biofilm formation by both the laboratory strain MG1655 and clinical isolates of *Escherichia coli*, by reducing the expression of the csgBAC operon encoding curli fimbriae subunits. Conversion of 5-FC into fluoronucleotides is necessary for its antibiofilm activity, as inactivation of the upp gene, required for UMP synthesis from uracil, prevented 5-FC-dependent inhibition of biofilm formation and curli gene expression. To identify molecular targets of 5-FC, we screened an *E. coli* genomic overexpression library and found that a short fragment of the mrcB gene was able to restore curli production in the presence of 5-FC. Likewise, overexpression of the whole mrcB gene, encoding penicillin-binding protein 1B (PBP1B), involved in peptidoglycan biosynthesis, could counteract 5-FC inhibition of curli production. Interestingly, mrcB transcription is upregulated in the presence of 5-FC, suggesting a direct link connecting peptidoglycan biosynthesis with curli production and biofilm formation.

OTS11/5 - Peptide-guided immune system activation

Presenting Author - *Yael Belo, Faculty of Dental Medicine, Hebrew University of Jerusalem, Israel*

Author/s - *Yael Belo, Einav Malach, Zvi Hayouka*

Abstract Content

The immune system plays a critical role in protecting the host against various types of pathogens, including bacteria, viruses, and parasites. However, many pathogens have evolved mechanisms to evade the immune system, such as by altering their surface proteins or producing enzymes that can interfere with the immune response. These evasion strategies allow the pathogens to evade detection and destruction by the immune system, which can allow them to establish serious infections. Thus, there is an urgent need for developing new antimicrobial agents. Here, we propose a novel strategy for targeting pathogens, by labeling them with peptide bacterial binder conjugate to a protein tag that is recognizable by the immune system, thereby activating the immune system against the targeted pathogen. Using our methodology, we were able to eradicate 90% of *E. coli* as a model bacteria by activating the immune system response towards it. This work represents a new basis for a therapeutic approach which leads to immune system activation towards pathogenic bacteria to eliminate it rapidly and efficiently.

OTS11/6 - Combating citrus canker with environmental friendly alternatives to copper

Presenting Author - *Dirk-Jan Scheffers, University Of Groningen, Netherlands*

Author/s - *Dirk-Jan Scheffers, Lúcia Bonci Cavalca, Peter Deuss, Henrique Ferreira*

Abstract Content

Background: The growth of citrus fruits is one of the most profitable commodities in the world. Bacterial diseases pose a constant threat to citriculture leading to substantial yield losses in all major cultivation areas. Citrus canker is one of the most relevant bacterial diseases, which affects all the commercially important citrus varieties worldwide, and is caused by the Gram-negative bacterium *Xanthomonas citri* subsp. citri (*X. citri*). Currently, the control of citrus canker is done by frequent sprays of copper formulations, which can protect leaves and fruit from bacterial infection. However, constant application leads to accumulation of this copper in the soil at toxic levels, affecting several important ecological interactions in the environment. Also, copper can be leached to surface water. In addition, resistance to copper has already emerged in *X. citri*.

Objectives: The development of envrinmental friendly alternatives to minimize/suppress the use of copper for a safer and more sustainable agriculture.

Methods: Chemical synthesis, mode of action studies, toxicity assays, field tests

Results: This presentation will summarize the work we have done over the past ten years to develop a variety of antibacterial compounds that are derived from natural organic building blocks, such as bagasse, a rest product from the sugarcane industry. The natural hydrocarbon origin should ensure that these compounds, unlike copper, are degraded in the soil after application to plants. These compounds include curcumin derivatives, chalcones, alkyl gallates and hydroxybenzoates. The mode of action, toxicity and effectiveness in protecting citrus plants in the greenhouse will be discussed.

OTS11/7 - In vitro evolution of phages improves their anti-biofilm activity in in vivo-like conditions

Presenting Author - *Luciana Meneses, University of Minho, Portugal*

Author/s - *Luciana Meneses, Diana P. Pires, Sílvio B. Santos, Aurélie Crabbé, Tom Coenye, Joana Azeredo,*

Abstract Content

Background: The use of (bacterio)phages, is considered a promising strategy to treat biofilm-related infections, including lung infection in cystic fibrosis (CF). However, complete eradication of biofilms is difficult to achieve.

Objectives: The aim of the present study was to improve the anti-biofilm effect of a phage through adaptive evolution and to evaluate its anti-biofilm effect in physiologically relevant conditions.

Methods: Phage PE1 was allowed to evolve in 24h-old *Pseudomonas aeruginosa* biofilms (CF isolate) grown in lysogeny broth (LB) in 24-well plates. 24h after infection, phages were recovered and added to fresh 24h-old biofilms. The procedure was repeated for 8 days. Afterwards, evolved phages were recovered, characterized, and tested against *P. aeruginosa* biofilms formed in LB, synthetic CF sputum medium (SCFM2), and in a three-dimensional (3-D) lung epithelial model.

Results: After evolution, the phages showed increased anti-biofilm activity (reduction of aggregates and viable cells) compared to the wild type phage. The evolved phages also showed improved efficiency-of-plating (EOP) against different clinical *P. aeruginosa* strains, and two single nucleotide polymorphisms in genes associated with host recognition and binding (coding for tail-fiber and baseplate proteins) were identified. Increased anti-biofilm activity was observed in SCFM2 and in the 3-D lung model (while not being cytotoxic). Given the increased EOP and the mutations identified, the improvement of the biofilm-killing efficacy of the phages may be due to an increased recognition of different variants within the heterogenous biofilm population. Our study confirms that it is possible to improve the anti-biofilm activity of phages using adaptive evolution.

OTS12/1 - Vibrio natriegens – a superior host for the heterologous production of redox proteins

Presenting Author - *Helena Fuchs, TU Bergakademie Freiberg, Germany*

Author/s - *Sabrina Hedrich, Sophie R. Ullrich*

Abstract Content

Since many acidophilic microorganisms produce only low biomass, heterologous protein production can be a way to accelerate protein characterization and interaction studies. The heterologous production of proteins from acidophilic microorganisms in neutrophilic expression hosts presents a challenge mainly due to the difference in extracellular and periplasmic pH, which can affect protein folding and co-factor integration. The Gram-negative acidophile *Acidithiobacillus ferrooxidans* is capable of iron reduction through exocellular electron transfer. Its electron transport chain spanning across both membranes and the periplasm consists of three c-type cytochromes and the copper redox protein rusticyanin. In order to investigate the functionality of this electron transfer pathway, it was reconstructed in three different *Escherichia coli* expression strains and *Vibrio natriegens* Vmax X2. The periplasmic, cytoplasmic, and membrane protein fractions of the hosts were screened for the presence and redox activity of the four redox proteins. All cytochromes were produced with an integrated heme c co-factor in all expression hosts and showed redox activity. The periplasmic cytochrome c Cyc1 and rusticyanin were correctly translocated into the periplasm of the neutrophilic hosts, while the inner and outer membrane-bound c-type cytochromes were present in the membrane fraction exclusively. Results indicate that V. natriegens Vmax X2 produces more mature c-type cytochromes and rusticyanin than all *E. coli* expression hosts, suggesting that *V. natriegens* Vmax X2 may be a superior host for the heterologous production of soluble and membrane-bound redox proteins from acidophiles.

OTS12/2 - The effect of *Salmonella* biofilm matrix on antimicrobial tolerance

Presenting Author - *Jolien Meesters, KU Leuven, Belgium*

Author/s - *Tom E. R. Belpaire, Bram Lories, Bart Smeets, Hans P. Steenackers*

Abstract Content

Introduction: Bacteria are thought to predominantly reside in biofilms, which are sessile, multicellular communities that are encompassed by a self-produced extracellular matrix. The biofilm lifestyle is notorious for immense increases in bacterial tolerance to multiple antimicrobials, which is often attributed to this extracellular matrix. However, it remains unclear how the quantity and structure of biofilm matrix influences therapeutic efficacy.

Objective: We aim to elucidate how the production of matrix at single-cell level can shape overall biofilm architecture and consequent tolerance to antimicrobials.

Methods: We engineered *Salmonella enterica* Typhimurium to produce different levels of biofilm matrix via constitutively expressing CsgD, the biofilm master regulator. The emergent biofilm structures were characterized using confocal microscopy in combination with in silico agent-based models of biofilm growth. Tolerance to cefotaxime was quantified using both cell counting and microscopy after 4 hours.

Results: Increasing levels of biofilm matrix resulted in increasingly sparse biofilm structures. Moreover, our results indicate that an increase in matrix production does not necessarily increase the protection against antimicrobials, but can even result in a decreased tolerance, in the case of cefotaxime. These differences in tolerance between the different matrix-producing strains remain even when the overall biofilm is disrupted in smaller clusters.

OTS12/3 - Engineering phagemid-based intercellular communication for distributed computing in engineered *Escherichia coli* consortium

Presenting Author - *Hadiastri Kusumawardhani, University of Lausanne, Switzerland*

Author/s - *Yolanda Schaerli*

Abstract Content

Background: Building large synthetic genetic circuits or combining multiple circuits in a single bacterial cell is a major challenge as they become too complex and inflict a high metabolic burden on the host cell. Distributing the circuit's function in a multicellular consortium can address this issue. This system will have further potential applications beyond biocomputing, for example, in biomedical therapy and bioprocess technology.

Objectives: In this project, we aim to engineer distributed computing in a consortium of *Escherichia coli*. Our system combines 1.) cascaded CRISPR-interference (CRISPRi) gene regulation to create single and multi-input logic gates and 2.) M13 phages for establishing intercellular communication in *E. coli*.

Methods: Intercellular communication is achieved by sending M13 phages carrying a single guide RNA (sgRNA) on a M13 phagemid between the donor and receiver cell populations. The donor cells will transmit this sgRNA constitutively or in the presence of chemical inducer. In combination with the catalytically dead Cas9 protein (dCas9), the transmitted sgRNA created transcriptional inhibition or activation of a reporter gene in the intended receiver cell population.

Results: Using this system, we constructed six orthogonal NOT gates. Upon receiving M13 phagemid encoding the corresponding sgRNA, the receiver cells successfully inhibit transcription 13- to 25-fold with negligible off-target interactions in more than 95% receiver cell population within 4 hours of co-incubation with the donor cells. Moreover, we have successfully layered NOT gates to build larger circuits, such as YES/buffer gates, NOR gates and OR gates, which also exhibit high degree of robustness and orthogonality.

OTS12/4 - AccSRT regulatory system controls carbon catabolite repression of the anaerobic degradation of benzoate in *Aromatoleum* sp. CIB

Presenting Author - *UNAI FERNÁNDEZ, Centre for Biological Research Margarita Salas, Spain*

Author/s - *J. Andrés Valderrama, Helena Gómez-Álvarez, Gonzalo Durante-Rodríguez, Eduardo Díaz*

Abstract Content

Background: Aromatic compounds are major environmental pollutants, and they are usually degraded through the benzoyl-CoA central pathway (bzd genes) in anaerobic bacteria. The β-Proteobacterium *Aromatoleum* sp. CIB has been used as a model system to study the regulation of the bzd genes, including the carbon catabolite repression (CCR) by some organic acids, e.g.,succinate. Thus, the AccS sensor histidine kinase and the AccR response regulator constitute a two-component regulatory system controlling the activity of the PN promoter, which drives expression of bzd genes, when CIB cells grow in the presence of benzoate and succinate.

Objectives: To expand our knowledge on the multicomponent regulatory system that mediates CCR of the bzd genes in *Aromatoleum* sp. CIB.

Methods: Biochemical characterization of AccT and its ligands was performed by ultracentrifugation and thermal shift assays. Gene expression studies were performed by qRT-PCR and β-galactosidase assays of PN:lacZ fusions, in wild-type, accS and accT CIB mutants, as well as in recombinant *E. coli* cells.

Results: We have identified the accT gene product within the acc cluster from *Aromatoleum* sp. CIB that is needed for the CCR of the bzd genes. AccT is a periplasmic binding-protein that recognizes short-chain keto acids and derivative and likely interacts with the periplasmic domain of AccS modulating its autokinase activity and, hence, the activity of the Pn promoter by phosphorylating the AccR transcriptional repressor. Thus, AccT constitutes a third component of the AccSR regulatory system that senses not only cytoplasmic signals (redox state) but also extracytoplasmic signals involved in CCR mechanisms.

OTS12/5 - *Pseudomonas aeruginosa* infection drives complex host responses in a cystic fibrosis-derived airway model

Presenting Author - *Claudia Antonella Colque, Denmark*

Author/s - *Signe Lolle, Filipa B. Simões, Søren Molin, Helle K. Johansen*

Abstract Content

Background: *Pseudomonas aeruginosa* (PA) is one of the leading pathogens of human pulmonary infections, especially in immune compromised patients.

Objectives: This study aimed to profile the host-microbe responses underlying lung infections utilizing a human airway-derived model to monitor PA colonization.

Methods: Primary epithelial cells and the BCi-NS1.1 cell line were cultured under Air-Liquid Interface (ALI) conditions and inoculated with PA isolates collected from patients at the onset and after several years of infection. Morphological changes of differentiated cells were monitored by confocal microscopy, epithelial integrity, and cytokine secretion. In addition, bacterial populations and host tissue responses were evaluated through dual-species transcriptomic sequencing (RNA-seq).

Results: There is a coupling between the state of PA airway adaptation and the actual infection process in the ALI model. Early isolates, like environmental strains, colonize the epithelium as large colonies, reduce the epithelial integrity, and penetrate the cell layer. In contrast, well adapted isolates do not damage the epithelium and mainly exhibit single-cell distribution. RNA-seq analyses document significant changes in the transcriptomic profiles of both human and bacterial cells after infection. Infected human cells exhibit a dysregulation of the calcium signaling pathway as well as an activation of signal transduction mechanisms resulting in upregulation of certain inflammatory cytokines and genes with antibacterial activity. Simultaneously, several iron uptake protein systems were significantly upregulated in the ALI associated bacteria. Interestingly, genes related to oxidative stress and central carbon metabolism, previously shown to be differentially expressed in vivo, also showed a significant change in the ALI model.

OTS13/4 - Virome of irrigation water as a tool for surveillance of plant pathogens

Presenting Author - *Olivera Maksimovic, National Institute Of Biology (Slovenia), Slovenia*

Abstract Content

High throughput sequencing (HTS) has enabled in-depth analysis of the virome of many ecosystem components, including various aquatic systems. Broad-spectrum information obtained from such studies covers both known and previously unknown viruses. The approach is still underutilized in the context of plant viruses in aquatic samples. Using HTS, we performed a metagenomics study of irrigation water from selected Slovenian tomato farms, which resulted in detection of nucleic acids from several known and putative new plant virus species. We observed that the source of irrigation water influenced the observed diversity of detected viruses. Bioinformatics analyses revealed *Tobamovirus* and *Tombusvirus* genera as the most prevalent. In a separate study, samples of tomato and weed plants from the same areas were also examined. Some of the viruses were found in both plant and water samples. Complementing the sequencing results enabled us to better understand the distribution of some viruses in the area. For example, a novel virus originally isolated from a single weed plant was later detected in numerous water samples from various places, demonstrating that it is present in a wide area, despite its recent discovery. Several full genomes of new plant viruses were assembled from water samples indicating the applicability of the method for new plant virus discovery. Our findings suggest that different information about plant viruses circulating in the environment can be obtained by studying the virome of water samples. This approach can serve as a foundation for developing water-based epidemiology for the surveillance of emerging pathogens in plants.

OTS13/5 - Integrated Research Infrastructure services as a new approach to Pandemic Preparedness in Europe

Presenting Author - *Martin Houde, European Research Infrastructure for Highly Pathogenic Agents, Belgium*

Abstract Content

Well before the current COVID-19 pandemic, health experts from around the world identified major gaps in global preparedness for infectious diseases and important needs to invest more in critical capacities to prevent, detect, and respond to epidemic and pandemic threats.

In the European Union, among others, investment was made in life sciences research infrastructures and infectious disease networks that federate facilities offering scientists access to cutting-edge research services, and that could be mobilized in times of crisis.

As one of the first actions of the European Health Emergency preparedness and Response Authority, is ISIDORe, "Integrated Services for Infectious Disease Outbreak Research", launched in 2022.

ISIDORe is a new approach to epidemic preparedness and response research in Europe. It assembles and provides free access to an unprecedented One Health-driven integrated portfolio of cutting-edge research resources, dedicated to the study of any epidemic-prone diseases.

ISIDORe involves all the major European Research Infrastructures and networks in the field of biomedical research, from the most fundamental (e.g. structural biology) to the most applied (e.g. vaccine development and clinical trials), including social sciences.

During its first year ISIDORe supported a number of projects targeting epidemic and pandemic-prone pathogens such as coronaviruses, Monkeypox, vector-borne pathogens, Risk-Group 4 pathogens. The free of charge access is offered through open calls for proposals of ISIDORe.

This new mechanism enables conducting transdisciplinary projects, to accelerate innovation during times of emergencies and improving preparedness.

We will showcase the results of ISIDORe-supported projects and ongoing opportunities open to researchers.

OTS13/6 - Bacteriophage-modulated production of intestinal immunomodulatory metabolites is associated with protection in Allo-SCT

Presenting Author - *Jinling Xue, Technical University Munic, Germany*

Author/s - *Erik Thiele Orberg, Elisabeth Meedt, Andreas Hiergeist, Paul Heinrich, Sakhila Ghimire, Melanie Tiefgraber, Sophia Göldel. Department of Medicine III, Technical University of Munich (TUM), School of Medicine, Klinikum rechts der Isar TUM, Munich, Germany*

Tina Eismann. Department of Medicine III, Technical University of Munich (TUM), School of Medicine, Klinikum

Abstract Content

The impact of the intestinal microbiome on the outcomes of patients undergoing allogeneic hematopoietic stem cell transplantation (allo-SCT) is well established. However, the contribution of bacterial, fungal, and viral communities, as well as microbiota-derived metabolites, is not fully understood. In this study, we aimed to gain a deeper understanding of this relationship by conducting a prospective, longitudinal study that analyzed the intestinal microbial communities of 78 allo-SCT patients. The study combined analysis of bacteria, fungi, and viruses with targeted metabolomics, and revealed a microbiome signature of bacteria from the Lachnospiraceae and Oscillospiraceae families and their corresponding bacteriophages, which were found to be correlated with the production of immunomodulatory metabolites. A BCoAT gene in phage contigs was observed that could contribute to the synthesis of butyric acid, a protective metabolite. The sustained production of these protective metabolites after allo-SCT was linked to improved survival and reduced transplantation-related mortality, while exposure to antibiotics significantly impacted metabolite expression. The study also demonstrated that the transfer of metabolite-producing bacterial consortia via fecal microbiota transplantation (FMT) could reverse a single taxon domination and depletion of metabolites in a patient with graft versus host disease (GvHD). These findings provide a basis for the development of microbiome-based therapies, such as engineered metabolite-producing consortia and defined metabolite combination drugs.

OTS13/7 - Improved bacteriophage cocktail design using a whole genome functional screen in *Salmonella*

Presenting Author - *Luke Acton, Quadram Institute, United Kingdom*

Author/s - *Hannah Pye, Gaetan Thilliez, Rafal Kolenda, Haider Al-Khanaq, Evelien Adriaenssens, Robert Kinglsey,*

Abstract Content

The World Health Organization estimates that 600 million people are affected by Foodborne Illness each year. A significant number of these infections are associated with *Salmonella enterica* – suggesting that current methods, used to exclude pathogens from food products are ineffective. Therefore, the development of novel antimicrobial agents is crucial to reduce the burden caused by bacterial diseases to the public. Bacteriophages offer a promising alternative to conventional antibiotics. Implementation of Bacteriophages for industrial use is critically dependant on robust understanding of every aspect of their infection cycle – including how bacteria evolve resistance to the bacteriophages.

A saturating transposon insertion library-based functional screen with Transposon Directed Insertion Site Sequencing (TraDIS) was used to identify bacterial host genes involved in infection by newly isolated *Salmonella* Bacteriophages. The bacterial library was produced with a complexity of ~600,000 unique insertions using *Salmonella enterica* serovar Typhimurium strain 4/74 – the highest complexity TraDIS library in *Salmonella* Typhimurium. TraDIS successfully identified host genes involved in sensitivity and resistance to newly isolated *Salmonella* bacteriophages. A collateral sensitivity dynamic, where resistance to one phage, resulted in increased sensitivity to a different phage, was initially highlighted by TraDIS and later confirmed using single isogenic mutants. This collateral sensitivity mechanism led to reduced frequency of phage resistance when used as a phage cocktail. This work is of importance to Food Safety and phage therapy as increased understanding of bacteriophage-host interactions will help design more effective phage cocktails.

OTS13/8 - Newly isolated bacteriophage multidrug-resistant *Acinetobacter baumannii* inhibition in ex vivo murine precision cut lung slices

Presenting Author - *Rita Costa, Helmholtz Munich, Germany*

Author/s - *Luz Quebrada, Jasmin Aicher, Wanqi Huang, Jinling Xue, Mohammadali Khan Mirzaei, Li Deng,*

Abstract Content

The spread of multidrug-resistant Acinetobacter baumannii in hospital-acquired infections and treatment complications, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)–associated pneumonia, is a global health threat. New therapeutics are urgently needed. Bacteriophages (phages) represent an alternative for critically ill patients. However, the understanding of molecular mechanisms of phage-mammalian cells interaction remains limited. Also, the translation of novel therapeutic interventions from model systems into clinical use remains a major challenge.

With this study we aimed at a preclinical evaluation of *A. baumannii* bacteriophage (vB_Ab_HMGU1) lytic capacity against *A. baumannii* ATCC 17978, using human lung epithelial cells and murine precision cut lung slices (PCLS) as experimental models of lung infection.

A. baumannii bacterial clearance was achieved with a phage to bacteria cells ratio/multiplicity of infection (MOI) of 10 and 100 in the lung epithelial cells. In contrast, in the murine PCLS, an MOI of 1 was sufficient to neutralize bacteria, given the presence of immune competent cells. On the transcript level, phage therapy reduced the gene expression of IL1B, IL6 and IL8 in the infected epithelial cells and Tnf in the PCLS. Finally, we observed that phage treatment alone increased the expression of inflammatory cytokines, Il1b, Il6, Cxcl1 and Tnf, in the murine PCLS, showing a higher sensitivity to endotoxins present in the phage solution.

Viable murine PCLS exhibited preserved lung function which translated into an improved bacteria neutralization after phage therapy. Importantly this model closely resembles *in vivo* environment and is an optimal tool for preclinical assessment of phage therapy.

OTS13/9 - Discovery of ΦKZ phage proteins that target host ribosomes

Presenting Author - *Milan Gerovac, University of Wurzburg, Germany*

Author/s - *Jörg Vogel*

Abstract Content

The Gram-negative bacterium *Pseudomonas aeruginosa* is a major cause of nosocomial infections due to its intrinsic antibiotic resistance, large genome, and wealth of regulatory mechanisms. We asked how cellular complexes between proteins and RNAs change upon application of a biological stress in form of a phage infection. We selected the giant bacteriophage ΦKZ whose genome encodes hundreds of uncharacterized proteins. To illuminate how the phage proteome merges with cellular complexes, we used the Grad-seq technique in which cellular lysates are fractionated using glycerol gradients prior to analysis with RNA-seq and mass spectrometry (Gerovac et al. 2021, Smirnov et al. 2016). This way, we discovered phage proteins in ribosomal fractions that do not match with known translation factors and hence might mediate new modes of regulation. We identified several early expressed phage factors to directly interact with the large ribosomal subunit throughout the translation cycle. It is tempting to speculate that some anti-phage defence systems might act to antagonize the targeting of the host translation machinery by phage-encoded factors.

OTS14/3 - A new method for a new bacterium

Presenting Author - *Héctor Carmona Salido, University Of Valencia, Spain*

Author/s - *López-Hontangas José Luis, Carmen Amaro*

Abstract Content

The genus *Vibrio* comprises a group of gram-negative bacteria naturally occurring in aquatic environments. Some species of the genus have the ability to infect humans, with *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* being the most studied. Due to climate change, cases of V. cholerae infections are skyrocketing in recent years. According to the WHO, the current situation represents a resurgence of the ongoing seventh cholera pandemic, which began in 1961. A number of species within the cholera clade have recently been described. One such species is *V. metoecus*, a species closely related to *V. cholerae* that co-inhabits the same environments.

In this work we report the first case of human death caused by *V. metoecus*. The bacterium was isolated from blood, its genome was sequenced with Illumina and minION and the species was identified by Average Nucleotide Identity (ANI). We have analysed the genomes of this and other strains of the same species and belonging to *V. cholerae* and identified genes involved in virulence as well as species-specific genes. With all this information, we have developed both a specific enrichment medium for the isolation of *V. metoecus* from the environment and a PCR for its identification. We are currently validating the complete protocol with lake and brackish water samples from our geographical environment.

OTS14/4 - Comparison of the genome of *Staphylococcus aureus* strains isolated from persistent and intermittent carriers in pharynx and nose

Presenting Author - *Samuel González-García, Universidad Autónoma Metropolitana, Mexico*

Author/s - *Samuel González-García, José Felix Aguirre-Garrido, Julia Pérez-Ramos, Anaid Bustos-Hamdan, Aida Hamdan-Partida, Jaime Bustos-Martínez*

Abstract Content

Background: About 20% of people are persistent carriers of *Staphylococcus aureus* and about 30% are intermittent carriers. It has been reported that persistent carriers are generally colonized by a particular strain, while intermittent carriers may have different strains.

Objectives: Identify differences at the genome level of *S. aureus* strains isolated from persistent and intermittent pharyngeal and nasal carriers.

Methods: A five-year follow-up of workers in a cold meat packing house was carried out, looking for the presence of *S. aureus* in the pharynx and nose, and 9 nine strains were selected, 4 non-persistent (3 from the pharynx and 1 from the nose), and 5 persistent strains (2 strains from a patient isolated from the pharynx and nose in the same sampling and 2 strains isolated from the pharynx in different samplings) from which DNA was extracted. Whole genome sequencing was performed using Illumina MiSeq technology. The sequences were assembled de novo with the Geneious Prime and aligned against reference genomes with Bowtie2. The presence of virulence and resistance genes was analyzed with MAUVE, VFDB, CGE, MLST, etc. platforms.

Results: 3 complete genomes and 6 draft genomes were assembled, all of which are already accepted at NCBI with the BioProject: PRJNA833862. No differences were found in the presence of adhesin genes, biofilm formation, toxins, among others. Strains isolates from the nose and isolates from the pharynx clustered specifically when MLST gene sequence alignment was performed.

OTS14/5 - Occurrence of hypervirulent *Klebsiella pneumoniae* in a German academic medical center

Presenting Author - *Eyuep Dogan, University of Greifswald, Germany*

Author/s - *Elias Eger, Stefan E. Heiden, Christian Kohler, Karsten Becker, Katharina Schaufler, Evgeny A. Idelevich*

Abstract Content

Background: Hypervirulent *Klebsiella pneumoniae* (hvKP) isolates have been increasingly reported from various countries. This pathotype causes invasive and metastatic infections and often leads to treatment failures and relapses.

Objectives: This study aimed to determine the prevalence of hvKp in a German university hospital, thus elucidating the need for specific diagnostic and treatment strategies.

Methods: K. pneumoniae isolates recovered from clinical samples between June 1 and August 31, 2022 were prospectively collected. Only the first isolate of a patient was included and screening samples were excluded. All isolates were subjected to whole genome sequencing (WGS). The phenotypic characteristics were assessed by the string test (ST) and mucoid-staining plates (MSP).

Results: A total of 128 *K. pneumoniae* strains were included. In two isolates, both phenotypic hypermucoviscosity assays – ST and MSP – were positive, while only ST and only MSP were positive in 19 and 5 isolates, respectively. WGS revealed typical markers of hypervirulence including iucABCD, iutA, iroCDN, clbAQ, ybtAX, irp1, irp2, fyuA, and rmpA in four isolates exhibiting MLST sequence types 66, 380, 380 and 1333. They were all largely antibiotic-susceptible; three of them were positive for ST and none was positive for MSP. Two *K. pneumoniae* isolates that did not have hypervirulence markers were carbapenem-resistant, one carrying blaNDM-5 and the other blaOXA-181.

Conclusion: The study revealed that 3.1% (4 out of 128) of all clinical *K. pneumoniae* isolates included were hvKp. Thus, this high-risk pathogen poses a significant threat in Germany, warranting further studies and surveillance measures.

OTS14/6 - Temporal composition of the cervicovaginal microbiome correlates with hrHPV infection outcomes in a longitudinal study

Presenting Author - *Mariano Molina Beitia, Radboud University Nijmegen, Netherlands*

Author/s - *Mariano A. Molina, William P. J. Leenders, Martijn A. Huynen, Willem J. G. Melchers, Karolina M. Andralojc*

Abstract Content

Background: Persistent infections with high-risk human papillomavirus (hrHPV) can cause cervical squamous intraepithelial lesions (SIL) that may progress to cancer. The cervicovaginal microbiome (CVM) composition correlates with SIL, but the dynamics of the CVM after hrHPV infections have not been fully clarified. Objectives. This study aimed to determine the association between the CVM composition and infection outcome after hrHPV infection diagnosis.

Methods: We applied high-resolution microbiome profiling using circular probes-based RNA sequencing on a longitudinal cohort of cervical smears obtained from 192 hrHPV DNA-positive women with normal cytology at first visit, of whom 74 were diagnosed by cytology with SIL six months later.

Results: Here we show that women with *Lactobacillus*-dominated microbiomes have more stable microbial communities that associate with protection against SIL development, while women with the microbial community state type IV-A at first visit, characterized by high diversity and low *Lactobacillus* abundance, have a higher risk of developing SIL at second visit. Analyses at the species-level demonstrate that increased abundance of *Gardnerella vaginalis* and *Atopobium vaginae* in the microbiome correlate with adverse infection outcomes. Our results suggest that the CVM can potentially be used as a predictive biomarker for cervical disease and SIL development after hrHPV infections with implications on cervical cancer prevention strategies and treatment of SIL.

OTS15/1 - Is soil microbial biomass depleted of ribosomes under short-, medium-, and long-term warming?

Presenting Author - *Andrea Söllinger, UiT The Arctic University Of Norway, Norway*

Author/s - *Laureen S. Ahlers, Mathilde Borg Dahl, Pall Sigurðsson, Liabo L. Motleleng, Eva Marie Breines, Christoph Gall, Victoria S. Martin, Coline Le Noir de Carlan, Biplabi Bhattarai, Bjarni D. Sigurðsson, Ivika Ostonen, Erik Verbruggen, Andreas Richter, Alexander T. Tveit*

Abstract Content

Background: How soil microorganisms respond to global warming is a key question in microbial ecology, eminently relevant for terrestrial carbon cycling and the climate system.

Objectives: We aim to reveal how seasonal dynamics (spring – summer – autumn – winter) and medium- to long-term warming affects soil microbiomes and the bacterial protein biosynthesis machinery (i.e., ribosomes).

Methods: We made use of the longest lasting in situ soil warming experiment worldwide, ForHot, in which Icelandic grassland and forest soils have been exposed to natural geothermal warming. We compared warmed soils and non-warmed controls of 15 replicated soil temperature gradients sampled throughout several years and seasons. We extracted DNA, RNA, and microbial biomass (carbon) from grassland and forest soils and generated 48 metatranscriptomes from grassland soils.

Results: RNA contents per unit of DNA tended to be lower in the warmed soils across all seasons except winter, independent of warming duration (6 weeks, 8-14 years, >50 years), warming intensity (+3-8.5°C), and soil type (grassland, forest). At higher seasonal temperatures, the RNA contents per unit of microbial carbon obtained from grassland soils were lower, indicating that soil microbial biomass is depleted of ribosomes under warming. Such cellular changes may have substantial effects on soil carbon dynamics, as indicated by significantly increased CO2 emissions per unit of RNA and hour in the warmed grassland soils. We propose that such accelerated activities are facilitated by a reduced investment in the protein biosynthesis machinery and will show and discuss first insights obtained via seasonal metatranscriptomics.

OTS15/2 - Seasonality of the bacterial and archaeal community composition of the Northern Barents Sea

Presenting Author - *Stefan Thiele, University of Bergen, Norway*

Author/s - *Oliver Müller, Anna Vader, Stuart Thompson, Karoline Saubrekka, Elzbieta Petelenz, Hilde Rief Armo, Lasse Olsen, Gunnar Bratbak, Lise Øvreås*

Abstract Content

Background: The Barents Sea is a transition zone between the Atlantic and the Arctic Ocean and is particularly vulnerable due to the high variability of the ecosystem, especially with respect to sea ice coverage. With global warming and retreating sea ice, also the Northern Barents Sea is becoming accessible.

Objectives: In order to improve our understanding of the pelagic marine microbial ecosystem in this area we investigated the bacterial and archaeal communities in different seasons (early and late winter, spring, and summer) as well as in different years (summers 2018, 2019, and 2021) along a transect through the Barents Sea into the Arctic Ocean.

Methods: We used 16S rRNA gene sequencing for community analyses, and inferred functions from a genome database using PiCRUST2.

Results: Winter samples were dominated by members of the SAR11 clade and a community of nitrifiers, including *Cand.* Nitrosopumilus and LS-NOB (Nitrospinia). During spring and summer successions of different members of the *Gammaproteobacteria* and *Bacteroidia* were seen, based on their utilization of different phytoplankton derived carbon sources but varied over the years with respect to sea ice coverage. This indicates that Arctic marine microbial ecosystems in this region switch from carbon cycling in spring and summer to nitrogen cycling in winter. Due to global warming, these nutrient cycles might change with unknown consequences for the ecosystem of the Barents Sea.

OTS15/3 - Metabolic adaptions of the trace gas oxidizer *Methylocapsa gorgona* MG08 to increasing methane mixing ratios in the atmosphere

Presenting Author - *Oliver Schmidt, UiT The Arctic University Of Norway, Norway*

Author/s - *Oliver Schmidt, Tilman Schmider, Alexander T. Tveit*

Abstract Content

Methylocapsa gorgona MG08 is a soil bacterium that can grow by co-metabolizing the atmospheric trace gases CH4, H2 and CO at mixing ratios present in ambient air (presently 1.9, 0.5, and 0.1 ppm for CH4, H2, and CO, respectively, on average). However, pre-industrial atmospheric CH4 mixing ratios have been ≤ 0.7 ppm and it must be expected that CH4 continues to accumulate in the atmosphere. Here, we designed an experimental setup based on the floating filter technique and customized controlled gas flow chambers that allows us to study metabolic adaptions of *M. gorgona* (or other trace gas oxidizers) to increasing atmospheric CH4 mixing ratios. First results indicate that small changes in CH4 mixing ratios (0.7 vs. 1.9 vs. 2.5 ppm CH4) have only minor effects on the colony growth of M. gorgona. In contrast, further increasing CH4 mixing ratios to 50 ppm (a CH4 mixing ratio that might be temporarily and locally reached in aerated soils during anoxic conditions following heavy rain events) or increasing the CO mixing ratio from 0.1 to 0.5 ppm (a CO mixing ratio that can be reached in urban areas with polluted air) did significantly speed up colony growth. Ongoing experiments aim at determining oxidation kinetics of CH4, H2, and CO by *M. gorgona* cells incubated at contrasting CH4 mixing ratios. This data will be combined with that from proteomic analyses as well as cellular protein, RNA, and DNA quantitation analyses to unravel cellular metabolic adaptions of *M. gorgona* to increasing atmospheric CH4 mixing ratios.

OTS15/4 - *Serendipita indica* confers drought tolerance in tomato by stimulating the expression of drought stress-related genes in leaves

Presenting Author - *Valentina Lazazzara, Scuola Superiore Sant'Anna, Italy*

Author/s - *Laura Ercoli, Elisa Pellegrino*

Abstract Content

Tomato (*Solanum lycopersicum* L.) is considered one of the most economically important horticultural crops worldwide. Abiotic stresses, such as drought, have a negative impact on tomato development and productivity. *Serendipita* (=*Piriformospora*) *indica* is a biotrophic mutualistic root endosymbiont that can colonize many plant species and benefits host plants with growth-promotion, disease resistance, and abiotic stress tolerance. Recent studies reported the ability of *S. indica* to enhance tolerance to drought to various crop species. However, scarce information is available on the role of *S. indica* on tomato tolerance to drought.

The aim of this study is to investigate the physiological and molecular effects of *S. indica* inoculation in tomato on drought stress tolerance.

Tomato plants cv. Moneymaker were grown in a sterile substrate, and they were inoculated with a GFP-labelled S. indica isolate. Control plants were mock-inoculated with the autoclaved *S. indica* isolate. *S. indica*- and mock-inoculated plants were stressed for five days. All pots were irrigated to reach a complete saturation (T0). Then, irrigation water was daily applied to replace 100% transpiration (well-watered) and to replace 50% transpiration (drought stress, DS) (T1) by pot weighting.

S. indica root colonisation was confirmed by fluorescent and light microscopy. Physiological measurements clearly revealed differences between *S. indica*- and mock-inoculated drought-stressed plants. Moreover, gene expression analysis performed on leaves at T0 and T1 revealed that *S. indica* was able to enhance the expression of drought tolerance- and phytohormone-related genes, suggesting a prominent role of this fungus to make tomato more tolerant to drought.

OTS15/5 - Role of extracellular Polysaccharide (EPS) secretion in *Alteromonas*: from environmental genomics to laboratory models.

Presenting Author - *Carla Perez Cruz, AZTI Foundation, Spain*

Author/s - *Carla Perez-Cruz, Uxue Arrizabalaga, Raquel Liébana, Eli Bilbao, Laura Alonso-Sáez*

Abstract Content

Background: *Alteromonas* are ecologically relevant microorganisms, which are widespread in marine environments and can proliferate in response to pulses of organic matter. Furthermore, they feature a wide ability to degrade polymers, such us carbohydrates generated during algal blooms. A few studies have described the secretion of extracellular polysaccharides (EPS) by *Alteromonas* in a biotechnological contex, but their potential role in natural habitats is poorly understood. As EPS may have a key role in nutrient acquisition, the analysis of this cellular mechanism can be relevant to understand their ability to degrade different carbon sources.

Objectives: To study the regulation of EPS synthesis in response to different environmental conditions, we studied two *Alteromonas* strains isolated from deep oceanic waters with different capabilities of polysaccharide degradation.

Methods: EPS secretion patterns of two *Alteromonas* strains were characterized when growing under different carbon sources (i.e. alginate, laminarin and glucose) by combining high-resolution microscopy, molecular (genomics, transcriptomics, proteomics) and chemical (monosaccharide composition) approaches.

Results: By genome analysis we identified four gene clusters potentially involved in EPS synthesis in both *Alteromonas* strains. EPS clusters showed a high homology degree with closest known phylogenetic relatives. Additionally, we searched for EPS marker genes from *Alteromonas* in metagenomes and metratranscriptomes from the global ocean to address the potential ecological relevance of this mechanism. Our results aim at expanding our understanding of the role of EPS secretion in *Alteromonas* in the remineralization of carbon produced by phytoplankton and their adaptation to different environmental conditions.

OTS15/6 - Metagenomics and infrared imaging resolves microbial responses to permafrost thaw

Presenting Author - *Neslihan Tas, , United States*

Author/s - *Neslihan Tas, Nancy Conejo, Hoi-Ying Holman*

Abstract Content

Permafrost microbial communities are complex, diverse, and active at subzero temperatures. While carbon turnover at depth is proposed to be slower than the surface, especially the fate of carbon in deep permafrost, which is currently protected from the warming climate, is uncertain. Permafrost microbiome is a seed bank of mostly novel organisms that have diverse and broad metabolic potential. The microbial response to thaw in arctic environments is not uniform and the relationship between permafrost microbiomes and greenhouse gas emissions is not well understood. Following thaw, redistribution of water is a key event that conditions the permafrost for microbial decomposition. We initiated batch-scale permafrost incubation experiments in dry, natural, and saturated moisture states and under microaerophilic or anaerobic headspaces. We couple omics methods with analysis of soil chemistry via synchrotron Fourier transform infrared spectral imaging at the Berkeley Infrared Structural Biology beamline of the Advanced Light Source. Analysis showed that a variety of organic compounds and metabolites were accumulated in thawed permafrost soils. Especially under saturated conditions while carbohydrates were depleted, dry soils accumulated aliphatic compounds. We found strong trends that under dry conditions *Dormibacteria* and *Chloroflexi* were able to survive over multiple years and retain carbon stocks as carbohydrates and microbial biomass. In contrast, saturated conditions gave rise to *Actinobacteria*. This study uses laboratory-scale incubators, metagenomics and spectral imaging approaches to examine how microbial processes and soil moisture interact during permafrost thaw in an Alaskan location to determine how these factors drive biogeochemical cycles in arctic soils.

OTS15/7 - Soil microbial legacy of extreme weather influences microbial but not plant communities

Presenting Author - *Lingjuan Li, University Of Antwerp, Belgium*

Author/s - *Qiang Lin, Ivan Nijs, Gerrit Beemster, Han Asard, Kris Laukens, Erik Verbruggen,*

Abstract Content

Soil microbial communities regulate and maintain ecosystem functions and services in a changing world. The changes in precipitation may affect soil microbial communities and have long-lasting effects on plant and microbial responses to subsequent precipitation regimes. Currently, little is known about whether previous extreme weather-induced changes in the soil microbial community will cause a legacy, influencing plant productivity and microbial community composition under new conditions. To assess the role of microbes in soil legacy, we performed a two-phase mesocosm experiment. In the first phase, twelve plant species were grown under either an ambient (1 day wet-dry alternation precipitation regimes) or an extreme PR (30 days consecutive wet–dry alternation precipitation regimes) for one year to generate “conditioned” soil. In the second phase, new plant communities were established with soil inoculums from the first phase, and subjected to either the ambient or extreme PR for 60 days. Our results show that microorganisms conditioned by past regimes significantly affected the subsequent development of soil microbial communities, but did not trigger soil legacy effects on plant performance in the context of subsequent regimes. Soil microbial legacies had larger impacts on fungal communities than bacterial communities, and were amplified over time for fungal communities. This study provides environmental evidence for a soil microbial legacy of climate extremes on responding to subsequent climate extremes, helping to understand and predict the effects of future climate change.