World Microbe Forum: FEMS Abstract Book - Table of Contents

FEMS iPosters Presented at World Microbe Forum

FEMSP100 FEMS: Environmental Microbiology and Ecology

Control Number:	2021-A-7438-MICROBE
Session Type:	**iPoster**
Session Number:	**FEMSP100**
Session Title:	**FEMS: Environmental Microbiology and Ecology**
Topic 1:	FEMS - Environmental microbiology and ecology
Keyword 1:	Oil spills
Keyword 2:	metagenomics
Keyword 3:	alkane degradation
Abstract Title:	**Identification Of Hydrocarbon-degrading Bacteria And Key Metabolic Pathways From Genomes Reconstructed From An Isotopically-labelled Community**
Author Block:	**M. Vázquez Rosas Landa**[1], G. Waldram[2], V. De Anda[1], A. Angelova[2], T. Gutierrez[2], B. Baker[3]; [1]The Univ. of Texas, Port Aransas, TX, [2]Heriot-Watt Univ., Edinburgh, United Kingdom, [3]The Univ. of Texas at Austin, Port Aransas, TX
Abstract Body:	Microbes can use petroleum as a source of energy; therefore, they are essential players in the bioremediation efforts. Here we explored the biodiversity and metabolisms of microbial communities to degrade petroleum hydrocarbons in the Faroe-Shetland Channel (FSC), a heavily used waterway for petroleum shipping. Forty-two new genomes were reconstructed from isotopically labeled hexadecane microbial enrichments from 5 and 700m in the water column. Phylogenomic analyses revealed the metagenome-assembled genomes (MAGs) fall within nineteen genera. Alcanivorax was dominant at both depths; however, Lentibacter and Thalassolituus were more dominant in the shallow (5m) enrichment, while Dokdonia and Olleya were more prevalent at 700m. Alcanivorax, Marinobacter, Thalassolituus, and Oleibacter code for complete pathways for alkane degradation via beta-oxidation; however, only Marinobacter, Thalassolituus, and Oleibacter carried the type VI secretion systems genes (T6SS), which has been considered an adaptation towards hexadecane degradation in Marinobacter. Bacteria in the shallow water code several pathways for sulfur cycling, while the bacteria at 700m contain more genes for nitrogen cycling. These findings advance our understanding of hydrocarbon degradation in the North Atlantic Ocean and the metabolic machinery involved in the adaptations of alkane degradation in different bacterial lineages.

Control Number: 2021-A-7681-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: microplastics

Keyword 2: biofilm

Keyword 3: salinity gradient

Abstract Title: **The Travelling Particles: Changes In Microplastic Biofilm Community Structure Across A Salinity Gradient**

Author Block: **J. Song**[1], L. Beule[2], A. Wichels[1], G. Gerdts[1]; [1]Alfred Wegener Inst. Helmholtz Ctr. for Polar and Marine Res., Helgoland, Germany, [2]Georg-August Univ. Göttingen, Göttingen, Germany

Abstract Body: **Background**: An increasing number of studies report on microplastics (MP) in nature and their interactions with microbial communities. Rivers, as major transport routes for MP into marine systems and as reservoirs for bacteria, are important focal points in understanding these interactions. **Objectives**: This study aims to investigate MP as substrates for bacterial colonization and their potential to transport these communities from riverine to marine environments. **Methods**: This transport was simulated via a sequential incubation experiment, where 3 sample types, synthetic (HDPE, tyre wear) and natural (wood) particles, were enclosed in a cage and successively incubated at four sites, from freshwater (Weser estuary) to coastal seawater offshore (North Sea). After each incubation period, a subset of each particle type and surface waters were sampled and the cage moved to the subsequent site. A second cage was simultaneously incubated at the offshore site for the entire period. **Results**: Bacterial communities differed significantly between sample types at each site and across the salinity gradient. Communities detected on HDPE were the most diverse, while those on tyre wear were the least. Percentage similarities between sample types were low overall while similarities within each type decreased across the salinity gradient. Of the total detected ASVs, only 0.7 % were successively found on HDPE across all sites, none of which were detected on HDPE from the second cage offshore. Our results show an almost complete turnover of bacterial communities on MP transported from riverine to marine systems.

Control Number: 2021-A-7701-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: Tropical peat swamp forest

Keyword 2: Carbon cycle

Keyword 3: Soil microbiome

Abstract Title: **Soil Prokaryotic Community Structure Association To Biogeochemical Processes In Tropical Peat Dome Of Sarawak, Malaysia**

Author Block: **S. Dom**[1], M. Ikenaga[2], S. Lau[1], S. Radu[3], F. Midot[1], M. Yap[1], M-Y. Chin[1], M. Lo[1], M. Jee[1], N. Maie[4], L. Melling[1]; [1]Sarawak Tropical Peat Res. Inst., Kota Samarahan, Malaysia, [2]Kagoshima Univ., Kagoshima, Japan, [3]Universiti Putra Malaysia, Serdang, Malaysia, [4]Kitasato Univ., Towada, Aomori, Japan

Abstract Body: **Background:** Tropical peat swamp forest is characterized as a water-logged, anoxic and acidic environment that contains large amount of carbon and is home to a diverse range of prokaryotic communities involve in various nutrient cycling processes. **Objectives:** This study was conducted to characterize soil prokaryotic communities in tropical peat swamp forest in relation to carbon nutrient cycling. **Methods:** Peat soil samples were collected at two depths (0-20 cm and 30-50 cm) in three primary forest types in a tropical peat dome in Sarawak, Malaysia namely, Mixed Peat Swamp (MPS), Alan Batu (ABt), and Alan Bunga (ABg) forests and analyzed via 16S meta amplicon sequencing using Illumina Miseq. Soil chemical properties and environmental condition such as soil pH, total carbon (C), total nitrogen (N), C/N ratio, loss on ignition, pyrophosphate solubility index and the water table were also measured. **Results:** It was observed that anaerobic and fermenter microbial groups such as Acidobacteria, Proteobacteria, Actinobacteria and Firmicutes comprised a large portion (80-90%) of the soil prokaryotic population. In addition, the prokaryotic community composition varied based on different forest types and soil depths. Specifically, the composition of prokaryotic community in MPS forest was influenced heavily by the higher humification level and lower pH conditions but for ABt and ABg forests, the prokaryotic community composition was shaped by the less acidic condition and higher organic matter content. Moreover, ABt and ABg exhibited higher prokaryotic diversity and abundance compare to MPS forest. Furthermore, *Methylovirgula ligni*, a major species of methanotroph in MPS could contribute to the low methane (CH_4) gas emissions in this forest type as this microorganism has the ability to consume CH_4. As for ABt and ABg, the major species were *Aquitalea magnusonii* and *Paraburkholderia oxyphila* respectively which can degrade aromatic compounds. This study demonstrated the effects of forest types and soil depths in shaping the prokaryotic community composition in tropical peat swamp forest. The information from this study provide better understanding of the below-ground biogeochemical processes in the tropical peatland ecosystem.

Control Number:	2021-A-7718-MICROBE
Session Type:	**iPoster**
Session Number:	**FEMSP100**
Session Title:	**FEMS: Environmental Microbiology and Ecology**
Topic 1:	FEMS - Environmental microbiology and ecology
Keyword 1:	Vibrio cholerae
Keyword 2:	tropics
Keyword 3:	estuary
Abstract Title:	**Environmental Determinants Of *Vibrio Cholerae* In A Tropical Monsoonal Estuary**
Author Block:	**K. Krishna**; CSIR Natl. Inst. of Oceanography, Kochi, India

Abstract Body:

Background : The argument between localist (water to human) and contagionist (human to human) mode of *Vibrio cholerae* transmission sustain in cholera endemic regions along Indian subcontinent, wherein outbreaks are often driven by hydrological factors such as rainfall and river discharge. Spatiotemporal variability in outbreaks further stress the need to understand how these hydrological factors influence environmental prevalence of *Vibrio cholerae*. **Objectives:**We monitored occurrence of *Vibrio cholerae* in response to range of physiochemical and biological factors in one of the largest tropical monsoonal estuary along south west coast of India viz. Cochin estuary. **Methods:**Water and phytoplankton collected during monthly (November 2008 - September 2009) sampling expedition from 10 stations, were enriched in alkaline peptone water and subsequently screened for presence of *Vibrio cholerae* and its toxigenic variants using PCR probes *toxR* and *ctxA* genes respectively. **Results:***Vibrio cholerae* in the study area were mostly found free living rather than plankton attached and were detected maximum during a lean discharge period. On the contrary, prevalence of this bacteria was reduced to minimum during heavy discharge. Logistic regression reveals prevalence of *Vibrio cholerae* in both fractions were determined primarily by temperature and dissolved oxygen, and its association with plankton was in addition driven by dissolved inorganic phosphate. Seasonal variability in temperature was in turn was regulated by rainfall ($r = -0.49$, $p < 0.005$). Overall, our study, highlight how physiochemical variables act in concert to orchestrate localist mode of *Vibrio cholerae* transmission observed during a least discharge period in a tropical monsoonal estuary.

Control Number: 2021-A-7772-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: Bacterial and Archea halophil

Keyword 2: carotenoids

Keyword 3: cancer cell lines

Abstract Title:

Author Block: **S. Shahbazi**; Extremophiles Lab. Dept. of Microbiol. Sch. of Biology Coll. of Sci. Univ. of Tehran, Tehran, Iran, Islamic Republic of

Abstract Body: Background: Extreme environments are proved to be interesting as a valuable source for industrially and biotechnologically important bacteria which produces enumerable active biomolecules. Saline environment are chemically close to human blood plasma and could provide microbial products and enzymes that could be safer having no or less toxicity or side effects when use for human therapeutic application. The halophilic and halotolerant microorganisms, live in these environments and contain high concentrations of various carotenoids with notable medical abilities. Therefore, today halophilic bacteria are promising candidates for medicinal purposes. The purpose of this study was to Identification of carotenoids from halophile and halotolerant microorganisms isolated from different saline environments of Iran, with anti-cancer cell activity. Objectives: The aim of this study was to evaluate the anti-cancer effect of carotenoids isolated from native Iranian halophilic microorganisms with the ability to inhibit breast and prostat cancer cell lines. Methods: 40 strains of halophilie and halotolerant strains were selected. carotenoids were extracted with methanol. Their identification of carotenoids was performed at a wavelength of 450 nm by UV-VIS spectroscopy and confirmed by thin layer chromatography (TLC). MTT assaye was used to evaluate the effect of carotenoid extract on viability breast and prostat cancer cell lines, MCF_7 and PC3, respectively. A mesenchymal cell line were used as control cell line. Using Real time PCR technique, the expression of genes specific for apoptosis or cell cycle, including *CASP3*, *KI67* genes in the presence or absence of carotenoids was examined and *GAPDH* gene was used as a control. Results: Among all strains, carotenoids from two strains had the most potent cytotoxic effect on breast and prostate cancer cell lines (IC50 = 0.0625 mg/mL) without any effects on normal cells. It significantly increased both early and late apoptosis in both cell lines with the down-regulation of *KI67* gene expression.

Control Number: 2021-A-7800-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: metal resistance

Keyword 2: mobile genetic elements

Keyword 3: adaptive resistance

Abstract Title: **Insertion Sequence Elements Promote Adaptation Of *Cupriavidus Metallidurans* To Toxic Metal Concentrations**

Author Block: **R. Van Houdt**[1], A. Aertsen[2], N. Leys[1]; [1]Belgian Nuclear Res. Ctr. (SCK CEN), Mol, Belgium, [2]KU Leuven, Leuven, Belgium

Abstract Body: Background: Metal homeostasis is important for bacteria since they have to react to both scarcity and excess of essential or toxic metals. To defend themselves, bacteria depend on multiple metal resistance mechanisms, including efflux pumps, proteins changing the oxidation state of metals, and intra- or extracellular sequestration of metals. *Cupriavidus metallidurans* was one of the first bacteria to be isolated from industrial sites characterized by an extremely high metal content and contains an unprecedented number of genes involved in the resistance and processing of metals.Objectives: While the metal resistance mechanisms of *C. metallidurans* have been well established, much less is known about its adaptive potential to metal stress. In this study we scrutinized its adaption to zinc stress.Methods and Results: A mutant (CH34^{ZnR}) of *C. metallidurans* CH34 adapted to high zinc concentrations was generated. Characterization revealed that it was also more resistant to cadmium, and that it incurred 7 insertion sequence (IS)-mediated mutations. Among these, an IS*1088* disruption of the *glpR* gene (encoding a DeoR-type transcriptional repressor) resulted in the constitutive expression of the neighboring ATP-binding cassette (ABC)-type transporter. Deletion of *glpR* or the ABC transporter and complementation of CH34^{ZnR} with the parental *glpR* gene further demonstrated that loss of GlpR function and concomitant derepression of the adjacent ABC transporter is pivotal for the observed resistance phenotype. Upregulation of this ABC-type transporter is therefore proposed as a new adaptation route towards metal resistance.Next, adaptation to zinc stress of a derivative devoid of the main zinc resistance determinant was scrutinized. Specifically, upon exposure to toxic zinc concentration, resistant variants arose in which a compromised *cnrYX* regulatory locus caused derepression of CnrH sigma factor activity and concomitant induction of the corresponding RND-driven *cnrCBA* efflux system. Late-occurring zinc resistant variants likely arose in response to the selective conditions, as they were enriched in *cnrYX* disruptions caused by specific IS elements whose transposase expression was found to be zinc-responsive. Interestingly, deletion of *cnrH*, still enabled adaptation by transposition of IS elements that provided outward-directed promoters driving *cnrCBAT* transcription. Thus, transposition of IS elements can be induced by stress conditions and play an important role in the adaptation to stress.

Control Number:	2021-A-7824-MICROBE
Session Type:	**iPoster**
Session Number:	**FEMSP100**
Session Title:	**FEMS: Environmental Microbiology and Ecology**
Topic 1:	FEMS - Environmental microbiology and ecology
Keyword 1:	filamentous fungi
Keyword 2:	surface sampling
Keyword 3:	Rhodotorula
Abstract Title:	**Fungi Cultured From Canine Coats Share A Filamentous Growth Classification**
Author Block:	**A. J. Reese**[1], A. Conrath[1], L. C. Busch[1], J. H. Kreigline[2], A. Metzger[1], A. Gould[1], F. Namous[2], J. J. Ory[1]; [1]Univ. of Hlth.Sci. & Pharmacy in St. Louis, SAINT LOUIS, MO, [2]Cedar Crest Coll., Allentown, PA
Abstract Body:	**Background:** How easy is it to pick up fungi from our surroundings? **Objectives:** We wanted to determine the prevalence of mold and yeast species on dog coats as dogs spend time outdoors and are in close contact with their owners. **Methods:** Members of a college community collected samples by rubbing dog coats several times with a sterile cotton swab over fall break and storing them in sterile collection vessels until processing. Samples were transferred to Sabouraud agar plates, incubated for three to seven days at 34 °C, plates photographed, and growth morphologies cataloged phenotypically. Representative mold samples were collected and genomic DNA extracted for further analysis. Potential yeast samples were streaked onto yeast peptone dextrose agar plates with chloramphenicol, further re-streaked for isolation, and genomic DNA extracted. The internal transcribed spacer (ITS) regions of genomic DNA of molds and yeasts were amplified using standard and modified polymerase chain reaction conditions, and the resulting amplicons were sequenced. The resulting trace reads were used to type the strains against the NCBI ITS database. **Results:** Of the seventy-three dog samples collected and analyzed, the most common molds identified were *Epicoccum*, *Curvularia*, *Alternaria*, *Moesziomyces*, and *Penicillium*. The most common yeasts were *Rhodotorula* and *Hannaella*. All of the genera described are classified as having filamentous growth forms. These results demonstrate some of the genera of fungal cells and spores that dogs can carry on their coats. Most of the species found were identified as plant pathogens, consistent with their environmental sampling from outdoor plants. A few, *Rhodotorula* and *Alternaria,* can have possible negative impacts on human health. This work illustrates that dog coats can harbor a wealth of molds and yeasts from outside environments, some of which could impact

human health. Understanding microbial reservoirs is necessary for predicting transmission risks from dog coats to individuals who may be immunocompromised.

Control Number: 2021-A-7841-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: environmental microbiology

Keyword 2: machine learning

Keyword 3: microbial ecology

Abstract Title: **Analysing The European Lake Microbiome Using Machine Learning Methods: A Study In Complexity**

Author Block: **T. Sperlea**[1], D. Beisser[2], G. Hattab[1], J. Boenigk[2], D. Heider[1]; [1]Philipps-Univ. Marburg, Marburg, Germany, [2]Univ. of Duisburg-Essen, Essen, Germany

Abstract Body:

Background: The development of Next-Generation Sequencing (NGS) methods opened the doors to observe and study objects that were not accessible before. Metabarcoding, for example, made it possible to, concurrently, record the hundreds of thousands different microbial operational taxonomic units present in environmental samples. In contrast to their wet-lab counterparts, which stem from well-controlled experimental settings, environmental sequencing datasets describe complex and chaotic systems. The underappreciation of this distinction might act as a roadblock for microbiome research.

Objectives: Analysing the lake microbiome represented in a large metabarcoding dataset generated by sampling taken from around 200 lakes across Europe, we encountered several theoretical and methodological obstacles regarding the distinction between experimental and environmental datasets. For example, with the latter, it is often impossible to control for confounders because not all relevant variables have been measured. This makes a re-interpretation of correlative analyses essential.

Methods: We combine machine learning methods and bioindicator analyses in such a way as to take the aforementioned obstacles into account and to work around them.

Results:

We have quantified the co-variation of the European lake microbiome with regard to a large set of physico-chemical parameters and the land use surrounding the lakes. Building on these results, we identified parameters that, indirectly or directly, act as strong determinants of the microbial community structure. Furthermore, we established so-called multi-task bioindicators, i.e., microbial taxa that respond to changes in many of the parameters studied here and, from these, we were able to gain insights into the latent structure of the microbiome. Taken together, these results show that, in spite of the obstacles discussed before, there are ways to analyse datasets from complex environments that result in meaningful statements.

Control Number: 2021-A-7907-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: metagenomics

Keyword 2: veterinary epidemiology

Keyword 3: veterinary pathogens

Abstract Title: ***Providencia Alcalifaciens* In The Canine Gut Microbiota**

Author Block: **A. M. Aardal**, E. Skancke, K. M. V. Herstad, S. F. Nørstebø, A-K. Llarena; Norwegian Univ. of Life Sci. (NMBU), Ås, Norway

Abstract Body:

Background: During the autumn 2019, an outbreak of acute haemorrhagic diarrhea syndrome (AHDS) affected dogs in an urban area of Norway. Several dogs were affected by the condition which ended in death in some cases. *P. alcalifaciens* was isolated from faeces in 62.0% of the investigated dogs that were affected (n=127), while only 2.5% of healthy control dogs (n=158) tested positively for this bacterium. This led to the hypothesis that *P. alcalifaciens* was involved in the aetiology of AHDS.

Objectives: To further investigate the role of *P. alcalifaciens* in AHDS, we used data from a large, cross-sectional study of a healthy canine population. We aim to answer the following research questions: What is the prevalence of *P. alcalifaciens* in healthy dogs? And, is the presence of *P. alcalifaciens* associated with shifts in the gut microbiota?

Methods: We characterised the gut microbiota in 133 dogs by analysing shotgun whole genome metagenomic datasets. Dried faecal smears on cellulose matrix were collected by the owners, and following DNA extraction and library preparation, the samples were sequenced using the NovaSeq 6000 platform (Illumina), yielding paired-end reads of 150 bp. The bioinformatic analysis was based on the Talos pipeline for shotgun metagenomic analysis and included low quality trimming and adapter trimming using Trimmomatic, removal of low complexity reads, removal of phi X reads and removal of human reads using BBTools and taxonomic classification using Kraken2.

Results: The clean samples had an average of 19.6 million forward and reverse reads, and an average of 35.22% reads were classified per sample (range: 10.75 to 68.13%). Faecal samples from 132 dogs had reads classified as *P. alcalifaciens*, albeit at low read counts (median: 15; range: 1 to 4296 reads). Other species of *Providencia* spp. were also identified. Further bioinformatic work to identify shifts in microbiota for samples with higher amounts of *P. alcalifaciens* is underway.

Control Number: 2021-A-7912-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

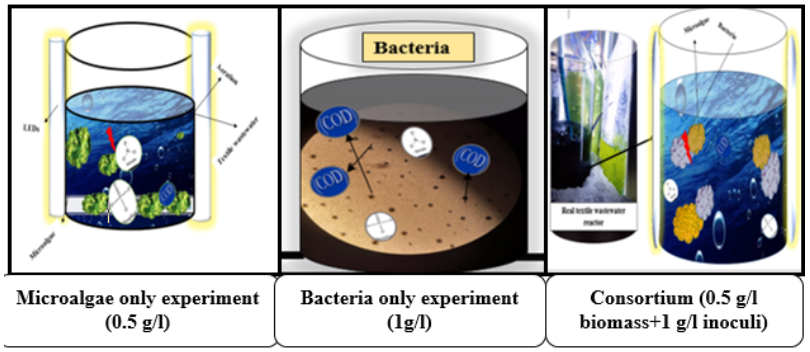
Keyword 1: Nutrients removal

Keyword 2: consortium

Abstract Title: **Bioremediation Of Real Textile Waste Water With Consortium**

Author Block: **N. RAZA**[1], M. Rizwan[2]; [1]Mehran Univ. of Engineering and Technology, HYDERABAD, Pakistan, [2]US Pakistan Ctr. for Advanced Studies in Water M, Jamshoro, Pakistan

Abstract Body: **Bioremediation of Real Textile Waste Water with Consortium**Abstract The unchecked disposal of textile wastewater containing high concentration of nitrogen (N), phosphorus (P) and Chemical oxygen demand (COD) responsible of causing Eutrophication resulting the loss of precious aquatic life and water pollution. Worldwide eutrophication issue highlighted the need of cost effective and eco-friendly method for removal of nutrients from textile wastewater. Bioremediation is such a technique used in 21th century in this regard. The Batch scale experiment was conducted in three different experiments Vis, single stage, two stage and consortium to remove nutrients with maximum biomass production. The results indicated that consortium of microalgae-bacteria provide maximum removal efficiency as 58.57 % nitrate, 86.42 % phosphate, 91.5% COD, respectively. The native *Chlorella sp*. Of micro-algae with *Styphloococus sp. of bacteria* was used in experiment observed under florescence microscope. Hence, results indicated that this method not only treat textile waste water cost-effectively but also, produces chlorophyll as biomass which could be utilized to overcome energy deficit and fertilizer production.



Control Number: 2021-A-7922-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: Streptomyces

Keyword 2: staurosporine

Keyword 3:

Abstract Title: **Potential Elicitation Of Staurosporine Production**

Author Block: **M. Stevanovic**[1], M. Mojicevic[1], P. M. D'Agostino[2], T. A. M. Gulder[2], J. Nikodinovic-Runic[1], S. Vojnovic[1]; [1]Inst. of Molecular Genetics and Genetic Engineering, Belgrade, Serbia, [2]Technical Univ. of Dresden, Dresden, Germany

Abstract Body: Streptomycetes are known for their exceptional biosynthetic potential, which is reflected in genomes with tens of different biosynthetic gene clusters (BGCs), but only small fraction of those clusters is expressed in laboratory conditions. Co-cultivation has become an emerging tool for activation of cryptic BGCs or elicitation of already active BGCs. In the previous research, an isolate from our microbial collection, *Streptomyces* sp. BV410[1], was found to produce staurosporine, a potent inhibitor of protein kinases. However, further research revealed that soil isolate BV410 was a mixture of two co-isolated streptomycetes, named BV410-1 and BV410-10[2]. The aim of this work was to identify the staurosporine producer and investigate the possibility that co-isolated *Streptomyces* sp. influence the production of staurosporine. Extracted genomic DNA was submitted for sequencing using the PacBio platform, assembled and submitted to RAST for genome annotation. The genome of BV410-10 was 11.4 Mb while that of BV410-1 was smaller with a genome size of 9.5 Mb. Annotated genomes were submitted to AntiSMASH which identified 29 BGCs from BV410-10 and 28 BGCs from BV410-1, including that responsible for staurosporine biosynthesis. Both BV410-1 and BV410-10 were cultivated in the previously described liquid medium, under the optimized conditions. The analysis of ethyl acetate extracts of whole cultures confirmed that *Streptomyces* sp. BV410-1 is the producer of staurosporine. Cross-streak experiments revealed that BV410-1 has an inhibitory effect on the growth of BV410-10, which increases with time interval between inoculations. Also, it was shown that the staurosporine yield in the BV410-1 monoculture was 5-fold lower compared to the yield of the mixed culture in the previous research. Possible elicitation of staurosporine production was tested by co-cultivation of the two streptomycetes and by adding either the cell free supernatants from BV410-10 monoculture or BV410-10 ethyl acetate extract to the growing culture of staurosporine producer.

Control Number: 2021-A-7946-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: metagenomics

Keyword 2: soil microbiology

Keyword 3: biochar

Abstract Title: **Differential Effects Of Various Biochar On The Soil Microbiome**

Author Block: **P. Kerner**[1], J-E. Lee[2], E. Struhs[3], A. Mirkouei[3], X. Liang[4], K. Sharma[1], K. Aho[1], K. Lohse[1], R. Dungan[5], Y. You[2]; [1]Idaho State Univ., Pocatello, ID, [2]SUNY Coll. of Environmental Sci. and Forestry, Syracuse, NY, [3]Univ. of Idaho, Idaho Falls, ID, [4]Univ. of Idaho, Aberdeen, ID, [5]Northwest Irrigation and Soils Res. Lab., Kimberly, ID

Abstract Body: Background: Biochar is a multifunctional soil amendment capable of improving soil health and mitigating agricultural pollution. Yet the underlying mechanisms are not fully understood, as are the influential factors that determine the overall performance of biochar amendment. This hinders precision biochar applications on a larger scale. Objectives: We aim to disentangle the influence of biochar, particularly the feedstock selection and application rate, on the soil microbiome, with an ultimate goal to bridge microbial phylotype and soil phenotype. Methods: Biochar was produced by slow pyrolysis at 350 °C from cattle manure, corn stover, and pinewood, and amended to an agricultural soil at either high or low level in controlled microcosm experiments. Over ten weeks, archaeal, bacterial and fungal communities were monitored using amplicon sequencing; community-level substrate utilization was assessed using the EcoPlate assay; greenhouse gas generation was estimated from headspace measurements. Results: The soil microbiome displayed sequential changes in alpha and beta diversity following a single amendment of biochar, with stronger responses seen among prokaryotes than fungi. By week 10, the prokaryotic communities clustered significantly based on biochar feedstock and application rate ($p < 0.001$ by PERMANOVA), while the fungal communities displayed a similar but insignificant pattern ($p > 0.050$). Biochar addition, regardless of feedstock, enriched plant-beneficial bacteria likely involved in symbiotic nitrification, denitrification, decomposition of cellulose and lignocellulose, and suppression of soil-borne pathogens. In line with these shifts in microbial community structure, biochar altered community-level substrate utilization, stimulating the usage of amino acids, carbohydrates, carboxylic and ketonic acids, phenolic compounds, and polymers at varying microcosm stages. Feedstock repeatedly emerged as the primary driver of differential patterns of community usage of substrates and production of CO_2, CH_4, and N_2O. The fact that biochar applied at a low rate did not significantly change soil properties but still modified the soil microbiome suggests direct biochar-microbiome interactions as the mechanism underlying beneficial effects of biochar soil amendment. Overall, this study highlights the importance of feedstock selection in magnifying desired microbial responses for sustainable agriculture.

Control Number: 2021-A-7996-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: HABs

Keyword 2: Arctic lake blooming

Keyword 3: Microbiome

Abstract Title: **Environmental Factors And Dynamics Of Microbial Communities During Summer Algal Bloom In Large Eutrophic Arctic Lake**

Author Block: **A. Bekkelund**[1], N. Kashulin[2], T. Kashulina[1]; [1]Creek-Bio AB, Oslo, Norway, [2]INEP, RAS, Apatity, Russian Federation

Abstract Body: **Background.** Lake Imandra is the largest arctic freshwater lake located in the Kola Peninsula, Russia. Regular cyanobacterial HABs in Lake Imandra Started in 2000 and since are always associated with fish mortality. All prior studies of the phytoplankton in Lake Imandra were performed using traditional manual taxonomic identification techniques and thus provided limited data on the entire biodiversity and co-occurrence of the bloom-forming species. **Objectives.** The objective of our study was to perform an analysis of eutrophication dynamics of Lake Imandra and depict earlier undescribed biodiversity and dynamics of microbial communities during summer HAB. **Methods.** Hydrochemical analysis and visualization of TC/TN/TP/Chl-a ratios: Water samples were collected between 1990 - 2017 and analysed according to standardised procedures in ISO17025:2009 compliant laboratory certified by national Agency on Technical Regulation and Metrology. Chlorophyll-a concentrations and key hydrochemical parameters were visualized in the ternary graphical system suggested by Smith et al. Linear regression analysis and PCA as well as visualization of the TC/TN/TP/Chl were performed in R. DNA preparation and processing: The surface water samples were collected from a boat using sterile one-litre bottles, filtered through 0.22 µm membranes, and used for DNA extraction with a DNeasy PowerWater Kit (QIAGEN). 16S amplicons were generated with primers specific to the V1-V3 hypervariable region (27F - AGAGTTTGATCCTGGCTCAG and 534R - ATTACCGCGGCTGCTGG) using the Nextera XT DNA Library Prep Kit (Illumina). The selected primers were suitable for identification of the plastids as well as bacteria. The 2 x 300 bp amplicons are available through NCBI BioProject PRJNA526000. **Results.** HABs in Lake Imandra occur outside optimal Redfield ratios, have a nonlinear association with P concentration, and begin as long as the P concentration reaches critical values specific to each part of the lake. The recent summer HABs in Lake Imandra occur as simultaneous blooms of a diatom *Aulacoseira* sp. and cyanobacteria *Dolichospermum* sp. We have depicted temporal dynamics of microbial communities during the beginning, peak, and end of the bloom and identified 37 bacterial phyla. *Aulacoseira* sp. and *Dolichospermum* sp. have genetic traits requisite for diatom-

diazotroph associations, the ability to store nutrients, and form dormant stages. All these factors will ensure further development of the HABs in Lake Imandra and dispersal of both bloom-forming species to neighboring lakes.

Control Number: 2021-A-8015-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: methanogenesis

Keyword 2: geochemical modelling

Keyword 3:

Abstract Title: **An Active Microbial Community In Boom Clay Pore Water Collected From Piezometers Impedes Validating Predictive Modelling Of Ongoing Geochemical Processes**

Author Block: **K. Mijnendonckx**[1], M. Honty[1], L. Wang[1], J. Elke[1], A. Provoost[1], M. Mysara[1], K. Wouters[1], M. De Craen[2], N. Leys[1]; [1]SCK CEN, Mol, Belgium, [2]ESV Euridice, Mol, Belgium

Abstract Body: Safe geological disposal of radioactive waste requires a detailed understanding of the geochemical conditions present in the host formation. Consequently, analysis of pore water is essential, as its composition determines among others, the speciation and solubility of radionuclides. In Belgium, Boom Clay is considered a potential host formation. Although the elemental composition of Boom Clay pore water is relatively well known, the real mechanisms controlling the pCO_2 (g) and the pH, the two most important parameters, are not completely understood. Currently, these parameters are under investigation based only on inorganic chemistry. Borehole waters of different Underground Research Facilities (URF) harbour an active microbial community; however, their possible impact on the geochemistry of Boom Clay pore water extracted from piezometers is not yet examined. The present study discusses the evolution of the geochemistry and the microbial community in the pore water from the piezometers around the PRACLAY gallery of the HADES URF during 7 years after installation of the piezometers. Overall, the elemental composition seemed to vary during the first 4 years, while afterwards it remained quite stable. However, the pCO_2 values varied substantially over time, while the pCH_4 increased in all filters. The presence of an active microbial community in the piezometers, could explain why experimental pCO_2 - pH data do not correspond to the data obtained by predictive modelling, hampering validation of current predictive models of the ongoing geochemical processes. Moreover, the nature of the sampling equipment and the sampling procedure possibly stimulated the present microbial community, resulting in increased methane production rates. To improve predictive modelling, microbial processes are needed to be taken into account together with inorganic geochemistry considered at the current stage, which necessitates detailed microbial and geochemical monitoring in future studies.

Control Number: 2021-A-8018-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: Salinity gradient

Keyword 2: Microbial Structure-function relationship

Keyword 3: Greenhouse gases

Abstract Title: **Characterization Of Inland Saline Lake Types From The Structure And Metabolic Function Of Prokaryotic Communities Using Molecular Methods**

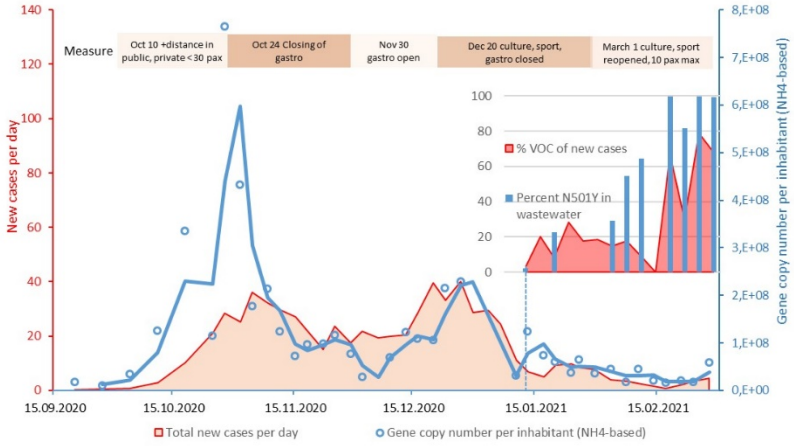
Author Block: **J. Miralles Lorenzo**, A. Picazo, C. Rochera, D. Morant, A. Camacho; Univ. of Valencia, Valencia, Spain

Abstract Body:

In this study we analysed the seasonal variation of the taxonomic structure and metabolic function of the prokaryotic communities inhabiting water and sediment of 15 inland saline lakes by 16S rRNA gene MiSeq Illumina sequencing. We selected the main subtypes on a salinity gradient, from brackish soda lakes to hypersaline lakes. The results of our study provided new insights on the factors affecting the structure and function of the prokaryotic communities of the saline inland lakes. Salinity was the main environmental driver that differentiated lake subtypes, but other factors are also relevant, such as seasonality, pH or the degree of anthropogenic alterations. Variability within some subtypes was high due to the strong seasonal changes, resulting in partial subtypes overlapping. Each subtype showed specific prokaryotic taxa with a low core community. In some lakes, completely different prokaryotic assemblages appear at different seasons. Anthropogenic alterations of some lakes also affected their prokaryotic communities by increasing the variability within each subtype. Besides the community structure, we studied the metabolic capabilities focusing on prokaryotic taxa that participate in some of the C-related metabolisms related to the lake C-balance, namely photosynthesis, aerobic methanotrophy and methanogenesis. Methanogenic archaea showed a high variability in their relative abundance among the studied lakes, which also affected the distribution of aerobic methanotrophic bacteria resulting in a complex balance of methane emissions. Primary producers showed strong seasonal variations, and were influenced by anthropogenic alterations. Our results highlight the high variability of inland saline lakes, where not only the lakes differed according to their subtype, but also the individuality and special characteristics of each lake shown by specific environmental variables has to be considered. Thus, further scientific studies and a proper management of this kind of wetlands must consider the high dynamism of inland saline lakes with a complex microbial community assembly and complex metabolic interactions.

Control Number:	2021-A-8025-MICROBE
Session Type:	**iPoster**
Session Number:	**FEMSP100**
Session Title:	**FEMS: Environmental Microbiology and Ecology**
Topic 1:	FEMS - Environmental microbiology and ecology
Keyword 1:	SARS-CoV-2
Keyword 2:	Wastewater
Keyword 3:	Wastewater epidemiology
Abstract Title:	**SARS-CoV-genes In Wastewater Serve As A Proxy For Prevalence, But Viral Variants Blur The Signal**
Author Block:	R. Markt[1], F. Amman[2], A. Bergthaler[2], L. Endler[2], D. Grünbacher[1], N. Kreuzinger[3], M. Mayr[1], M. Pedrazzini[4], E. Peer[1], W. Rauch[1], A. Wagner[1], **H. Insam**[1]; [1]Univ. of Innsbruck, Innsbruck, Austria, [2]Austrian Academy of Sci., Vienna, Austria, [3]Technical Univ. of Vienna, Vienna, Austria, [4]Regierung des Fürstentums Liechtenstein, Vaduz, Liechtenstein
Abstract Body:	The surveillance of SARS-CoV-2 through individual testing is biased by the testing strategy and thus alternative options for disease surveillance are desirable. In the past few months, progress has been made in SARS-CoV-2 wastewater (WW) epidemiology. We studied 25 WW plants in Austria and its neighbourhood, many of them for almost a year. We found that SARS-CoV viral gene copy numbers in wastewater served as early indicators of upcoming rises in disease incidence and thus gene copy numbers in WW may serve as a proxy for disease prevalence. Challenging predictor models, novel virus variants may, however, change the fecal shedding of viral fragments. As an example, for the Principality of Liechtenstein (38.000 inhabitants) the figure below shows the number of new cases, also those of the variant of concern (VOC, B.1.1.7.), the viral WW signal and the percent N504Y gene in the inflow of the WW treatment plant Bendern that serves the entire country. When looking at the WW signal, in the period of early November, the individual testing underestimated the disease prevalence compared to late December testing which we attribute to the doubling of the number of tests from Nov 19. In both periods, the WW signal started to increase prior to the incidences based on individual samples. With the appearance of B.1.1.7. by Feb 13, 2021, the WW signal increased compared to the incidences, albeit testing frequency remained constant. We attribute this to increased shedding by B 1.1.7. Our data corroborate the view that WW may serve as an early indicator and we conclude that (i) testing is unreliable concerning the estimation of prevalences and (ii) viral variants change fecal shedding and

thus predictor models need to be adapted.



Control Number: 2021-A-8045-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: adaptation

Keyword 2: niche

Keyword 3: Campylobacter

Abstract Title: **Interspecies Recombination In Agricultural *Campylobacter*: Is It The Song Or The Singer?**

Author Block: **E. Mourkas**[1], K. Yahara[2], S. Bayliss[1], J. Calland[1], B. Pascoe[1], S. Sheppard[1]; [1]Univ. of Bath, Bath, United Kingdom, [2]Natl. Inst. of Infectious Diseases, Tokyo, Japan

Abstract Body: Hypothesis:Evolutionary analyses of bacteria often consider lineages and species that inhabit different niches. For example, within the genus *Campylobacter* there are species that inhabit the gut of different animals and species that inhabit different niches within a single animal. The maintenance of these species as discrete entities depends on barriers to genetic exchange between them. These can be physical - with species inhabiting different niches, or adaptive - implying selection against hybrid lineages, but quantifying the relative importance of these barriers can be challenging. Considering genes, rather than lineages, as units of selection provides a theoretical solution to this. While understanding clonal population structure and phylogenetics remains important, new theoretical approaches consider the genes that underlie the collective functions of a microbiome (songs) rather than the lineages in which they are found (singers).Summary/Results:In this study, we analysed >600 genomes of multiple *Campylobacter* species isolated from birds, mammals and reptiles. We characterized interspecies core and accessory genome recombination in isolates from the same and different hosts and quantified the extent to which genes, rather than lineages, inhabit the niche. Specifically, for some species pairs there was ~0.6 times more recombination between cohabiting isolates than host segregated ones. Conclusions:By broadly defining the limits of interspecies recombination and the function of mobile genes, we provide real-world data by which to interrogate influential theories about the levels of the biological hierarchy (genes, lineages, species) at which selection operates to maintain what we know as ‘species’.

Control Number: 2021-A-8069-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: bacteria interactions

Keyword 2: pesticide degradation

Keyword 3:

Abstract Title: **Interspecies Interactions Of The 2,6-dichlorobenzamide Degrading *Aminobacter* Sp. Msh1 With Resident Sandfilter Bacteria: Indications For Mutualistic Interactions That Improve Micropollutant Degradation**

Author Block: **S. DU**, J. Vandermaesen, A. Wouters, M. Glorieux, B. Horemans, D. Springael; KU Leuven, Heverlee, Belgium

Abstract Body: Background: Bioaugmentation involves an invasion process requiring the establishment and activity of a foreign species in the resident community of the target system. Direct social interactions with residents, either antagonistic or cooperative, are suspected to play a role in invasion but to which extent is currently unknown. *Aminobacter* sp. MSH1 mineralizes the groundwater micropollutant 2,6-dichlorobenzamide (BAM) and is used for bioaugmentation of sand filters exploited in drinking water treatment plants to avert BAM-contamination. Objectives: To examine the nature of social interactions between MSH1 and sand filter resident species is a first step to decipher the role of direct interactions in bioaugmentation using a bottom-up synthetic biology approach. Methods: In dual- and triple-species assemblies containing MSH1 and 13 sand filter residents in sand filter microcosms, behaviors of MSH1 regarding growth, survival and BAM mineralization capacity were determined. Alongside, the cell densities of sand filter resident bacteria were counted using colony forming units determination on selective media. Results: The BAM mineralization by MSH1 was affected by the residents without though always impacting MSH1 cell densities indicating that interactions can also affect cell physiology. Exploitative competition explained most of the effects but also indications for interference competition were found. Two residents improved MSH1's mineralization in dual species assemblies, apparently in a mutual collaboration, and even overruled negative effects by other residents in triple species systems, suggesting strong beneficial effects. In conclusion, our study shows that sand filter communities can contain species that support MSH1 mineralization in sand filter environments. Such "benefactor" species might be used as co-inocula for improving the bioaugmentation of MSH1.

Control Number: 2021-A-8075-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: microbial ecology

Keyword 2: community assembly

Keyword 3: network analysis

Abstract Title: **Highly Specialized And Competitive Communities Emerge On Activated Sludge Bioreactors In A Succession Governed By Deterministic Processes**

Author Block: **M. de Celis**[1], J. Duque[1], D. Marquina[1], H. Salvadó[2], S. Serrano[1], L. Arregui[1], A. Santos[1], I. Belda[1]; [1]Complutense Univ. of Madrid, Madrid, Spain, [2]Barcelona Univ., Barcelona, Spain

Abstract Body:

Background: The performance of wastewater treatment plants (WWTPs) promotes the differentiation of niches needed for carbon, nitrogen, and phosphorous removal. These processes are carried out by the microbial communities inhabiting the bioreactors.

Objective: To study the structure and assembly mechanisms of microbial communities inhabiting activated sludge bioreactors of WWTPs.

Methods: We assessed the microbial community dynamics and assembly mechanisms of two WWTP bioreactors with microbial communities in different stages of adaptation, via 16S rRNA sequencing. We used a probabilistic model to calculate the co-occurrence and co-exclusion associations between microbial taxa and built metacommunity networks. These networks include every potential association in the system, allowing us to infer the associations occurring in individual samples, and thus, to infer their local network properties. Besides, we used phylogenetic turnover to assess the balance of stochastic/deterministic ecological processes involved in the assembly of these WWTPs microbial communities.

Results: The local network properties of the microbial community adapted to wastewater environment (FF-WWTP) showed variation over time, but not described a clear pattern. However, in the microbial community in adaptation to wastewater environment (SU-WWTP) the modularity and co-exclusion proportion showed a constant increase in detriment of clustering coefficient, reflecting the niche specialization the community is going towards. The FF-WWTP microbial community was divided in two subcommunities, showing a seasonal alternation related to the temperature variation over the year. This succession is observed in the ordination plot of beta diversity, where we observed how the microbial communities experiment an annual cycle governed by the temperature. However, the microbial community of SU-WWTP was divided in three subcommunities. One of them clearly correlated with nutrient removal rates, being the functional subcommunity. The dynamics of this community was marked by the detriment of the subcommunity governing the initial samples, in accordance with the increasing abundance of the functional subcommunity, indicating the functional adaptation

of the community to the environment. The described dynamics are the result of the selective pressure the wastewater is exerting, with a marked dominance of deterministic ecological processes governing community assembly.

Control Number: 2021-A-8120-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: adaptation mechanisms

Keyword 2: Rhodococcus

Keyword 3: pharma pollutants

Abstract Title: **Adaptation Strategies Of Rhodococci And Impact On The Ibuprofen Biodegradation**

Author Block: **E. A. Tyumina**, G. A. Bazhutin, I. B. Ivshina; Perm Federal Res. Ctr. of the Ural Branch of the Russian Academy of Sci.; Perm State Natl. Res. Univ., Perm, Russian Federation

Abstract Body: **Background**. Pharmaceutically active compounds (PhACs) are a large group of emerging contaminants found ubiquitously in aquatic and terrestrial ecosystems. Some of the most abundant PhACs in the environment are non-steroidal anti-inflammatory drugs (NSAIDs). NSAID molecules are highly reactive and stable. This determines their resistance to biodegradation, ecotoxicity, persistence and therefore threat to the environment. Among the microorganisms that carry out the processes of natural attenuation from anthropogenic xenobiotics, including PhACs, are actinobacteria of the genus *Rhodococcus*, which have the greatest variety of degradable pollutants and a wide range of adaptive capabilities. **Objectives**. To study the kinetics and regularities of the biodegradation process of ibuprofen, a monocyclic NSAID, often detected in the environment; to investigate the mechanisms of action of this pharmaceutical pollutant on bacterial cells. **Methods**. The mechanisms of adaptation of rhodococci exposed to ibuprofen were investigated using an atomic force microscope coupled with a confocal laser scanning microscope. The metabolic activity of bacteria was studied by high-resolution respirometry. Ibuprofen and its metabolites were detected using chromatographic and spectral methods. **Results**. The *Rhodococcus cerastii* strain capable of efficient biodegradation of ibuprofen at concentrations up to 100 mg/L was isolated from urban soil. The kinetic regularities of the ibuprofen bioconversion were studied. The initial stages of ibuprofen bioconversion with the formation of hydroxylated and carboxylated derivatives were described. In the presence of ibuprofen and its metabolites, a formation of specific multicellular aggregates accompanied by a pronounced morphological anomaly of cells (changes in their shape and size, parameters of cell surface roughness), a shift in the ζ-potential towards more negative values and a decrease in the permeability of cell membranes were revealed.

Control Number:	2021-A-8126-MICROBE
Session Type:	**iPoster**
Session Number:	**FEMSP100**
Session Title:	**FEMS: Environmental Microbiology and Ecology**
Topic 1:	FEMS - Environmental microbiology and ecology
Keyword 1:	microbial communities
Keyword 2:	antibiotic resistance
Keyword 3:	marine ecosystem
Abstract Title:	**Antibiotic Resistance In Black Sea Microbial Communities**
Author Block:	**M. Pavlovska**, I. Prekrasna, A. Dzhulai, E. Dykyi; Natl. Antarctic Scientific Ctr., Kyiv, Ukraine
Abstract Body:	**Background.**Marine environment pollution with antibiotics can become a serious ecological concern in the region turning the water body into the natural pool for the distribution of antibiotic resistance genes. **Objectives.**A large-scale study of antibiotic resistance in microbial communities has been performed in the Black Sea, both in the coastal and offshore regions. **Methods.**The quantitative distribution of the genes responsible for the inactivation of the beta-lactam (blaCMY, blaSHV), vancomycin (VanA, VanB), macrolides (erm) and colistin (mcr) was assessed with real-time quantitative PCR. **Results.**The present study revealed the distribution of antibiotic resistance genes targeting the response to all antibiotics included in our analysis at various locations across the Black Sea. Notably, the genes coding for the resistance to the last resort antibiotics, such as vancomycin and colistin were detected reaching $2,9\times10^5$ and $3,1\times10^6$ gene copies in 5000 ng of DNA/ml respectively. The less abundant gene was erm encoding macrolide-lincosamine resistance amounting for up to $8,0\times10^4$ gene copies in 5000 ng of DNA/ml. The abundance of antibiotic resistance genes was significantly higher (p-value = 0.05) within Ukrainian coastal area when compared to the offshore stations. This certainly highlights the presence of an alarming problem of antibiotic resistance in the region and calls for regular monitoring of antibiotic resistance in both marine and

freshwater bodies.

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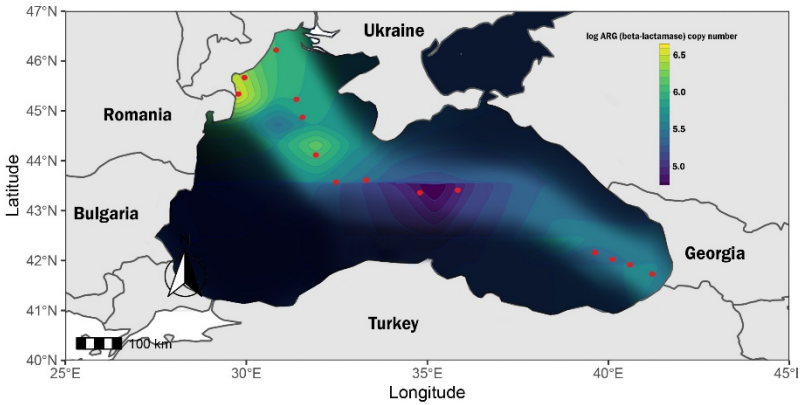

Control Number: 2021-A-8160-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: Methane Production

Keyword 2: Microbes

Keyword 3: Freshwater ecosystems

Abstract Title: **Methane Production: The Role Of Microbes In Freshwater Ecosystems**

Author Block: **S. Khatun**; Univ. of Dhaka, Dhaka, Bangladesh

Abstract Body: Methane production has known to be a common phenomenon in freshwater ecosystems. Although methane is conventionally reported to produce by anaerobic methanogens in sediments, methane oversaturation in the oxygenated water columns has been observed in many freshwater ecosystems. Thus, it is crucial to elucidate the plausible sources of dissolved methane in freshwater ecosystems. It has been observed that the stoichiometric balance of biogeochemical elements might dictate the structure of planktonic microbial communities and thereby control the methane production in these ecosystems. Here, we will discuss all the potential microbes that are relating to aerobic and anaerobic methane production in freshwaters. Although studies have suggested methanogenic and planktonic bacteria and algae that carry C-P lyase genes may responsible for aerobic methane production in oxic water, we hypothesize that other freshwater microbes might be responsible for this phenomenon in many freshwater systems. We, therefore, review the previous studies relating to oxic methane in the aerobic water column of different freshwaters and it is likely that other unknown pathways might co-exist to stimulate the accumulation of methane in the oxic water column of freshwater ecosystems. We will then discuss the role of anaerobic microbes present in both oxic and anoxic environments to produce methane production in such an environment. Finally, the obtained data will present the importance of different microbes to advance our understanding of freshwater methane dynamics as well as the interaction of microbial communities in freshwaters.

Control Number: 2021-A-8194-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: microbiome

Keyword 2: AMR

Keyword 3: chicken

Abstract Title: **Characterization Of Chicken Caecal Microbiota And Their AMR Gene Profile: An In-house Longitudinal Study**

Author Block: **A. Hinsu**[1], K. Panchal[1], A. Golaviya[1], H. Paleja[1], S. Jakhesara[1], D. Blake[2], F. Tomley[2], P. Koringa[1]; [1]Anand Agricultural Univ., Anand, India, [2]Royal Vet. Coll., North Mymms, United Kingdom

Abstract Body:

Background

Increased preference of chicken as meat source has surged its production and zoonoses risk. Chicken caecum microbiota have been linked with chicken health and production. The widespread use of antibiotics in poultry has raised concern of antimicrobial resistance (AMR). However, AMR genes could also be obtained by horizontal gene transfer from other passing microbes. Here, we have studied the longitudinal changes in caecal microbiota and their AMR gene content in the absence of any antibiotics supplement.

Objectives

The study was conducted to track the changes in microbiota development as well as their AMR gene content across the production cycle.

Methods

Commercial broiler Cobb400 were raised in six pens each containing 30 birds at university poultry station. Standard diet was fed to birds without any feed additives. Caecal content was collected each week from one bird per pan from 0th day to 42nd day totalling 42 samples from 7 collections. V3-V4 region of 16S rRNA gene was amplified from metagenomic DNA and sequenced on Illumina MiSeq sequencer. Same DNA was used to target 493 AMR genes part of AMR AmpliSeq Panel and sequenced on Illumina MiSeq sequencer. The 16S data was analysed using DADA2 pipeline and taxonomy was assigned using GTDB r95. Data was further processed in R environment using Phyloseq and other packages. AMR Ampliseq data was mapped to custom reference using BWA and summarised average coverage per target gene was converted to TPM (Transcripts per million) for analysis. The data was analysed in R environment using various packages.

Results

Despite the sterile gut hypothesis, we were able isolate low concentration DNA from 0-day old chicks sample. The 16S data was

classified in total 3274 ASVs. Significant differences were observed in alpha diversity metrics among all collections even after excluding 0-day. Further, an increasing trend was observed for Shannon diversity with respect to age/collection. Similarly, NMDS plot also showed separate cluster for Collection-1, while rest of the samples showed horse-shoe like pattern. However, PERMANOVA test showed significant differences among all collections with and without Collection-1. Firmicutes_A followed by Bacteroidota phyla were the most abundant from 24 detected phyla. 82 genes from 15 different Antibiotic resistance classes were detected from AmpliSeq data with mapped reads >5. Majority of the reads belonged to tetracycline-resistant genes followed by aminoglycoside resistance genes. Further, 14 genes were observed to differ significantly across Collection-2 to 7.

Control Number: 2021-A-8221-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: psychrophiles

Keyword 2: microbial communities

Keyword 3:

Abstract Title: **Investigating The Structure Of Cold Microbiomes: The Chesapeake Bay In Winter And Arctic North Slope In Summer**

Author Block: **C. Sweet**[1], C. Koo[1], L. Treaster[1], A. Barker[2], T. Douglas[2], J. Smith[1], S. Colston[3], S. Gallaher[1]; [1]United States Naval Academy, Annapolis, MD, [2]Cold Regions Res. Engineering Lab., Fort Wainwright, AK, [3]Naval Res. Lab, Washington, DC

Abstract Body: Background: The bacterial community of Arctic rivers (the planktonic microbiome) and of temperate rivers during winter is of interest to ecologists and microbiologists as unique collections of organisms adapted to psychrophilic (cold-growth) conditions. Methods: We characterized these microbiomes by both culture-based and DNA extraction water sampling, identification, and taxonomic analysis in both the Kuparuk River and Sagavanirktok River of North Slope Borough (AK) in summer 2019 and the Chesapeake Bay main stem and Severn River (MD) in winter of 2020. Objectives: 16S identification of cultured bacteria and shotgun metagenomic characterization of the whole microbial community were used to assess two hypotheses: 1.) That there is commonality in microbiome structure and composition between the summer arctic river watersheds and the winter Chesapeake/Severn River and 2.) That the headwaters-origin hypothesis of river microbial communities is representative of the microbiome structure in arctic riverine watersheds. Results: Our preliminary data do not support the commonality hypothesis between the arctic summer and Chesapeake winter microbiomes, and suggest that the headwater-origin hypothesis for river microbial populations discussed in other studies only partially explains development and structure of the arctic riverine microbiome. The analysis presented here shows differences in taxonomic distribution by location independent of headwater origin, and potential correlation to the hydrology and/or geochemistry of the arctic watersheds. We are working to establish sampling and analysis programs for vertical year-over-year characterization of these microbiomes as well as further horizontal comparison between these watersheds.

Control Number: 2021-A-8222-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: geosmin

Keyword 2: chemical ecology

Keyword 3: aposematism

Abstract Title: **The Ubiquitous Terpene Geosmin Is A Warning Chemical**

Author Block: **B. Findlay**; Concordia Univ., Montreal, QC, Canada

Abstract Body: Known as the smell of earth after rain, geosmin is an odorous terpene detectable by humans at picomolar concentrations. Geosmin production is heavily conserved in actinobacteria, myxobacteria, cyanobacteria, and some fungi, but its biological activity is poorly understood. We theorized that geosmin was an aposematic signal used to indicate the unpalatability of toxin-producing microbes, discouraging predation by eukaryotes. Consistent with this hypothesis we have found that geosmin and the related terpene 2-methylisoborneol reduced predation of *Streptomyces coelicolor* and *Myxococcus xanthus* by the bacteriophagous *Caenorhabditis elegans.* Predation was restored by the removal of both terpene biosynthetic pathways or deletion of the *C. elegans* ASE sensory neuron, and resulted in the death of the nematodes. By itself geosmin was non-toxic, but did lead to *C. elegans* movement with both roaming and dwelling characteristics. This is the first warning chemical to be identified in bacteria or fungi, and suggests molecular signalling affects microbial predator-prey interactions in a manner similar to the well-studied visual markers of poisonous animal prey.

Control Number: 2021-A-8288-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: plant-microbe interactions

Keyword 2: Micromonospora

Keyword 3: bioinformatics

Abstract Title: **A New Tool For The Selection Of *Micromonospora* Strains Associated To Plants With Potential For Plant Growth Promotion**

Author Block: **R. Riesco**, M. Ortúzar, M. E. Trujillo; Univ. de Salamanca, Salamanca, Spain

Abstract Body: **Background** "Plant Growth Promoting Bacteria" or PGPB is a common term in plant-microbe interaction microbiology that can englobe multiple lifestyles and include a wide range of bacteria that interact with the plant in very different ways. Given the complexity of these microbe-host interactions, it is surprising that the most of genome-based PGPB studies focus only on commonly known PGP factors (IAA, siderophores, etc.) and overlook the rest of the genomic content. **Objectives** Using *Micromonospora* as a model microorganism, we developed a new bioinformatic pipeline to select for plant-associated strains with potential for plant growth promotion, based on the enrichment of several gene functions. Experiments were designed to test and validate the pipeline proposed using *Medicago* and *Arabidopsis* for *in-planta* trials. **Methods** Seventy-eight *Micromonospora* strains were inoculated in *Medicago sativa*, both in the presence and absence of the *Medicago*-nodulating rhizobium *Sinorhizobium meliloti* Sm1021. Also, to test if the *Micromonospora* strains promoted plant growth outside legume plants, all *Micromonospora* strains were also inoculated in *Arabidopsis thaliana*. After four weeks, several plant growth parameters were measured (root and aerial length, number of secondary roots, leaves, nodules, flowers, etc.). All results were compared with un-inoculated plant controls and correlated with the *in-silico* predictions. **Results** *In- vitro* inoculation tests support that our bioinformatic workflow has the capacity to select for *Micromonospora* strains with plant growth promotion potential with high accuracy, based only on their genomic content.

Control Number: 2021-A-8296-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: plant-microbe interactions

Keyword 2: Micromonospora

Keyword 3: endophytes

Abstract Title: **From Roots To Leaves: The Capacity Of *Micromonospora* To Colonize Different Host Legume Tissues**

Author Block: **P. Benito**, L. Carro, M. Ortúzar, M. Trujillo; Univ. of Salamanca, Salamanca, Spain

Abstract Body: **Background**. An important number of *Micromonospora* strains have been reported from nitrogen-fixing root nodules of legume and actinorhizal plants. It was recently demonstrated that *Micromonospora* can colonize the internal nodular tissues of its original host and other legumes. However, the question of whether this bacterium can also be found in other parts of these plants remains unanswered. **Objective**. To determine the capacity of *Micromonospora* to colonize upper plant tissues in addition to nitrogen-fixing nodules. **Methods**. Bacterial isolations were carried out from different plant tissues of *Lupinus angustifolius* and *Pisum sativum***.** Enzymatic activity assays for amylases, cellulases, chitinases, pectinases and xylanases were determined for all isolates identified as *Micromonospora*. To assess the ability of *Micromonospora* to reach colonize different parts of the host plant, *gfp*-tagged *Micromonospora* ML01 and nitrogen-fixing bacteria were co-inoculated on the axenic roots of *P. sativum*. Leaf tissues were monitored by light and fluorescence microscopy for 30 days. **Results**. Over 150 strains were recovered from different *L. angustifolius* and *P. sativum* tissues including leaves, stems, roots, and nodules. Ninety-seven percent of the isolates were identified by 16S rRNA gene sequence in the target genus and were associated with 27 different *Micromonospora* species. The most abundant species were *M. noduli* and *M. saelicesensis*, representing 49% of the total strains and these were recovered from multiple tissues of all plants. In addition, BOX-PCR profiles revealed that several strains recovered from different tissues within the same plant potentially represented clones. Plant-polymer degrading enzymes are suspected to play a role in the colonization of plants. Enzymatic assays showed that all strains produced xylanases and pectinases, while 98.6%, 98.0%, and 94.6% of them produced amylases, cellulases, and chitinases, respectively. The most productive strains included seven isolates from *P. sativum* and one from *L. angustifolius*. Strain *Micromonospora* ML01-*gfp* was used to determine its capacity to colonize different plant organs. The first evidence of the presence of *Micromonospora* in the foliar tissues was observed 11 days post-inoculation (dpi), but bacterial cells were not easily visible inside stomata and in the surrounding leaf cells until 24 dpi. These results strongly suggest that *Micromonospora* is able, not only to infect nitrogen-fixing nodules, but also of reaching other parts of the host plant, especially the leaves.

Control Number: 2021-A-8298-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: leafroll 3

Keyword 2: native grapevine

Keyword 3: genetic diversity

Abstract Title: **Glrav-3 Genetic Variants In Indigenous Vines Of Mediterranean Croatia**

Author Block: **K. Hancevic**; Inst. for Adriatic Crops and Karst Reclamation, Split, Croatia

Abstract Body: **Background** Grapevine leafroll-associated virus 3 (GLRaV-3), the main causal agent of leafroll disease, is one of the most important grapevine virus pathogens worldwide, as also proved for the Mediterranean vineyards in Croatia (Vončina et al., 2019). There, it is continuously detected mostly in mixed infections with other viruses, but always as the most predominant one. (Hančević et al., 2021). Since the first report on GLRaV-3 population structure (Turturo et al., 2005), genetic variability has been brought to the wider attention, so new divergent variants are continuously discovering all around the globe. **Objectives and Material** In order to identify and characterize GLRaV-3 variants present within each isolate, thirty-three selected GLRaV-3 positive samples from most widespread native grapevine varieties were analyzed on a molecular basis. Samples included sixteen varieties from Croatian Adriatic coast, now present in the germplasm collection vineyard located at the Institute for Adriatic Crops in Split, Croatia. **Methods and Results** A fragment (545 bp) of the HSP70h gene of GLRAV-3 was amplified using LC1F and LC2R primer pairs (Turturo et al., 2005) to detect GLRaV-3 presence in all grapevine varieties tested. The PCR conditions were as reported by Turturo et al., except for the final extension, which in our case was prolonged to 15 min. To separate different genomic variants presumably present in each isolate, amplicons were cloned in the in E. coli competent cells. Whenever possible, 20 transformed colonies per sample (in total 586 clones) were randomly selected and analyzed by single-stranded conformational polymorphism (SSCP; Černi et al, 2008) and sequenced. The phylogenetic analysis of all 139 sequenced variants obtained showed clustering into GLRaV-3 phylogenetic groups I and/or II supported by high bootstrap values. Genomic variants from 55% analyzed samples clustered into phylogenetic group II, while those from 27% samples clustered into phylogenetic group I. Approximately one fifth of samples, 18%, were mixed infected with both variants clustering into both phylogenetic groups I and II. This study emphasizes the predominant presence of GLRaV-3 phylogenetic groups I and II, in indigenous vines of Mediterranea Croatia, either individually or in combination.

Control Number:	2021-A-8306-MICROBE
Session Type:	**iPoster**
Session Number:	**FEMSP100**
Session Title:	**FEMS: Environmental Microbiology and Ecology**
Topic 1:	FEMS - Environmental microbiology and ecology
Keyword 1:	Metagenomics
Keyword 2:	Plant
Keyword 3:	Microbiome
Abstract Title:	**Seasonal Variations Of The *Lupinus Angustifolius* Microbiome**
Author Block:	**M. Ortúzar**, J. Niño-Ramírez, M. Trujillo; Univ. de Salamanca, Salamanca, Spain
Abstract Body:	**Seasonal variations of the *Lupinus angustifolius* microbiome**Maite Ortúzar, Jairo Niño-Ramírez and Martha E Trujillo*Department of Microbiology and Genetics, Edificio Departamental, University of Salamanca, Salamanca, Spain. maiteortuzar@usal.es* **Background***Lupinus angustifolius* is a world widely distributed legume of significant interest in agriculture due to its nutritional value for both animals and humans. Previous studies have shown the large diversity of cultivable microbiota associated with this plant, isolated either from within the plant (nodules, roots, ...) as well as the rhizosphere or the associated soil. It is essential to describe and understand the functional role of the microbiome associated with a host plant as this information will be useful to biotools for a more sustainable agriculture. **Objectives**This work was designed to study in different seasons (spring and autumn), the microbiome associated with the rhizosphere and the soil where *Lupinus angustifolius* grows wild. **Methods**Soil and rhizosphere samples of *Lupinus angustifolius* were collected from two different locations in the region of Salamanca (Spain) in different seasons (spring and autumn); being the first location Cabrerizos (40º 58’ 38.9” N; 5º 35’ 47.7” W) and the second one Salamanca (40º 57’ 38.7 “; N 5º 41’ 34.8” W). DNA was extracted with the FastDNA® Spin Kit for soil and sequenced with the Illumina MiSeq platform for the identification of Bacteria (16SrRNa gene, V3-V4 region) and Eukarya (ITS2 region). **Results and discussion**The surrounding microbiome of *Lupinus angustifolius* varies greatly depending on the type of soil and the season when the samples are collected. In both locations the most dominant bacterial genera are *Massilia*, *Mucilaginibacter*, *Pseudomonas* and *Sphingomonas*. In the first location (Cabrerizos) *Streptomyces* is quite abundant, while

in the second (Salamanca) the most abundant genus is *Bradyrhizobium*; which is an expected result because this bacteriumresponsible for nitrogen fixation in symbiosis with *Lupinus*. However, eukaryote diversity is very different between the two locations. In the first location (Cabrerizos) the fungal genera *Penicillium*, *Pleosporales* and *Fusarium* stand out; while in the second location (Salamanca) *Entoloma*, *Gelidatrema* and *Helotiales* are found in a higher proportion.

Control Number: 2021-A-8321-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: Pseudomonas putida

Keyword 2: Biological control

Keyword 3: T6SS

Abstract Title: **Screening Of Type VI Secretion System Genes In The Environmental Isolates Of Pseudomonas Putida**

Author Block: **T. Pavlovic**, I. Nikolic, O. Stanojevic, T. Beric, S. Stankovic; Univ. of Belgrade, Faculty of Biology, Belgrade, Serbia

Abstract Body: *Pseudomonas putida* represents a metabolically versatile saprotrophic bacterium, ubiquitous in soil, water and closely associated with plants. It can suppress the growth of phytopathogenic bacteria, which highlights its application potential in the biological control of diverse plant diseases. An important mechanism underlying its biocontrol potential and its persistence in competitive polymicrobial environments is the Type VI secretion system (T6SS) which delivers toxic effectors directly into recipient cells in a contact-dependent manner. A baseplate protein TssA, encoded by the *tssA* gene, is one of the core components of T6SS, essential for its activity. On the other hand, the *tagX1* gene is a conserved accessory gene, and the hallmark of strains with K1-T6SS cluster, a potent antibacterial device. This study aimed to detect *tssA* and *tagX1* genes among the environmental strains of *P. putida*, isolated from fresh basins of Sava and Danube Rivers in Serbia and previously identified by amplification of Repetitive Extragenic Palindromic (REP) elements. The collection of 34 isolates was screened for the presence of T6SS genes by Polymerase Chain Reaction (PCR). Primer3 software was used to design primers from *tssA* sequence of *P. putida* DOT-T1E and *tagX1* sequence from *P. putida* KT2440, obtained from the Pseudomonas Genome Database (https://pseudomonas.com). The primer sequences to amplify *tssA* gene were 5'-GAAGAGGTCACTCGGTTGCT-3' and 5'-GGCAATGGCCTCTTCATAGA-3', and those for *tagX1* gene were 5'-ATCAAGCTGCGTAGCCAAAC-3' and 5'-ACCGAGTCGTCGGGAAGT-3'. Both amplified DNA fragments of *P. putida* D1/8 isolate were sequenced and revealed 99.5% and 98.6% of similarity with *tssA* gene from *P. putida* DOT-T1E and *tagX1* from *P. putida* KT2440, respectively. Based on the detection of *tssA* gene, the T6SS was present in 29 isolates, while *tagX1* was detected in 28 isolates, which indicated that these isolates had K1-T6SS. Both *tssA* and *tagX1* were absent in 5 strains, mainly isolated from the Danube River. These results suggest that the environmental strains of *P. putida,* which possess T6SS, may have promising potential in combating phytopathogens, primarily in the K1-T6SS-dependent manner. The sources of irrigation water, such as rivers, represent natural reservoirs of plant pathogens. An ecologically friendly approach to reducing the dispersal of phytopathogens into agricultural fields is needed. Therefore, further

investigation of the T6SS-dependent biocontrol potential of *P. putida* strains from such habitats is important and should be followed up.

Control Number: 2021-A-8322-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: soil

Keyword 2: methane oxidation

Keyword 3: biochar

Abstract Title: **Response Of Methane Oxidation And Soil Microbial Community Structure To Biochar Application**

Author Block: A. Kubaczyński[1], A. Walkiewicz[1], **A. Pytlak**[1], J. Grządziel[2], A. Gałązka[2], M. Brzezińska[1]; [1]Inst. of Agrophysics, Polish Academy of Sci., Lublin, Poland, [2]Inst. of Soil Sci. and Plant Cultivation State Res. Inst., Puławy, Poland

Abstract Body: Climate change is having an increasingly severe impact, making the development of countermeasures one of the greatest global challenges. One of the main problems to be solved is the sharp increase in greenhouse gases (GHGs) emissions. Particular attention should be paid to CH_4, which has 28 times higher global warming potential (GWP) than CO_2 and is highly dependent on anthropogenic sources. An important determinants shaping the global GHG balance are soil microbial processes which lead to both GHG production and elimination. Recently, amendment with biochar has become a means to improve soil quality by increasing pH, moisture-holding capacity, cation-exchange capacity and carbon sequestration. There is an ongoing debate about the biochar impact on GHGs emission. Some of the available data show that biochar amendment doesn't significantly affect emissions or even increase GHGs production in soil. However, there is growing evidence that biochar addition can also reduce emission of CH_4 and CO_2 to the atmosphere. Our research focused on the effect of biochar addition to *Haplic Luvisol,* representing the soil type commonly found in temperate zone and is often utilized for agricultural purposes. Short-term and long-term response of the soil microbial community to biochar application were studied using methane oxidation assay (GC) and next generation sequencing (NGS).

Control Number:	2021-A-8323-MICROBE
Session Type:	**iPoster**
Session Number:	**FEMSP100**
Session Title:	**FEMS: Environmental Microbiology and Ecology**
Topic 1:	FEMS - Environmental microbiology and ecology
Keyword 1:	endophytes
Keyword 2:	core microbiome
Keyword 3:	seeds
Abstract Title:	**Current The Composition Of Wheat Seed Microbiome Determined Culture Independent Technique Microbiota**
Author Block:	**A. Kuzniar**[1], K. Włodarczyk[1], J. Grządziel[2], W. Goraj[1], M. Woźniak[2], K. Furtak[3], A. Gałązka[2], E. Dziadczyk[1], E. Skórzyńska - Polit[1], A. Wolińska[1]; [1]The John Paul II Catholic Univ. of Lublin, Lublin, Poland, [2]Inst. of Soil Sci. and Plant Cultivation, Puławy, Poland, [3]Inst. of Soil Sci. and Plant Cultivation, Lublin, Poland
Abstract Body:	Endophytes are related with host plants throughout their life history from seed germination to fruit development. One of the most significant plant organs colonized by endophytic microbiota is the seed. The aim of this study was to determine the structure of the seed core microbiota exist in the endosperms and embryos of eight wheat cultivars with the use of a culture-independent technique. The seeds of *Triticum aestivum* L. cv. Hondia, Wilejka, STH, Opcja, Tybalt, Euforia and *Triticum spelta* L. cv. Rokosz and Schwabencorn (producer: Plant Breeding Strzelce Sp. z o.o. Group IHAR) were studied. Rokosz and Hondia wheat were cultured *in vitro* and *in vivo* to identify obligatory bacterial endophytes. A restrictive analysis of reads originating from the *in vitro* plants has demonstrated that the bacterial genera *Paenibacillus* and *Propionibacterium* inhabiting Rokosz and Hondia wheat plants have a status of obligatory endophytic microorganisms. Greater biodiversity of seed-borne endophytic bacteria was determined in the seed endosperms than in the embryos. The multiple comparison analysis of the OTU abundance showed that the seed part significantly influenced the relative abundance. The seed-born microbiome is not statistically significantly dependent on the wheat cultivars; however, it cannot be claimed that every wheat seed is the same. *Research financed by the National Center for Research and Development as part of the Lider IX project- contract No. Lider/7/0024/L-9/17/NCBR/2018.*

Control Number: 2021-A-8331-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: Microbes

Keyword 2: Coculture

Keyword 3: Secondary metabolites

Abstract Title: **Exploring The Variety Of Interactions Between Fungi And Bacteria**

Author Block: **G. Abeysinghe**, M. Shunsuke, N. Takaya, N. Takeshita; Univ. of Tsukuba, Japan, Tsukuba, Japan

Abstract Body: Microbial communities play key roles in agriculture, human health, and ecosystem services. These complex microbial communities are formed through the molecular interplay of the diverse microbial species in the environment. Fungi and bacteria comprise a large fraction of biomass in the soil and since they interact with each other, bacterial-fungal interactions are crucial for understanding the microbial ecosystem. It is apparent that microbial interactions promote the activation of cryptic biosynthetic pathways leading to the production of bioactive compounds which drive defense functions, cell to cell communication, and other interactive dynamics. Coculturing has been proven to be an effective method to mimic the conditions existing among the microbial interactions in the natural environment, hence may be of potential to facilitate the production of novel bioactive compounds like antimicrobials as well as facilitator molecules. Our recent study characterized the mutualistic relationship between the filamentous fungus *Aspergillus nidulans* and gram-positive bacterium *Bacillus subtilis* (Abeysinghe et al. 2020) providing evidence to show their spatial and metabolic interaction that facilitates the communication in between species to explore untraversed environmental niches and obtain nutrients. Appealing to this interactive nature, the current study comprised of co-culturing of different combinations of environmental fungi and bacteria to observe the interactive dynamics in bacteria and fungi. Cocultures were incubated prior to microscope imaging. Parameters such as the effect on the fungal growth, the affinity of the bacterial cells to the fungal hyphae, bacterial cell dispersal distance, and the velocity of movement of bacteria were analyzed to define the interaction specificity. Depending on the nature of interactions, the combinations were then classified as positive, negative, and neutral. Selected combinations were then subjected to LCMS analysis and subsequent transcriptomic analysis to visualize their genomic potential and expression in coexistence compared to their monoculture state. Our approach gains more perspective on an ecological context on the diversity of interactions of the environmental microbiota since there is a high potential to increase the metabolic capacity of chemically prolific microorganisms.

Control Number: 2021-A-8336-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: viral ecology

Keyword 2: algae

Keyword 3: carbon flux

Abstract Title: **Viral Lysis Essential To Explain Seasonal Carbon Mass Balance Of Antarctic Phytoplankton**

Author Block: **C. P. D. Brussaard**, T. E. G. Biggs; NIOZ Netherlands Inst. for Sea Res., Den Burg, Netherlands

Abstract Body: Viral infection induced mortality of marine microorganisms affects not only the microbial community composition differently than predation, but especially also the flow of carbon and energy. While viral lysis rates of the unicellular algae (phytoplankton) in the field indicate their ecological importance, virtually no data are available on Antarctic phytoplankton. The polar regions are highly sensitive to global climate change and changes in community composition are predicted. Temporal studies are therefore highly warranted. We present here for the seasonal dynamics of viral lysis rates of key phytoplankton groups in Antarctic waters and relate these to the specific grazing rates. Group-specific responses show *Phaeocystis* and picoeukaryotes to display a negative correlation between grazing and viral losses, while cryptophyte losses were dominated by viral lysis. The seasonal population dynamics of the smaller-sized diatoms was not strongly affecte by viral activity, conversely, viral lysis 'events' of the larger-sized diatoms dominated algal carbon flow and shunted large amounts of carbon production towards the microbial loop. Overall, viral lysis was responsible for 58% of seasonal carbon losses and was found critically important for explaining temporal dynamics of phytoplankton in a key area for global pCO_2 drawdown. Our study shows for the first time the importance of viral lysis to obtain a complete seasonal mass balance.

Control Number: 2021-A-8346-MICROBE
Session Type: **iPoster**
Session Number: **FEMSP100**
Session Title: **FEMS: Environmental Microbiology and Ecology**
Topic 1: FEMS - Environmental microbiology and ecology
Keyword 1: degradation
Keyword 2: effluent
Keyword 3:
Abstract Title: **DEGRADATION OF PAINT AND TEXTILE INDUSTRIAL EFFLUENTS BY INDIGENEOUS BACTERIAL ISOLATES**
Author Block: **S. Adebajo**[1], A. Ojo[1], A. Oladotun[1], A. Akintokun[1], E. Ogunbiyi[2], A. Bada[2]; [1]Federal Univ. of Agriculture, Abeokuta, oru-Ijebu, Nigeria, [2]Federal Univ. of Agriculture, Abeokuta, Abeokuta, Nigeria
Abstract Body: **ABSTRACT Background** Civilization, urban growth and some other mankind activities have resulted to pollution as well as reduced crop productivity. Untreated paint and textile effluents discharged from industries cause serious environmental threats to fauna and flora. **Objectives**This study thus, investigated the potential of different indigenous bacterial cells for the degradation of paint and textile effluents. **Methods** Paint and textile effluents samples were aseptically collected from paint and textile industries. Physical, chemical, heavy metals properties and microbial load of the effluent were investigated using standard methods. Enrichment, Isolation and identification of bacterial isolates were determined using the standard microbiological methods. Screening of paint and textile effluents degraders was carried out using solid phase screening method. Degradation of paint and textile effluents were determined by Fourier-transform infrared (FTIR) analysis and spectrophotometrically.

Results Forty-seven bacterial isolates were obtained from the paint and textile effluents samples. Screening of the 47 isolates showed that 21 isolates possessed the ability to grow on agar plates amended with paints and textile dyes. Out of the 21 positive isolates, 12 isolates were obtained from paint samples while nine isolates were recovered from the textile samples. Highest degradation activity was recorded by *Bacillus subtilis* (94.37 % ±5.35) obtained from textile effluent sample while *Micrococcus* sp (46.55 % ±8.30) from paint polluted soil sample had the lowest degradation activity. FTIR analysis showed the transformation and disappearance of peaks in the treated paint and textile effluents by *Bacillus subtilis*. This study revealed the ability and effectiveness of *Bacillus subtilis* in the removal of toxic materials in paint and textile effluents, thus could be applied for industrial use.
Keywords: effluent, spectrophotometer, FTIR, degradation, *Bacillus subtilis*

Control Number: 2021-A-8350-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: Biofilm

Keyword 2: Microalgae

Keyword 3: Aquaculture

Abstract Title: **Microalgae Microbiomes - A Natural Source For The Prevention And Treatment Of Diseases In Aquaculture**

Author Block: **L. Bergmann**, W. Streit, I. Krohn; Univ. Hamburg, Hamburg, Germany

Abstract Body: Aquaculture is one of the fastest growing food sectors in the world with land-based aquaculture gaining increasing interest. Diseases caused by bacteria like *Pseudomonas* sp. or *Flavobacterium psychrophilum* are an obvious challenge to the aquaculture industry exacerbated by many pathogen's biofilm building abilities. Antibiotics are still in use in many regions of the world, contributing to increased antimicrobial resistance. Our work builds on the hypothesis that a novel approach to disease treatment in aquaculture can be achieved by exploiting the healthy properties of microalgae and their associated microbiomes. Exploration, characterization and screening of microalgae microbiomes are meant to assemble and apply an advanced 'omics toolbox on the natural synergy of microalgae and microbial consortia to discover novel bioactive and prebiotic compounds for sustainable use in land-based aquaculture systems. Targeted classes include microbial biofilm and growth inhibiting enzymes, peptides and small molecules with large-scale application potential. By the use of various strain collections of microalgae and microorganisms (MZCH, SAG), we performed both functional and sequence-based screening of microalgae and microbial consortia. NGS and RNA sequence data will be analysed as well as *in silico* mining will be performed to find novel Quorum Quenching (QQ) and anti-microbial biomolecules. Within this framework the supernatants of about twenty different microalgae were screened in biofilm assays. Five promising candidates of microalgae which showed significant biofilm building reduction were chosen for further investigation. Ongoing metagenomes-, metaproteomes- and metatranscription analyses gave detail information on possible biofilm degradation and antimicrobial properties and offered insight into the divers symbiotic microalgae and bacterial communities.

Control Number: 2021-A-8378-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: Biocontrol

Keyword 2: Quorum-quenching

Keyword 3: ISR

Abstract Title: **Deciphering The *Pseudomonas Syringae* Pv. *Tomato*Biocontrol Mechanism Of Three Halotolerant Strains By Metabolomic And Enzymatic Approaches**

Author Block: **M. Rodriguez**[1], I. Llamas[1], V. Bejar[1], C. Cassan[2], G. Decros[2], A. Flandin[2], P. Pétriacq[2], Y. Gibon[2], I. Sampedro[1]; [1]Univ. of Granada, Granada, Spain, [2]Univ. of Bordeaux, Villenave d'Ornon, France

Abstract Body: *Pseudomonas syringae* pv. *tomato* DC3000, the causal agent of tomato bacterial speck, causes considerable economic losses worldwide. The control of this hemibiotrophic pathogen by chemical pesticides has supposed treatment resistances and environmental pollution. Recently, plant growth-promoting bacteria (PGPB) have been employed as a tool to combat bacterial infections through competence, lytic enzymes or the induction of plant systemic resistance (ISR)[1]. Moreover, *P. syringae* pv. *tomato* virulence is known to be regulated by quorum sensing (QS), an intercellular communication system in which gene expression, coupled with cell density, is mediated by the diffusion of signal molecules such as *N*-acylhomoserine lactones (AHLs)[2]. The enzymatic degradation of AHLs, termed quorum quenching (QQ), has proven to reduce virulence without affecting pathogen growth, which reduces the risk of resistances and constitutes a more efective strategy than treatments commonly used. In this study, we investigated the ability of three PGP-QQ strains, *Peribacillus* sp. N3*, Pseudomonas segetis* P6 and *Staphylococcus equorum* EN21 to act as biocontrol agents against *P. syringae* pv. *tomato*, and whether this biocontrol was mediated by ISR triggering or by QQ using metabolomics and enzymatic approaches. For ISR triggering mechanism, plants were irrigated with each PGP-QQ strain and then challenged with the pathogen by foliar spraying. To determine the effect of AHL degradation in the virulence, cocultures between each PGP-QQ strain and the pathogen were sprayed. After treatments, biomass and disease parameters were determined and fresh material was destined to its analysis at Bordeaux Metabolome facility (INRAE, University of Bordeaux) using spectrophotometric and LC-MS techniques for enzymatic and metabolic profiling[3], in the frame of EU H2020 EPPN2020 project. Fresh biomass and disease incidence and severity showed a significant variation between treatments. Regarding metabolomics and enzymatic analysis, plants from irrigation treatments exhibited a substantial increase in levels of enzyme capacities and metabolites involved in carbon metabolism, while there was a decrease for those involved in nitrogen metabolism in plants from coculture treatments. Profiling of stress phytohormones and redox compounds also showed different patterns between treatments. These results taken together suggest that the biocontrol mechanism of *P. syringae* pv. *tomato* might be ISR for *P. segetis* P6 and QQ for *S. equorum* EN21 and *Peribacillus* sp. N3.

Control Number: 2021-A-8400-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: nodule-inhabiting bacteria

Keyword 2: polymyxa complex

Keyword 3: taxonomy

Abstract Title: **Paenibacillus Farraposensis Sp. Nov. With Antagonistic Activity Against Phytopathogens, Isolated From A Legume Nodule**

Author Block: **D. M. Roldán**[1], A. Costa[1], V. Amarelle[1], J. Menes[2], E. Fabiano[1]; [1]Clemente Estable Biological Res. Inst. (IIBCE), Montevideo, Uruguay, [2]Faculty of Sci. & Faculty of Chemistry, Montevideo, Uruguay

Abstract Body: With the aim of studying the bacterial diversity in nodules of Uruguayan native legumes, samples of *Arachis villosa* were collected in the “Esteros de Farrapos” National Park. This is a natural protected area which consists of a system of riverine wetlands and islands that are permanently and/or temporarily flooded as a result of the flooding of the Uruguay River. Bacterial strains were isolated on YMA (Yeast-Mannitol Agar) using standard protocols and incubated at 30 °C. A Gram-stain-variable rod, strain UY79, belonging to the genus *Paenibacillus* was isolated and selected for further characterization. At the time of writing, 256 species of *Paenibacillus* are recognized, many of them isolated from soil samples. The *Paenibacillus polymyxa* complex is a group of species within this genus comprising *P. brasilensis, P. jamilae* (recently reclassified as *P. polymyxa*), *P. kribbensis, P. ottowii*, *P. peoriae, P. polymyxa* and *P. terrae* species. This complex is characterized by their biotechnological applications as plant growth-promoting bacteria, biofertilizers, biocontrol potentials and protection against abiotic stresses. Results obtained by our group show that strain UY79 has diverse traits involved in antagonistic activity against a broad spectrum of phytopathogenic fungi and it would be a potential and valuable strain to be further evaluated as a biofungicide. In this work, we determined the taxonomic position of UY79 strain by using a polyphasic approach. We found that UY79 strain is a facultative anaerobic, mesophilic, catalase positive, oxidase negative and non-motile rod. Growth was observed at 15-42 °C (optimum 30 °C), pH 5.0-9.0 (optimum pH 7.0-8.0), and in the presence of 0-3.0% NaCl (optimum 1.0-2.0%). 16S rRNA gene sequence analysis placed UY79 strain within the *Paenibacillus polymyxa* complex and showed the highest similarity to *P. ottowii* MS2379(T) (99.4%), *P. peoriae* BD-57(T) (99.0%), *P. polymyxa* ATCC 842(T) (99.0%) and *P. brasilensis* PB172(T) (98.9%). Additionally, *in silico* DNA hybridization analysis showed DDH percentages lower than 70% (27.8-48.7%) and ANI values lower than 95% (84.0-88.6%). Based on the data from polyphasic study, UY79 represents a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus farraposensis* sp. nov. is proposed.

Control Number: 2021-A-8406-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: plant-microbe interactions

Keyword 2: phytoremediation

Keyword 3: genomics

Abstract Title: **A Comprehensive Omics Assessment Of Microbial Assisted Plant Growth Promotion In The Presence Of Heavy Metals**

Author Block: **L. Carro**, C. Garbisu; NEIKER - Basque Inst. for Agricultural Res. and Dev., Basque Res. and Technology Alliance (BRTA), Derio, Spain

Abstract Body: **Background** Agriculture is currently confronting limitations of soil use due to, among other reasons, pollution levels above food safety threshold values. Some agricultural practices increase the heavy metal (HM) content of agricultural soil, representing an important threat for global agricultural development. The use of microorganisms as plant growth promoters has been increasingly studied for several years, but it has only recently been proposed to improve plant metal tolerance. **Objectives** The abovementioned challenges for agricultural production require the study of microbial-assisted plant growth promotion (PGP) mechanisms in the presence of HM to better promote a more efficient and sustainable agriculture. **Methods** In this study, the genome of an actinobacteria strain, *Micromonospora cremea* CR30^{T}, was mined to determine its PGP and HM resistance potential, which was later confirmed by *in vitro* tests. Both abilities were evaluated under greenhouse conditions. A transcriptomic analysis was used to identify the most relevant genes for those processes. **Results** We were able to confirm the PGP capacity of the rhizospheric strain, as well as its remarkable capacity to colonize the internal tissues of *Pisum sativum*. In addition, we have confirmed the presence of genes, and their corresponding physiological function (at the phenotype level), involved in IAA production, ACC-deaminase activity, and siderophore production, all of them implicated in plant development. Furthermore, high tolerance to HMs and their accumulation in plant internal tissues have been confirmed, and several genes implicated in these processes have been identified in the CR30 genome and transcriptome. Both traits, plant promotion and HM tolerance, were used to determine the capacity of CR30 to protect plant development under HM stress, finding out that plant development was influenced by CR30, whose presence considerably reduced HM-induced adverse effects on plant performance. Similarly, the presence of CR30 altered metal translocation to upper plant tissues. This finding opens the possibility of using CR30 for bacteria-assisted phytoextraction and or phytostabilization strategies depending on the specific HM under consideration.

Control Number: 2021-A-8455-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: taxonomy

Keyword 2: genome analysis

Keyword 3: 16S RNA

Abstract Title: **Taxonomic And Genomic Characterization Of *Comamonas Fluminis* Sp. Nov., A Vancomycin-resistant Bacterium Isolated From The Han River, South Korea**

Author Block: **E-H. Park**, C-J. Cha; Chung-Ang Univ., Anseong, Korea, Republic of

Abstract Body: A gram-stain-negative, aerobic and motile bacterial strain, designated CJ34^T, was isolated from the Han River water in South Korea. Colonies were circular, cream-colored, convex and entire margins. Strain CJ34^T grew optimally on tryptic soy agar at 30 °C and pH 7.0 in the absence of NaCl. Phylogenetic analysis based on 16S rRNA gene sequence showed that strain CJ34^T belonged to the genus *Comamonas* within the family *Comamonadaceae* and was most closely related to *Comamonas testosteroni* ATCC 11996^T and *C. thiooxydans* DSM 17888^T (both 98.63% similarity). The average nucleotide identity values between strain CJ34^T and two closely related type strains *C. testosteroni* ATCC 11996^T and *C. thiooxydans* DSM 17888^T were 82.77% and 82.73%, respectively. The major isoprenoid quinone of strain CJ34^T was ubiquinone Q-8. The major cellular fatty acids of strain CJ34^T were $C_{16:0}$, $C_{16:1}$ *ω6c* and/or $C_{16:1}$ *ω7c* and $C_{18:1}$ *ω6c* and/or $C_{18:1}$ *ω7c*. The predominant polar lipids of strain CJ34^T were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, and unidentified aminophopholipid. Whole genome sequencing revealed that strain CJ34^T had a genome of 4.9 Mbp and the G+C content of the genomic DNA was 59.73%. The genome contained several antimicrobial resistance genes including vancomycin resistance gene. On the basis of the polyphasic taxonomic study, strain CJ34^T represents a novel species in the genus *Comamonas,* for which the name *Comamonas fluminis* sp. nov. is proposed. The type strain is CJ34^T.

Control Number: 2021-A-8482-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: Bacillus amyloliquefaciens

Keyword 2: biocontrol

Keyword 3: antimicrobial compounds

Abstract Title: **Genetic Potential Of *Bacillus Amyloliquefaciens* For The Production Of Bioactive Compounds Effective In *Aspergillus Flavus* Supression**

Author Block: **V. Vlajkov**[1], I. Pajčin[1], M. Grahovac[2], M. Loc[2], D. Budakov[2], J. Grahovac[1]; [1]Faculty of Technology Novi Sad, Novi Sad, Serbia, [2]Faculty of Agriculture, Novi Sad, Serbia

Abstract Body: The future of plant disease management lies in the application of biocontrol agents, recognized as a promising eco-friendly alternative to the conventional usage of chemical pesticides. Members of the *Bacillus* genus are leading candidates in microbial-based biopesticides production due to their high antagonistic activity against a broad range of phytopathogens. A novel strain isolated from the rhizosphere soil of the *Phaseolus vulgaris*, identified as *Bacillus amyloliquefaciens* based on 16S rRNA gene sequencing, was evaluated for the suppressive effect on aflatoxigenic strains of *Aspergillus flavus*. After the cultivation (96 h), samples of cultivation broth and cell-free supernatant were tested for the antimicrobial activity against selected phytopathogens using a well diffusion method. The results interpreted through the measurement of the inhibition zones indicated the suppressive effect of both sample types. The antagonistic effect of cell-free supernatant pointed out the production potential of the strain for the synthesis of secondary metabolites with antimicrobial activity. Further analysis included PCR screening of the genes responsible for the production of lipopeptides belonging to the three main families: surfactins, iturins and fengycins. These compounds are well known for their strong antibacterial and antifungal activity, and conceded as bioactive products of great importance for the biological control of plant pathogens. The obtained results indicated the presence of the following genes: *srfAA, fenD, bacA, bacD, ituD, ituA*. The genetic characterization of the strain demonstrated the great potential to be considered for future investigation and further development of the bioprocess solution for the production of microbial-based biopesticide. Considering the genetic potential, besides the proven ability to suppress phytopathogenic fungi, it is to be expected that the selected strain will show antagonistic activity against bacterial pathogens as well. It is a promising starting point for developing the product for biological control applicable in the management of a wide range of plant diseases. Further investigation will focus on optimising the cultivation conditions to enhance the expression of the key genes for the production of the compounds with the highest antimicrobial activity against target strains. The final aim is to increase

the overall bioprocess productivity by creating a specifically optimized environment for the selected microorganism to produce the desired final product.

Control Number:	2021-A-8487-MICROBE
Session Type:	**iPoster**
Session Number:	**FEMSP100**
Session Title:	**FEMS: Environmental Microbiology and Ecology**
Topic 1:	FEMS - Environmental microbiology and ecology
Keyword 1:	rhizosphere microorganisms of tree
Keyword 2:	Microbiome
Keyword 3:	metabolic potential
Abstract Title:	**Microbiome And Metabolic Potential Of The Rhizosphere Microorganisms Of Black Alder *(alnus Glutinosa)*, Silver Birch *(betula Pendula)* And Scots Pine (*Pinus Sylvestris)***
Author Block:	**A. Galazka**; Inst. of Soil Sci. and Plant Cultivation - State Res. Inst., Pulawy, Poland
Abstract Body:	**Microbiome and metabolic potential of the rhizosphere microorganisms of black alder *(Alnus glutinosa)*, silver birch *(Betula pendula)* and scots pine (*Pinus sylvestris)*Anna Gałązka[1], Anna Marzec-Grządziel[1], Jarosław Grządziel[1], Jacek Niedźwiecki[2], Karolina Furtak[1], Karolina Gawryjołek[1]**Forest ecosystems differ significantly from agricultural ones. One of the key elements of both forest and agricultural soil are soil microorganisms. Microorganisms are an integral part of the soil environment and perform a number of positive functions in it. They affect the functioning of ecosystems, plant health, and soil structure and productivity. In forest and agricultural ecosystems, edaphic conditions, plants and soil microorganisms are closely related. The formation of specific features of forest habitats is determined by the physical, chemical and biological properties of the soil. The aim of the study was to determine the structural and functional biodiversity of soil microorganisms inhabiting the rhizosphere of three selected tree species: *Alnus glutinosa*, *Betula pendula* and *Pinus sylvestris*. Soil samples were collected in 2019 and 2020 from a mixed forest located near the Agricultural Experimental Station IUNG-PIB in Osiny, Poland. Samples were taken from tree root layers. Each sample was collected in three biological replicates every August from selected tree species. The basic physical and chemical parameters of soils were determined, as well as the determination of enzymatic activity and the assessment of the metabolic profile of soils (Biolog EcoPlates and FFPlates). The highest metabolic activity on EcoPlates plates was observed in soil collected from under black alder and warty birch. In turn, the soil collected from under Scots pine was characterized by a much lower biological activity and a lower metabolic potential. The results obtained on the FFPlate also showed the highest metabolic potential of fungi in samples taken from the root zone of

the black alder. The best metabolized compound was L-Phenylalanine, L-Asparagine, D-Mannitol and g-Hydroxy-Butyric Acid. *The research was carried out as part of the implementation of the topic No. 1.27 "Structural and functional characteristics of the biodiversity of soil microorganisms in the forest and agricultural ecosystem", (2019-2021).*

Control Number: 2021-A-8500-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: long-term use of straw

Keyword 2: Microbiome

Keyword 3: metabolic potential

Abstract Title: **The Impact Of Long-term Use Of Straw On Soil Microbiome, Metabolic Potential And Plant Yield - Postharvest Residues Of Winter Wheat, Winter Triticale And Winter Rape As The Waste Biomass From Primary Agricultural Production**

Author Block: **A. Galazka**; Inst. of Soil Sci. and Plant Cultivation - State Res. Inst., Pulawy, Poland

Abstract Body:

The impact of long-term use of straw on soil microbiome, metabolic potential and plant yield - postharvest residues of winter wheat, winter triticale and winter rape as the waste biomass from primary agricultural productionAnna Gałązka[1], Karolina Gawryjołek[1], Jarosław Grządziel[1], Jarosław Ciepiel[1], Anna Marzec-Grządziel[1], Marcin Przybyś[2], Janusz Smagacz[3]

The field experiment was carried out at RZD Grabów, Poland in 2017-2018 (harvest years) on lessive soil. They were established on the basis of a static crop rotation experiment existing since 1987 in a randomized block system, in 4 repetitions, and the size of plots to be harvested was 45 m^2. In the rotation: winter rape - winter wheat - winter triticale, cultivated in the fields of all plants at the same time, different frequency of straw plowing was used. The following variants were compared: 1) control - without the use of straw; 2) straw plowed in once in rotation (rape straw); 3) straw plowed twice in rotation (rape and wheat straw); 4) straw plowed three times in rotation (rape, wheat and triticale straw) 5) straw plowed three times in rotation (rape, wheat and triticale straw) without additional nitrogen fertilization for the straw. The cultivated cereal varieties were: in the 2016/2017 season - Sailor winter wheat, Trismart winter triticale, and in 2017/2018 - Desamo winter wheat and Meloman winter triticale. In the case of rape, the Monolit variety was grown in both seasons. Soil samples for the determination of the metabolic profile (Biolog EcoPlates) and structural biodiversity (Next Generation Sequencing), depending on the tested factors, were collected in both years in June.

For cereals (wheat and triticale) it was the phase of milk maturity of grain (BBCH 73-75), while for rapeseed it was in the ripening phase (BBCH 80-82). The soil was taken from the topsoil, 0-30 cm, and mixed in 2 replications for each object. The highest biological activity of soils was found in soil samples for the use of straw twice in rotation, both in the cultivation of winter rape, winter wheat and winter triticale. Moreover, a high variability in the structure of the microorganisms was observed, in particular in combination with straw in three rotations.

Control Number: 2021-A-8502-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: natural product

Keyword 2: PKS

Keyword 3: NRPS

Abstract Title: **Physicochemical Drivers Of The Natural Product Biosynthesis Potential Of Earth's Microbiomes**

Author Block: **A. Geers**, M. Bentzon-Tilia; Technical Univ. of Denmark, Kgs. Lyngby, Denmark

Abstract Body:

Background

The spread of antibiotic resistance is a global threat to human health, with a collective estimated annual death toll exceeding 10 million by 2050. The antibiotic discovery void defining the past half century represents a severe exacerbation of the situation. The majority of antibiotics used today are bioactive molecules produced by culturable microorganisms, or derivatives of such compounds. Targeted sequencing of conserved genes involved in the biosynthesis of compound classes such as polyketides and nonribosomal peptides have uncovered their extensive distribution in the unculturable majority of Earth's microbiomes. Nonetheless, the physicochemical drivers governing the biosynthesis potential of environmental microbiomes remain obscure and consequently, the most promising microbiomes likely remain hidden.

Objectives

The purpose of the present study was to uncover, which environmental parameters drive the natural product biosynthesis potential in different environmental microbiomes.

Methods

We investigated microbiomes of soil, marine coastal sediment and pelagic waters, which all exhibited spatial scale gradients of various physicochemical parameters such as pH, redox potential, and salinity. Using 16S and ITS, in combination with ketosynthase domain (KS) and adenylation domain (AD) amplicon sequencing, we assessed the effects of the physicochemical variables on taxonomic and biosynthetic diversity.

Results

Soil microbiomes exhibited the highest taxonomic diversity (16S Chao1 4281±1439, Shannon 6.7±0.5) and the composition of the bacterial soil microbiome was largely driven by pH with a p-value below 0.05 in a canonical correspondence analysis. By contrast, the

composition of the less diverse (16S Chao1 2470±1055, Shannon 4.8±0.8) aquatic pelagic microbiomes were driven by a range of variables, all of which were co-varying with salinity. Interestingly, sediment microbiomes (Chao1 1345±430, Shannon 5.1±0.4) did not seem to change despite the presence of a steep vertical oxygen gradient. The ongoing KS and AD sequence analysis will show if changes in the biosynthesis potential are driven by the same environmental determinants. These results will enable us to make predictions about the global microbial biosynthesis potential. Such comprehensive mapping of the biosynthetic natural product potential will facilitate a more focused approach in the hunt for structural novelty and hence truly novel antimicrobial compounds.

Control Number: 2021-A-8518-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: Tajikistan

Keyword 2: anaerobic bacteria

Keyword 3: keratin-degrading

Abstract Title: **KERATIN-DEGRADING THERMOPHILIC ANAEROBIC BACTERIA FROM HIGH-ALTITUDE GEOTHERMAL SPRINGS IN TAJIKISTAN**

Author Block: **M. Dzhuraeva**[1], N. Birkeland[2], C. Ratnadevi[2], K. Bobodzhanova[1]; [1]Tajik Natl. Univ., Dushanbe, Tajikistan, [2]Univ. of Bergen, Bergen, Norway

Abstract Body: **Background**:Wild birds shed once or twice a year, and abandoned feathers do not accumulate in nature, which suggests the existence of natural decomposers or users of feathers. Feathers consist of almost pure keratin protein (90%), which is insoluble and not degraded by most proteolytic enzymes. However, some microorganisms have keratinolytic enzymes that convert keratin to peptides. Studies of feather-decomposing microorganisms are mainly limited to animal diseases and biotechnology for processing large amounts of waste by-products in poultry processing plants. **Objectives:** The purpose of this study was to recover feather-degrading thermophilic anaerobic bacteria from hot springs in Tajikistan.The aim of our work was to culture and isolate keratinolytic bacteria that are active at elevated temperatures. **Methods and Results:** Active isolates degradation feather at 50 – 80 ^{o}C were recovered from the Tamdykul geothermal spring using a nutrient-rich anaerobic medium. At 80 °C, complete degradation of feathers could be seen after four days. Identification of isolates based on 16S rRNA gene sequences revealed relationships to members of *Caldanaerobacter* spp. The ecological significance of this thermophilic isolate in natural systems is unknown. It is not known how widely the ability to degrade feather is distributed among bacteria, as our basic knowledge of bacterial diversity and physiology is limited by the limitations of cultivation methods. Several studies have been conducted to investigate the effect of keratinolytic microorganisms in nature. Analysis of keratinolytic associations can reveal a significant and indescribable community for keratin processing and potentially influencing the characteristics of bird feathers. The identification of new isolates that degrade feather is necessary for the development of culture-independent methods for analyzing such communities. These efforts can also find potential applications in biotechnology and basic biology.

Control Number: 2021-A-8523-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: biocontrol

Keyword 2: yeasts

Keyword 3: aflatoxin

Abstract Title: **Evaluation Of The Influence Of Two Antagonistics Yeast Strains On Growth And Aflatoxin Production Of *A. Flavus*In Dried Fig-based Agar**

Author Block: **A. Galván Romero**[1], A. Hernández[2], M. Serradilla[3], S. Ruiz-Moyano[2], M. Vázquez[2], M. López-Corrales[1], M. Córdoba[2]; [1]Res. Ctr. Finca La Orden-Valdesequera (CICYTEX), Badajoz, Spain, [2]Univ. of Extremadura, Badajoz, Spain, [3]Agrifood technological Inst. of Extremadura (CICYTEX), Badajoz, Spain

Abstract Body: Dried figs are increasingly in demand due to their high nutritional value. Spain is the leader in European production, being Extremadura the main national producer of dried figs. However, there is an increasing concern about contamination and development of filamentous fungi in dried figs and the subsequent mycotoxin production. The most important postharvest toxigenic fungi found in dried figs is *Aspergillus flavus*, which under favorable environmental conditions can produce aflatoxins (AFs). The application of synthetic fungicides to control fruit spoilage is being limited due to their negative impact on human health and the environment. Therefore, biological control is one of the most promising alternatives. Yeasts are widely used for the control of filamentous fungal pathogens on fruit. Thus, the aim of this study was to evaluate the *in vitro* antagonistic capacity of two yeast strains (793 *Hanseniaspora uvarum* and 153 *Aureobasidium pullulans*) against the aflatoxigenic *A. flavus* strain M144 in a dried fig-based (DFB) medium at 0.99 water activity. A control batch without yeasts was also analysed. The DFB medium was prepared with lyophilized dried fig at 10 %. Yeasts (batches AF+793 and AF+153) or sterile water (control batch) were inoculated onto the DFB medium at a final concentration of 3.5×10^7 cells/mL. After drying the yeast inoculum, 3 µL of *A. flavus* at 10^5 conidia/mL were inoculated in the middle of the plate. The diameter of the mycelia was measured daily. Lag phase (days) and growth rate (mm/day) were calculated. AFs were extracted and the amounts (ppb) produced by *A. flavus* was determined on day 9 by HPLC. Results showed a reduction in growth in batch AF+153, and a total inhibition in batch AF+793, compared to the control. The growth rate was faster in the control (4.6 mm radius/day) compared to batch AF+153 (0.8 mm radius/day). As expected, the opposite occurred with lag phase, being longer in batch AF+793 (5.7 days) than in control batch (2 days). A drastic removal of AFB_1 and AFB_2 mycotoxins by yeast 153 was observed. In conclusion, *H.uvarum* 793 inhibited the growth and *A. pullulans* 153 reduced growth and mycotoxin production of *A. flavus* M144. Therefore, yeast biocontrol is a promising alternative to control mycotoxigenic moulds such as *A. flavus*.

Control Number: 2021-A-8536-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: microplastics

Keyword 2: AmpC beta-lactamases

Keyword 3: river

Abstract Title: **Microplastic Pollution And Associated Microbial Community Harbouring AmpC β-lactamase Genes In A South African River System**

Author Block: **T. G. Magome**, C. C. Bezuidenhout, C. M. S. Mienie; North-West Univ. - Potchefstroom Campus, Potchefstroom, South Africa

Abstract Body: **Background**: Microplastics as pollutants have shown that they are persistent, ubiquitous, and pose health risks to aquatic biota more especially in marine system. There are very limited studies about microplastics and microorganism interactions in freshwater systems, more worrisome is that microplastics may act as vectors of potentially pathogenic microorganisms which carry clinically relevant antibiotic resistance genes (ARGs) in the riverine system. **Objectives**: The objectives of this study were to detect microplastics and characterize the associated bacterial community composition (BCC). Moreover, to investigate the presence of integrase (intl1) and AmpC β-lactamase genes including the levels of AmpC β-lactamase gene groups in microplastics and water samples across five sites in a South African river system. **Method and Results:** 206 putative microplastics were collected from 120 L of surface running water and wastewater treatment effluent (WWTe) following in situ filtration through 300-75 µm pore size metal sieves. Metabarcoding 16S rRNA data analysis using LEfSe showed that *Bacteroidaceae, Enterobacteriaceae* and *Legionellaceae* families were significantly higher ($p < 0.05$) on microplastics than in water samples. These families are known to contain pathogenic species which are resistant to antibiotics in clinical settings. BCC in water was richer and diverse but less even than on microplastics. Conventional PCR revealed that the *intl1* gene was present in both microplastics and water samples from the sites G3, W4 and T5 and water samples from site O2, which all contained more than five AmpC β-lactamase gene groups. Overall, real-time qPCR revealed that the AmpC gene group copy number was greater in microplastics (31.59 ± 6.63 gene copies/16S rRNA) than in water samples from the river (7.83 ± 0.19 gene copies/16S rRNA). However, the gene copy number in WWTe water samples from site W4 was greater (612.77 ± 162.53 gene copies/16S rRNA) than in microplastics (48.82 ± 4.40 gene copies/16S rRNA) from this site. The study showed that the high abundance by certain bacterial families associated with antibiotic resistance and high gene copy number on microplastics, suggests microplastics as a possible hotspot for horizontal gene transfer and vector which may disperse microbials carrying resistance genes to areas downstream the river.

Control Number:	2021-A-8541-MICROBE
Session Type:	**iPoster**
Session Number:	**FEMSP100**
Session Title:	**FEMS: Environmental Microbiology and Ecology**
Topic 1:	FEMS - Environmental microbiology and ecology
Keyword 1:	endophytes
Keyword 2:	wheat
Keyword 3:	enzymes
Abstract Title:	**Hydrolytic Enzyme Bioprospection Of Endophytic Bacteria Associated With Spelta Wheat Plants**
Author Block:	**K. Wlodarczyk**[1], A. Kuzniar[1], A. Pytlak[2], A. Wolińska[1]; [1]The John Paul II Catholic Univ. of Lublin, Lublin, Poland, [2]Inst. of Agrophysics, Polish Academy of Sci., Lublin, Poland
Abstract Body:	**Hydrolytic enzyme bioprospection of endophytic bacteria associated with spelta wheat plants** Kinga Włodarczyk[1], Agnieszka Kuźniar[1], Anna Pytlak[2], Agnieszka Wolińska[1] [1] The John Paul II Catholic University of Lublin, Department of Biology and Biotechnology of Microorganisms, Konstantynów 1 I Str., 20-708 Lublin, Poland [2]Institute of Agrophysics, Polish Academy of Sciences, Doświadczalna 4, 20-290 Lublin Endophytes are plant-associated bacteria that live in internal tissues without harming the host. They have an important role in plant growth promotion, as they directly or indirectly stimulate the host growth. When endophytic bacteria colonize the plant surface, they produce enzymes that hydrolyze the plant cell wall. Further, endophytes secrete antibiotics or hydrolytic enzymes to prevent plant colonization with microbial pathogens. The capability of endophytes to release lytic enzymes facilitates decomposition of a wide range of polymeric compounds including chitin, proteins, cellulose, hemicellulose, and DNA. The aim of the study was to determine the ability of endophytic bacteria isolated from the tissues of two spelt species (*Triticum spelta* L. cv. 'Rokosz' and 'Schwabenkorn') to synthesize cellulolytic, xylanolytic and proteolytic enzymes. Analysis of the strains ability to secrete enzymes was performed by the plate method based on the assessment of hydrolysis zones on LB medium supplemented with 0.5% (w/v) carboxymethylcellulose, 0.5% (w/v) xylan and 5% (w/v) skim milk. Hydrolysis zones were analyzed after 48 and 72 hours. A total of 40 endophytic strains were analyzed. Cellulolytic activity was shown by 95% of the strains after 48 h and 97% of the strains after 72 h of culture. After 48 h and 72 h of the experiment, 97.5% of the tested strains showed xylanolytic activity and 92.5% showed proteolytic activity. The strains synthesizing hydrolytic enzymes belonged to the genera *Bacillus*, *Serratia*, *Microbacterium*, *Paenibacillus*, *Rothia* and *Pantoea*. Key words: endophytes, wheat, enzymes *Research*

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Control Number: 2021-A-8542-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: endophytes

Keyword 2: seeds

Keyword 3: core microbiome

Abstract Title: **Current The Composition Of Wheat Seed Microbiome Determined Culture Independent Technique**

Author Block: W. Goraj[1], **A. Kuzniar**[1], K. Włodarczyk[1], J. Grządziel[2], M. Woźniak[2], K. Furtak[2], A. Gałązka[3], E. Dziadczyk[1], E. Skórzyńska-Polit[1], A. Wolińska[1]; [1]The John Paul II Catholic Univ. of Lublin, Lublin, Poland, [2]Dept. of Agricultural Microbiol., Inst. of Soil Sci. and Plant Cultivation, Puławy, Poland, [3]Dept. of Agricultural Microbiol., Inst. of Soil Sci. and Plant Cultivation, Lublin, Poland

Abstract Body: Endophytes are related with host plants throughout their life history from seed germination to fruit development. One of the most significant plant organs colonized by endophytic microbiota is the seed. The aim of this study was to determine the structure of the seed core microbiota exist in the endosperms and embryos of eight wheat cultivars with the use of a culture-independent technique. The seeds of *Triticum aestivum* L. cv. Hondia, Wilejka, STH, Opcja, Tybalt, Euforia and *Triticum spelta* L. cv. Rokosz and Schwabencorn (producer: Plant Breeding Strzelce Sp. z o.o. Group IHAR) were studied. Rokosz and Hondia wheat were cultured *in vitro* and *in vivo* to identify obligatory bacterial endophytes. A restrictive analysis of reads originating from the *in vitro* plants has demonstrated that the bacterial genera *Paenibacillus* and *Propionibacterium* inhabiting Rokosz and Hondia wheat plants have a status of obligatory endophytic microorganisms. Greater biodiversity of seed-borne endophytic bacteria was determined in the seed endosperms than in the embryos. The multiple comparison analysis of the OTU abundance showed that the seed part significantly influenced the relative abundance. The seed-born microbiome is not statistically significantly dependent on the wheat cultivars; however, it cannot be claimed that every wheat seed is the same.

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Control Number:	2021-A-8551-MICROBE
Session Type:	**iPoster**
Session Number:	**FEMSP100**
Session Title:	**FEMS: Environmental Microbiology and Ecology**
Topic 1:	FEMS - Environmental microbiology and ecology
Keyword 1:	bacteria
Keyword 2:	maize
Keyword 3:	
Abstract Title:	**Fungal Indicators Of Sensitivity And Resistance To Long-term Maize Monoculture: A Field Experiment**
Author Block:	**K. Wlodarczyk**[1], A. Wolińska[1], J. Podlewski[2], A. Słomczewski[2], J. Grządziel[3], A. Sochaczewska[1], A. Kuźniar[1]; [1]The John Paul II Catholic Univ. of Lublin, Lublin, Poland, [2]Potulicka Fndn. Economic Ctr., Wojnowo, Poland, [3]Inst. of Soil Sci. and Plant Cultivation, Puławy, Poland
Abstract Body:	**Fungal indicators of sensitivity and resistance to long-term maize monoculture: a field experiment** Microbiota (bacteria and fungi) existing in the plant rhizosphere and endosphere are beneficial to plant nutrient acquisition, health, and growth. Currently, modern and culture-independent techniques, for example next generating sequencing (NGS), are recommended for using in agroecological research. Here, we studied fungal community in soils under different cropping regimes (intercropping mixture and long-term maize monoculture) in field experiment. Fungal communities in bulk soils were analyzed using metabarcoding (ITS1 and ITS2) with application of Illumina platform. Fungal sequences were clustered into operational taxonomic units (OTUs) based on a 97% similarity threshold. The soil materials were sampled from surface layer (0-20 cm) in three terms of growing seasons: Spring (25.03.2020), Summer (24.06.2020) and Autumn (19.11.2020). Two fields (marked as K20 and K21) were selected for the study. Field K20 covers an area of 5 ha and consists of a perennial maize monoculture, while in 2020 it was sown with a intercropping mixture (perennial ryegrass, incarnate clover and winter vetch) to improve soil quality. Field K21 covers an area of 24 ha and also consists of a perennial maize monoculture (no intercropping mixture was used here). We detected differences in fungal richness and diversity between intercropping mixture and long-term maize monoculture during studied seasons. Generally, fungal diversity at genera level were higher in bulk soil samples of long-term maize monoculture compared to intercropping mixture, however their abundance dependent on the term of vegetation season. It was evidenced that selected fungal genera (*Mortierella* and *unidentified_59*) displayed sensitivity to maize monoculture, whereas *Soliccocozyma*, *Exophiala* and *unidentified_88* seemed to be resistant to maize monoculture. Their sensitivity/resistance was confirmed by a decrease/increase in the number of OTUs in the soils originated from the field of intercropping mixture, in comparison

to the soils under maize monoculture. Importantly, mentioned dependences were present in each of the broadcast seasons. We would like to emphasize that the proposed genera were selected on the basis of samples taken from field experiments. Presented results contribute to the understanding how cropping regimes affect fungal communities soils (*in situ*). *This research was funded by Potulicka Foundation Economic Center, grant number UKDKW 2020/03/1.*

Control Number: 2021-A-8556-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: methane

Keyword 2: methane oxidation

Keyword 3: methanogenesis

Abstract Title: **Methane-related Biological Processes In A Miocene Saline Sedimentary Pool**

Author Block: **W. Goraj**[1], A. Szafranek-Nakonieczna[1], J. Grządziel[2], C. Polakowski[3], A. Gałązka[4], Z. Stępniewska[1], A. Pytlak[3]; [1]The John Paul II Catholic Univ. of Lublin, Lublin, Poland, [2]Dept. of Agricultural Microbiol., Inst. of Soil Sci. and Plant Cultivation, Puławy, Poland, [3]Inst. of Agrophysics, Polish Academy of Sci., Lublin, Poland, [4]Dept. of Agricultural Microbiol., Inst. of Soil Sci. and Plant Cultivation, Lublin, Poland

Abstract Body: Methane and carbon dioxide are one of the most important greenhouse gases. A substantial proportion of this gas originates from marine environments where both its biological formation and oxidation occur. This study presents methane-related biological processes in the Wieliczka Formation sediments in various moisture and aeration conditions. Microbial involvement in this processes were studied using activity measurements (respiration and methane oxidation in anaerobic and aerobic conditions and methane production) and high throughput sequencing. The research material were sedimentary rocks (W2, W3, W4) surrounding the salt deposit in the Wieliczka Salt Mine. It was found that biological activity was found in all of the studied samples but mainly under water-saturated conditions. Microbial respiration was higher in anaerobic conditions and ranged from 36 ± 2 ($W2_{200\%t.w.c}$) to 48 ± 4 ($W3_{200\%t.w.c}$) nmol CO_2 gdw^{-1} day^{-1}. Methanogenic activity was the highest in W3 (0.025 ± 0.018 nmol CH_4 gdw^{-1} day^{-1}), while aerobic methanotrophic activity was the highest in W4 (220 ± 66 nmol CH_4 gdw^{-1} day^{-1}). The relative abundance of methane oxidation microorganisms (*Methylomicrobium, Methylomonas, Methylocystis*) constituted 0.7-3.6% of all taxa. Methanogens were represented by *Methanobacterium* (0.01-0.5 %). The stable isotope composition of the CO_2 and CH_4 trapped in the sediments suggests that methane oxidation could have influenced methane $\delta^{13}C$, especially in siltstone and sandstone (W3), and siltstone with veins of fibrous salt (W4).
Funding: this project was partly financed by the National Science Centre, Poland (DEC-2014/15/N/NZ8/00315).

Control Number: 2021-A-8562-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: gut microbiome Hermetia illucens

Keyword 2: core microbiota

Keyword 3: antibiotic-disinfectant resistance genes

Abstract Title: **Changes Of The Gut And Feed Residue Microbiota During The Rearing Of *Hermetia Illucens* Larvae And Intraspecies Diversity In Its Core Microbiota**

Author Block: **Y. A. Cifuentes Triana**[1], J. Mvie[1], J. O. Bartz[1], A. Müller[2], H. O. Gutzeit[2], A. Vilcinskas[3], P. Kämpfer[1], S. Glaeser[1]; [1]Justus Liebig Univ. Giessen, Giessen, Germany, [2]Dresden Univ. of Technology, Dresden, Germany, [3]Justus Liebig Univ., Giessen, Germany

Abstract Body: Larvae of *Hermetia illucens* efficiently convert organic waste into nutrient-rich supplements [1]. The gut microbiome plays a key role in this process [2]. It is also expected that the gut microbiome thereby affects the abundance of bacteria in the remains of the applied waste-substrate which can be important, e.g., for the elimination of pathogens. We studied the dynamics of the gut microbiome of *H. illucens* larvae and bacterial communities in the substrate during a rearing experiment. We furthermore quantified the presence of antibiotic resistance and disinfectant genes in the gut and feed microbiota. To culture core microbes of the gut microbiota, two different cultivation methods were employed, dilution-to-extinction and direct plating. According to our results, the gut and feed residue bacterial communities were distinct throughout the rearing process. The gut microbiome remained more stable compared to the feed residue microbiome which varied in both alpha diversity and community structure during rearing. At least seven genera were identified in all growth stages in the gut microbiome including *Morganella, Klebsiella, Providencia, Enterobacter, Enterococcus, Bacillus*, and members of yet uncultured *Lachnospiraceae,* all proposed as possible core microbiota. From most of these genera representatives could also be isolated. Detailed genotypic characterization of the isolates showed a high diversity of phylotypes (16S rRNA gene sequence) and genotypes (genomic fingerprinting). Antibiotic-resistance genes were present in both, gut and feed residues, with a significant increase in pupae and residue samples. Disinfectant resistance genes were present in the feed residue and even increased during the rearing process but were not transferred to the gut microbiome. We conclude that *H. illucens* larvae have a stable gut microbiome that does not change significantly over the course of larval development, whereas bacterial communities in the feed residue were affected by rearing. An accumulation of resistance genes during rearing was indicated in the gut and residue community and of disinfectant genes in the residue, however this experiment was performed in the absence of added antibiotics and disinfectants.

Control Number: 2021-A-8591-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: Extremophiles

Keyword 2: Psychrophiles

Keyword 3:

Abstract Title: **Recoverability And Metabolic Activities Of Three Arctic Marine Psychrophiles Under Long-term Sub-zero Temperature Conditions**

Author Block: **K. Dhakar**, Erin Firth, Marcela Ewert, Shelly Carpenter, Bonnie Light, Brook Nunn, Karen Junge; Univ. of Washington, Seattle, WA

Abstract Body: Colder regions on Earth and extra-terrestrial places such as Europa are being investigated for signs of life because biological activities have been recorded at certain limits of low temperature on Earth. Life processes, such as enzymatic activity, motility and metabolic speed, can slow down due to ice emplacement and low kinetic energies, but are also known to persist during exposure to sub-zero temperatures. In the present study, one Arctic marine psychrophilic bacterial strain, *Colwellia psychrerythraea* str. 34H (Cp34H), and two Arctic marine psychrotolerant bacterial strains, *Psychrobacter sp.* str. P7E (P7E), and *Halomonas sp.* str. H3E (H3E) were investigated for their recoverability and metabolic activity (protein synthesis) in low-nutrient artificial saline ice formations at two sub-zero temperatures (-5 ℃ and -36 ℃) for 12 months using a Most probable number (MPN) recoverability assay, DAPI total cell counting, and tritiated Leucine incorporation protein synthesis assay. Recoverability for Cp34H and P7E declined to <1% at both temperatures after the 1year incubation period, whereas H3E maintained 29% ± 14.6% recoverability at -5 ℃ and <1% at -36°C, respectively. Total cell counts declined to 60-80% of the original count for Cp34H and remained stable for the other strains. All three strains showed high protein synthesis activity at -5 ℃ with maximum rates ranging from 1.0 $*10^{-4}$ to 4.5 $*10^{-4}$ $gC{\cdot}gC^{-1}$ h^{-1} over the course of the experiment, indicating maintenance metabolism at that temperature for all three strains. In comparison at -36 ℃, one of the strains (Cp34H) maintained some, albeit low, metabolic activity (maximum $2.3*10^{-6}\pm1.6*10^{-6}$ $gC{\cdot}gC^{-1}$ h^{-1}) possibly also indicating maintenance metabolism at that temperature according to definitions in Price and Sowers (2004). However, recoverability and total cell counts declined significantly for Cp34H at both temperatures indicating that the majority of Cp34H cells had died and/or became mostly non-culturable at the end of our experiment. Only H3E maintained culturability throughout the experiment at -5 ℃. The observed bacterial responses to extended low nutrient and sub-zero temperatures in-ice incubations adds to the limited body of literature on sub-zero microbial activities and provides insights towards life processes under similar conditions located on Earth or extra-terrestrial regions, such as Europa.

Control Number: 2021-A-8596-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: Diversity

Keyword 2: Acinetobacter

Keyword 3: MLST

Abstract Title: **Diversity Of Acinetobacter Spp. In Manure, Biogas Digestate Manure And A Municipal Wastewater Treatment Plant Without Hospital Waste.**

Author Block: **D. Pulami**, P. Kämpfer, S. Glaeser; Inst. of applied microbiology, JLU Giessen, Germany, Giessen, Germany

Abstract Body: Background: The clinical relevance of *Acinetobacter* spp. has increased significantly [1]. Modern livestock is one of sources of antibiotic resistant bacteria (ARB) and wastewater treatment plants (WWTP) are considered as hotspots for spread of ARB from human sources [2,3]. Anaerobic treatments e.g. of manure in biogas plants (BGP) and WWTP are often discussed as biotechnological barrier to prevent the release of ARB, thereby reducing microbial loads into the environment [4,5,6]. Objectives: The aim of this research was to study the abundance and the diversity of culturable *Acinetobacter* spp. in manure, anaerobically digested manure (BGP digestates) and WWTP (without hospital waste) using ChromAgar Acinetobacter^{TM} medium. We hypothesize that phylogenetically distinct *Acinetobacter* spp. are present in manure, BGP digestate and WWTP. Methods: *Acinetobacter* were isolated from above mentioned sources by direct plating and pre-enrichment methods using ChromAgar Acinetobacter^{TM} medium. To elucidate the phylogenetic diversity we combined 16S rRNA and *rpoB* genes sequencing, and *A. baumannii* isolates were further characterized by bla_{OXA-51} typing and MLST (mulitlocus sequence typing) for epidemiological relevance. Phenotypic susceptibility to antibiotics was determined by broth microdilution assay. Results: During cultivation-based studies, 68 *Acinetobacter* isolates were identified from BGPs and WWTP samples: 23 from BGPs and 45 from WWTP. Of these, 75% (51 of 68) were considered as *A. calcoaceticus-baumannii* (ACB) isolates following phylogenetic assignment, while others were isolates of species frequently found in natural environment (e.g. *A. indicus, A. bereziniae, A. gandensis, A. gerneri*). Of note, only 14.8% (10 of 68) ACB isolates were isolated from BGP digestate manure and treated effluent of WWTP. In addition, distinct phylogenetic differences (MLST types) were observed among manure, BGPs digestate and WWTP strains. Few MLST types related to IC7 (international lineages causing outbreaks) were detected in WWTP, however majority represented previously described and novel MLST types. All isolates were susceptible to quinolones, carbapenems and colistin antibiotics. This study evidences that phylogenetically diverse *Acinetobater* spp. are present in livestock manure, BGPs digestate and WWTP. Despite the majority of

ACB isolates belonged to *A. baumannii,* most of them were phylogenetically distinct from those causing outbreaks. The spread of ACB isolates were significantly reduced after anaerobic treatment.

Control Number: 2021-A-8606-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: Microbiome

Keyword 2: Rhizospheric engineering

Keyword 3: Salinity stress

Abstract Title: **Directing The Microbiome Towards Mitigation Of Salinity Stress: Case Study With Vigna Radiata**

Author Block: **S. DUBEY**, S. Sharma; IIT DELHI, NEW DELHI, India

Abstract Body:

Directing the Microbiome Towards Mitigation of Salinity Stress: Case Study With *Vigna radiata* Shubham Dubey, Shilpi Sharma*Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology Delhi, Hauz Khas, New Delhi 110016, INDIA*Corresponding author: shilpi@dbeb.iitd.ac.in

Abiotic stress factors like salinity stress stands out as one of the major challenging factors, negatively affecting productivity of various economically important crops like cereals and legumes. There is an urgent need to mitigate salinity stress using environmentally safe approaches. Rhizospheric microbiome engineering has emerged as an eco-friendly approach for improving plant health, stress tolerance and establishing sustainable agriculture. It has advantages over the traditional approach of amendment with bioinoculants, since rhizospheric microbiome as an inoculum is more robust and hence exhibits better survival and efficacy upon application. The microbial members of plant's rhizosphere tend to adapt efficiently under stress conditions across subsequent passaging cycles. Thus, multiple passaging of rhizospheric microbiome over successive rounds of plant growth ultimately leads to plant fitness even under stress conditions. The aim of this study was to evaluate the efficacy of a stress-acclimatized rhizospheric microbiome in salinity stress mitigation in *Vigna radiata*, using a multi-passaging approach. Specifically, salinity stress was ramped-up after every alternate passaging round to assess the efficacy of acclimatized microbiome under increasing salt levels. Over successive passages, plant biometric parameters and stress marker levels were monitored to track the efficacy of acclimatized microbiome in salinity stress mitigation. Further, 16S rRNA amplicon sequencing was employed to decode the alterations in the rhizospheric bacterial community under increasing salt levels over subsequent passaging rounds. Results indicated that the amendment of plants with acclimatized microbiome led to improved plant growth parameters and reduced stress marker levels. Next generation sequencing analysis revealed increased abundance of plant growth promoting rhizobacteria in the acclimatized rhizospheric microbiome over successive passages.

Overall, multi-passaging-based plant-mediated indirect selection of microbiome, resulted in obtaining an effective stress-acclimatized microbiome, capable of salinity stress mitigation in *Vigna radiata*.

Control Number: 2021-A-8613-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: Legionella pneumophila

Keyword 2: PCR method

Keyword 3: water system

Abstract Title: **Evaluation Of Culture-based &Lt Pcr Methods &Gt Detection Of *Legionella Pneumophila* From Water Samples**

Author Block: **n. naher**, s. ahmed, L. Bari; Dhaka Univ., Dhaka, Bangladesh

Abstract Body: **Background:** *Legionella* sp. an unusual respiratory pathogen are considered an emerging public health problem.Naturally, this pathogen is found in very low and undetectable concentrations at the surface water and in ground water. Temperature in the range 20-45 °C of water systems in food industry and hospitals, in particular cooling towers, ICU water systems which act as potential sources for the proliferation of *Legionella* spp. In recent years food industry and hospital have flourished significantly demand of growing population in the city and has increased the potential habitats of *Legionella* as well as risk of exposure to this pathogen. **Objective:** The current study aimed to evaluate the application of conventional culture method and molecular techniques for isolation and identification of *Legionella pneumophila* from hospital and food industry water samples. **Method:** A total of 114 water samples were collected from different locations of two hospitals and five food industry. All water samples were inoculated on *Legionella* specific medium directly (untreated) as well as following treatment with acid treatment (KCl-HCl solution, pH 2.2), heat treatment (30 min at 50°C) or combination of both. **Result:** When suspected colonies from each sample were subjected to Latex agglutination test, no agglutination was observed for any, as compared to agglutination by the *Legionella pneumophila* ATCC 33512. When tested by PCR using *Legionella pneumophila*16SrRNA specific primer, none of the suspected isolates produced any characteristic band. However, PCR assay of total genomic DNA from one liter water of each sample using the same *L. pneumophila*16SrRNA specific primer detected presence of *Legionella pneumophila* in four of 114 water samples. Two isolates were obtained from hospital ICU tap water and cooling tower water while the remaining two were isolated from food industry cooling tower water. Sequencing and analysis of the data using NCBI database showed 95% homology of all four isolates with *Legionella pneumophila* (GENBANK accession number of four isolates were MZ102255, MZ102256, MZ102257 and MZ102258). Increasing reports of pneumonia in the country ,the current study also emphasizes on regular screening of water systems for this pathogen. The 16SrRNA gene specific PCR method has been found to be an

easy, rapid and specific method for the screening as compared to conventional culture based method which is time consuming and might provide false positive result as well as might fail to detect the Viable but non-culturable state of *Legionella* in sample.

Control Number: 2021-A-8628-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: xerotolerance

Keyword 2: Exiguobacterium

Keyword 3: polyextremophile

Abstract Title: **Xerotolerance A New Property In *Exiguobacterium* Genus**

Author Block: **M. Castillo**, B. Galán; Spanish Natl. Res. Council, Madrid, Spain

Abstract Body: A high xerotolerant bacterium classified as *Exiguobacterium* sp. Helios isolated from a solar panel in Spain showed a close relationship to *Exiguobacterium sibiricum* 255-15 isolated from Siberian permafrost. Xerotolerance has not been previously described as a characteristic of this extremely diverse *Exiguobacterium* genus, but both strains Helios and 255-15 showed a superior xerotolerance to that of the reference xerotolerant model strain of *Deinococcus radiodurans*. Significant changes observed in the morphology of the Helios cells after their desiccation suggest that the structure of cellular surface plays an important role in its xerotolerance. Apart from its remarkable resistance to desiccation, Helios strain shows several polyextremophile characteristics that makes it a promising chassis for biotechnological applications. Helios cells produce nanoparticules of selenium in the presence of selenite due to its resistance mechanism. Using the *Lactobacillus* plasmid pRCR12 carrying a cherry marker we have developed a transformation protocol for this strain, being the first time that a bacterium of this genus has been transformed. The comparison of Helios and 255-15 genomes revealed several interesting similarities and differences. Both strains contain a complete set of competence related DNA transformation genes suggesting that they might have natural competence, and incomplete set of genes involved in sporulation although these strains do not produce spores, suggesting that these genes might be involved in xerotolerance.

Control Number: 2021-A-8636-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: Arthrobacter

Keyword 2: Desiccation

Keyword 3: Xerotolerant chassis

Abstract Title: ***Arthrobacter Sp.*** **Helios A New Strain Highly Resistant To Low Water Stress Conditions: A Robust Chassis For Biotech Applications**

Author Block: **G. Hernández**[1], M. Castillo[1], J. M. Navarro[2], J. L. García[1], B. Galán[1]; [1]Centro de Investigaciones Biológicas - CSIC, Madrid, Spain, [2]Univ. Complutense de Madrid, Madrid, Spain

Abstract Body: The genus *Arthrobacter* is well known among Actinobacteria as bacteria of this genus are one of the most commonly isolated from soils and polluted environments. They are highly ubiquitous due to their resistance to several stresses, such as desiccation, low temperatures or starvation. However, little is known about the molecular mechanisms that grant them a high survival under desiccation conditions, since *Arthrobacter* are known to be non-sporulating bacteria. Different studies suggest that their resistance might be based on a combination of different strategies involving the accumulation of compatible solutes, a high protection against reactive oxygen species and the expression of different chaperones. Bacteria belonging to this genus also present a great metabolic versatility, for example various *Arthrobacter* species have been described to metabolize different environmental pollutants. In this work, a new strain named *Arthrobacter sp.* Helios was isolated from a solar panel in Valencia, a Mediterranean city in Spain. Our work shows that it is highly resistance to desiccation, compared to the xerotolerant model strain *Deinococcus radiodurans.* This bacterium does not only stand out for its tolerance to water deficiency but for its resistance to other extremes conditions, since it is able to grow in a hypertonic medium at high salt concentrations, and in the presence of high concentrations of PEG 6000, apart from its ability to survive to high ultraviolet doses. It is worth noting that *Arthrobacter sp.* Helios is also capable of generating nanoparticles of selenium and has a high nutritional versatility, thereby it can metabolize some molecules recalcitrant to biodegradation such as cholesterol. This bacterium shows potential plant growth promoting properties since it can produce indole acetic acid and solubilize inorganic phosphates. These properties are typical of bacteria showing an endophytic lifestyle, and they can be very useful for different agricultural applications. Moreover, we have been able to create tools to genetically modify this bacterium using the pSEVA plasmid collection. Thus, we propose *Arthrobacter sp.* Helios could become a xerotolerant chassis that could be very used for numerous biotechnological applications (e.g., biofilters, plant growth promoter).

Control Number: 2021-A-8665-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: Synthetic consortium

Keyword 2: Synechococcus elongatus

Keyword 3: Bioplastics

Abstract Title: **A Synthetic And Light-Driven Consortium *Synechococcus Elongatus-Azohydromonas Lata* For Bioplastic Production**

Author Block: **S. Baldanta Callejo**[1], L. Agulló[1], B. Fernández[1], I. Martínez[2], J. Nogales[2], B. Galán[1], J. García[1]; [1]Centro de Investigaciones Biológicas-CSIC, Madrid, Spain, [2]Centro Natl. de Biotecnología - CSIC, Madrid, Spain

Abstract Body: Synthetic biology development has made possible to design and engineer microbial species that increase the efficiency of industrial processes, such as plastic production. Currently, microalgae are attracting more attention for biotechnological applications, since the requirements for their growth are minimal. Algal and cyanobacterial production of bioplastic precursors and other renewable compounds is increasingly being studied, which usually involves genetic optimization of metabolism within a single species of cyanobacteria or algae in order to achieve the overproduction of a target renewable. However, a monoculture approach usually requires extensive research and development to optimize each strain. Nowadays, cutting-edge biotechnological studies suggest synthetic microbial consortia as a more effective strategy for solving complex biotransformations. In this work, a light-driven consortium formed by the cyanobacterium *Synechococcus elongatus* and the heterotophic bacteria *Azohydromonas lata* has been developed to produce bioplastics. The engineered cyanobacteria *S. elongatus* strain SBG363 was used to produce sucrose from CO_2 and sunlight. This sucrose is secreted to the medium culture and support the growth of other heterotrophic bacteria with industrial interest. In our synthetic consortium, the sucrose produced by SBG363 was used to grow *A. lata*, which produces PHA. Prior to the co-culture, we optimized the growth conditions and bioplastic production by *A. lata* in the cyanobacterial medium BG11, using different concentrations of sucrose. We found that a medium with higher concentration of phosphate was enough to support a robust growth and good production of PHA. Our results display a good compatibility of consortium members in the same culture medium, showing that the synthetic consortium *S. elongatus* - *A. lata* is a promising tool for bioproduction of compounds of interest from CO_2.

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Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: Microbial Ecology

Keyword 2: Environmental Remediation

Keyword 3: Functional Redundancy

Abstract Title: **Microbial Indices For Monitoring And Evaluation Of Groundwater And Soil Bioremediation Process**

Author Block: **Y. Miao**; Univ. of California, Los Angeles, Los Angeles, CA

Abstract Body: Microbial indices are emerging as pillars of environmental monitoring programs because of their abilities to provide an accurate and specific diagnosis for active bioremediation processes and effective and holistic measurements for post-perturbation ecosystem health. In-situ bioremediation, including bioaugmentation and biostimulation, has always relied on contaminant concentrations and physicochemical variables to determine and adjust these strategies, while neglecting the makeup and function of microbial ecosystems. High-throughput sequencing technologies equipped with bioinformatics have become an increasingly recognized toolbox that are applied in a variety of fields, including complex groundwater systems containing emerging and refractory contaminant such as 1,4-dioxane.Taxonomic and functional analyses were conducted in groundwater microcosms containing 1,4-dioxane, which were bioaugmented with 1,4-dioxane-metabolizing bacterium *Pseudonocardia dioxanivorans* CB1190 or 1,4-dioxane-co-metabolizing bacterium *Rhodoccocus Ruber* ENV425, respectively, as well as biostimulated with oxygen and propane, respectively. The bioaugmented conditions triggered metabolic pathways that enhanced rapid and repeatable 1,4-dioxane biodegradation, while the overall taxa and functions rebounded to original compositions over time. The “metabolic buffer capacity”, termed as functional redundancy, blocked the oxygen or propane biostimulation efficiencies because of the unaffected housekeeping and self-defense functions in the community, and the consistent functions decoupled with the dynamic taxonomy. Only simultaneous addition of propane and nutrients promoted *Mycobacterium*- and *Methyloversatilis*-initiated 1,4-dioxane degradation.The utilization of metagenomics and bioinformatics provided comprehensive insights into molecular mechanisms in soil and groundwater treatment, and will be valuable to lead to cost savings and improve remedial outcomes in short-term active remediation as well as long-term changes to the ecosystem. Besides, metagenomics served as diagnostic tools to discover the potential defects in the treatment process from molecular aspects, that will be helpful for engineered remediation work to employ efficient and accurate adjustments when encountering unknown issues. Moreover, the advanced understanding of the relationship between the quantity of microorganisms and the ecological “phenotype” of that organism will guide microbiome engineering.

Control Number: 2021-A-8735-MICROBE
Session Type: **iPoster**
Session Number: **FEMSP100**
Session Title: **FEMS: Environmental Microbiology and Ecology**
Topic 1: FEMS - Environmental microbiology and ecology
Keyword 1: antimicrobial
Keyword 2: quaternary ammonium compound
Keyword 3: cetylpyridinium chloride
Abstract Title: **Assessment Of Environmental Bacteria For Resistance To Cetylpyridinium Chloride, Modes Of Potential Resistance Transmission, And Clinical Applications**
Author Block: **N. B. A. Higdon**, E. Gillock; Fort Hays State Univ., Hays, KS
Abstract Body: Projections indicate by 2050 the leading cause of premature death will be due to drug-resistant microbes. Antimicrobial resistance and its associated danger to human health increases daily with each new discovery of resistant pathogens. Opinions vary on how, and why, resistance is growing, but a new premise has gained traction. It is hypothesized that quaternary ammonium compounds (QACs) in the environment at subclinical levels may be promoting selection and differentiation of microbes and thereby contribute to resistance. We used cetylpyridinium chloride (CPC, $C_{21}H_{38}NCl$) to assess if CPC resistant bacteria are present in the environment. After collecting soil samples from 17 sites, 1:100 dilutions were made and used to create lawns on 0.35% CPC incorporated agar, and resistant colonies were streaked for isolation 3 times. 75 isolated bacterial colonies were obtained, and 10 were sent for 16S rRNA sequence analysis identification. This yielded three distinct genera, *Pseudomonas*, *Enterobacter*, and *Pluralibacter*, which have been shown to be pathogenic in humans, fish, and plants. Kirby-Bauer assay, MICs, sole carbon source, and isolation of transferable plasmid tests were performed. Our results show clinically relevant bacteria with CPC resistance are present in the environment, and further research on environmental QAC resistance should be conducted.

Control Number: 2021-A-8770-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: Wastewater

Keyword 2: Digital PCR

Keyword 3:

Abstract Title: **Development Of A Wastewater Surveillance Method For Sars-cov2**

Author Block: M. Rumpler[1], **K. Maynard**[2]; [1]Tennessee Dept. of Hlth., Nashville, TN, [2]Tennessee Dept. of Hlth., Lab. Services, Nashville, TN

Abstract Body: **Abstract:***Background* Wastewater-based epidemiology (WBE) for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can be a vital source of information for coronavirus disease 2019 (COVID-19) management during and beyond the pandemic. Environmental surveillance as a part of WBE of SARS-CoV-2 can provide an early, cost-effective, unbiased community-level indicator of circulating COVID-19 in a population. We present a fully validated analytical droplet digital PCR method for the detection and quantification of SARS-CoV2 in environmental wastewater specimens.*Methods*Composite sampling was performed by local utilities using a standardized approach. Raw wastewater specimens were concentrated by either centrifugation or electronegative filtration. Viral RNA extraction was performed using an automated 96 well magnetic particle purification. Analysis was achieved with a droplet digital PCR platform. Absolute viral load was used to approximate infection rate in the community served by the utility. *Results*The droplet digital PCR method was superior to qPCR methods for the analysis of SARS-CoV2 in wastewater because of reduced inhibitors, absolute quantification, and the lack of need for calibrators. Controls for wastewater analysis are difficult to source and should be carefully considered. The validation included and evaluation of sensitivity, accuracy, precision, and specificity. Challenges to wastewater testing include supply chain, sensitivity, sample inhibitors, and data interpretation. *Conclusion*Wastewater based surveillance of SARS-CoV2 is proving to be a valuable component of understanding the epidemiology of the coronavirus pandemic. It may be considered as one more tool in the public health laboratory arsenal to monitor corona virus outbreaks. Further, wastewater surveillance frameworks developed for estimating COVID-19 prevalence could be readily adapted to help to identify areas where vaccination is lacking and for long-term monitoring of disease prevalence. Uncovering such vaccination deficiencies will be crucial in ensuring the goal of herd immunity is expeditiously achieved.

Control Number: 2021-A-8775-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: microbial communities

Keyword 2: cell-cell interaction

Keyword 3:

Abstract Title: **The Study Of A Micro-community Of Four Riverine Bacteria Reveals Different Types Of Pairwise Interactions**

Author Block: **M. Bonal**[1], D. Gonze[1], K. Faust[2], I. George[1]; [1]Université Libre de Bruxelles, Brussels, Belgium, [2]Katolieke Univ.it Leuven, Leuven, Belgium

Abstract Body: **The Study of a Micro-Community of Four Riverine Bacteria Reveals Different Types of Pairwise Interactions** Heterotrophic bacteria play a key role in rivers' ''self-purification'' by their action of mineralization of organic matter and export of carbon to the atmosphere; plus, they form an important trophic link as they are involved in the microbial food web. However, studies conducted so far don't lead to a fine understanding of the intrinsic functioning of the microbiome (role of each species and how they interact). Therefore, we aimed in this research project to study the microbial interactions amongst a simplified model micro-community of 4 bacterial strains frequently found in rivers to get an insight of how the community is structured and how each strain interacts with the others. over a whole bacterial biological life cycle.We first followed each strain individually (mono-cultures) in batch conditions. The cell counts of these monocultures obtained by flow cytometry were used to calculate the value of the growth rate (μ) and the carrying capacity (K). We then followed the growth of each possible pairwise association (bi-cultures) in batch experiments over 72 h. Total cell counts and relative abundances of each strain were assessed by flow cytometry and 16S rRNA Illumina sequencing, respectively. Eventually, the community (quadri-culture) was also followed the same way as for the bi-cultures. Based on μ, our results show a varied panel of interactions: some pairs compete, others have no interaction, and others display exploitation (positive effect of A on B and negative effect of B on A). Interactions inferred based on K show only competitions with different intensities, the majority being "weak" competitions. Particularly one strain, *Janthinobacterium* sp., is always involved in the strongest observed competitions.The quadri-culture showed that the strains growing the best in our community are the same as in a 20-strain community previously studied at the lab [1]. This could indicate that interactions occurring in the community may be pairwise interactions rather than higher-order interactions involving more than two bacterial partners. Ongoing analyses will help investigate this question and results will be ready to be presented at the World Microbiology Forum.

Control Number: 2021-A-8800-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Environmental microbiology and ecology

Keyword 1: alcohol dehydrogenase
Keyword 2: oxidative resolution
Keyword 3: microbial biotechnology

Abstract Title: **Enzyme Chemistries Of Glacial River Isolates**

Author Block: **E. M. Ingvadottir**, S. M. Scully, O. Vilhelmsson; Univ. of Akureyri, Akureyri, Iceland

Abstract Body: Eighty pseudomonads originating from glacial rivers in Northern Iceland were screened for their ability to oxidize a range of primary, secondary, branched, cyclic and aromatic alcohols with the aim of identifying useful alcohol dehydrogenases (ADHs). These include secondary alcohol dehydrogenases (SADHs) which allow for the enantioselective oxidation of racemic alcohols and thus present a green route towards chiral resolution. Half of the pseudomonads screened exhibited robust ADH activity. Most notably, *Pseudomonas* strains JF29-01 and G34 showed a preference for secondary alcohols with the latter demonstrating further preference for (*R*)- over (*S*)-1,2-propanediol. Screening results will be presented and discussed in detail.

Control Number:	2021-A-8805-MICROBE
Session Type:	**iPoster**
Session Number:	**FEMSP100**
Session Title:	**FEMS: Environmental Microbiology and Ecology**
Topic 1:	FEMS - Environmental microbiology and ecology
Keyword 1:	bioplastic
Keyword 2:	extremophiles
Keyword 3:	psychrophiles
Abstract Title:	**Geothermally-heated Intertidal Pools: Hot Spots For Bioprospecting Salt-tolerate Polyhydroxyalkanoate-producing Bacteria**
Author Block:	**S. M. Scully**, L. Langin, L. Wrogemann, E. Ingvadottir; Univ. of Akureyri, Akureyri, Iceland
Abstract Body:	Polyhydroxyalkanoates (PHAs) are a promising biodegradable alternative to traditional plastics which are problematic, recalcitrant pollutants. PHA-producing bacteria are ubiquitous although most of the well-studied PHA producing strains are mesophilic, have a limited substrate range, and typically do not tolerate high concentrations of salt. The geothermally-heated intertidal pools found just north of Husavik (north central Iceland) have yielded a wide range of cold-active and salt tolerant PHA-producing bacteria with broad substrate spectra. As an example, strain C4A, isolated from a 40°C pool, demonstrates PHA accumulation on a wide range of substrates, including mannitol, and salt tolerance in excess of 5% w/v. The characterization of PHA-producing strains as well as the production of PHA from various substrates under a range of culture conditions will be discussed.

Control Number:	2021-A-8807-MICROBE
Session Type:	**iPoster**
Session Number:	**FEMSP100**
Session Title:	**FEMS: Environmental Microbiology and Ecology**
Topic 1:	FEMS - Environmental microbiology and ecology
Keyword 1:	bioplastic
Keyword 2:	extremophiles
Keyword 3:	psychrophiles
Abstract Title:	**Fishing For Cold-active Bioplastic-producing Pseudomonads In Iceland'S Glacial Rivers**
Author Block:	**S. M. Scully**, L. Wrogemann, O. Vilhelmsson, E. Ingvadottir; Univ. of Akureyri, Akureyri, Iceland
Abstract Body:	Microorganisms isolated from arctic environments represent a valuable reservoir of cold-active, catalytically promiscuous enzymes, including those which can be used for the production of polyhydroxyalkanoates (PHAs). Recently, cold-active Pseudomonas strains isolated from two glacial rivers in northern Iceland, Glera and Jökulsá á Fjöllum, were screened for their ability to accumulate PHAs from a variety of substrates. Several strains, such as Pseudomonas strain G48 and G58, demonstrated the ability to produce PHAs from a wide range of substrates in batch and fed-batch culture at ambient temperature. Furthermore, these strains show that they can accumulate a wide range of short- and medium-chain PHAs for which the optimization and characterization of these materials will be presented.

Control Number: 2021-A-7743-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP100**

Session Title: **FEMS: Environmental Microbiology and Ecology**

Topic 1: FEMS - Molecular microbiology and biochemistry

Keyword 1: Metagenomics

Keyword 2: 16S

Keyword 3: DNA Extraction

Abstract Title: **Automated Isolation Of Microbial DNA From Human Samples**

Author Block: **D. O'Neil**, H. Block, M. Sprenger-Haussels, S. Magyar; QIAGEN GmbH, Hilden, Germany

Abstract Body: **Background** The influence of the human microbiome on health has been demonstrated in multitudes of studies and is an important area of ongoing investigation for medicine and diagnostics. For these studies it is crucial to have a reliable, reproducible extraction method which is able to extract DNA of all microorganisms with comparable efficiency, while simultaneously depleting inhibitory substances which might disturb subsequent downstream analysis. In addition, the increase in number and size of microbiome studies necessitates automation of these extraction methods. Here, we present the results of automating an established extraction method on a magnetic bead handling instrument. **Methods** Human microbiome samples (stool, buccal swabs, and others) were homogenized and microbial cells (including gram-negative and gram-positive bacteria, fungi, and archaea) were rapidly and efficiently lysed by bead beating in conjunction with chemical lysis. In a subsequent step various inhibitory substances were removed from all kinds of inhibitor-rich sample types including stool and gut samples. Thereafter, inhibitor-free DNA was captured on magnetic silica beads, washed, and eluted on the QIAsymphony SP automated system. Extracted DNA quality was assessed for purity and yield, as well as tested in qPCR and 16S rDNA sequencing experiments. **Results** The automated DNA extraction method was able to efficiently extract microbial DNA from one to 96 samples per run, generating consistent results with no detectable crosscontamination. Extracted microbial DNA displayed no inhibition in qPCR with internal control. 16S rDNA sequencing revealed highly complex communities, measured by alpha diversity (observed operational taxonomic units (OTUs)), being comparable to the manual DNeasy PowerSoil Pro reference method, and higher than other tested methods. Beta diversity indicated that extraction of multiple replicates of the same sample were extremely consistent. **Conclusions** This workflow enables processing of up to 192 human or environmental samples and delivers high-quality DNA for downstream applications such as PCR amplification and NGS analysis in less than one day.

Control Number: 2021-A-8093-MICROBE
Session Type: **iPoster**
Session Number: **FEMSP100**
Session Title: **FEMS: Environmental Microbiology and Ecology**
Topic 1: FEMS - Molecular microbiology and biochemistry
Keyword 1: heat shock
Keyword 2: Sulfolobus
Keyword 3: Archaea
Abstract Title: **RNA-thermometer Mediated Post-transcriptional Regulation Of The Heat Shock Response In The Thermoacidophilic Crenarchaeon Sulfolobus Acidocaldarius**
Author Block: **R. Baes**, E. Peeters; Vrije Univ.it Brussel, Brussels, Belgium
Abstract Body:
Background:
Temperature stress is a crucial environmental factor for all living organisms, and is well studied in eukaryotes and bacteria. However, knowledge is lacking for archaea. This is striking, since many archaea are (hyper-)thermophiles, thriving in high-temperature habitats typified by large temperature gradients. For *Sulfolobus acidocaldarius*, living in volcanic hot springs and growing optimally at 75°C, heat shock (HS) response is characterized by an upregulation of heat shock proteins (HSPs). However, it is not known how this temperature-sensing and corresponding molecular regulation is established.
Objectives:
Our hypothesis is that the 5' end regions of the HSP-encoding transcripts contain RNA thermometers (RNATs), structured RNA elements inferring temperature-responsive post-transcriptional regulation by only allowing translation initiation at elevated temperatures by a melting of the secondary structure.
Methods & Results:
Aiming to identify RNAT-candidates, transcriptomics and proteomics have been carried out, investigating the effect of a mild HS. Results show a dynamic transcriptional reprogramming after 15 min of HS, whereas most differential protein expression is only occurring after 60 min, besides a few proteins showing a fast increase in abundance. A decrease in both transcripts and proteins with a function related to energy production, replication and certain metabolic pathways is observed.
In order to follow differential protein expression upon HS with Western blot, genetically engineered strains were constructed, expressing "tagged" putative RNAT-regulated proteins. Additional strains have been constructed in which the 5'UTR sequence is deleted, therefore abolishing any possible leader-associated regulation. Preliminary results confirm the importance of the 5'UTR as a determinant for protein expression levels of certain HSPs at the optimal growth temperature and the deletion impairs their HS-responsive upregulation.

In order to determine the RNA secondary structure of RNAT-candidates, both *in vitro* and *in vivo* SHAPE experiments were carried out, making use of a reagent which specifically modified unpaired RNA nucleotides. *In vitro* results confirm the predicted RNA secondary structure at 37°C. Preliminary *in vivo* experiments intents to characterize the secondary structure(s) of the RNAT-candidates in the high-temperature environment of the cell, both at the optimal growth temperature of 75°C (inhibitory structure) and upon HS (translation-permissive structure).

Control Number: 2021-A-7910-MICROBE
Session Type: **iPoster**
Session Number: **FEMSP105**
Session Title: **FEMS: Eukaryotic Microbiology and Biotechnology**
Topic 1: FEMS - Eukaryotic microbiology and biotechnology
Keyword 1: Mold-ripened cheeses
Keyword 2: filamentous fungi
Keyword 3: Penicillium roqueforti

Abstract Title: **Filamentous Fungi Isolated From Turkish Mold-ripened Cheeses And The Diversity Of *Penicillium Roqueforti* Isolates**

Author Block: **H. E. Kirtil**, B. Metin; Istanbul Sabahattin Zaim Univ., Istanbul, Turkey

Abstract Body:

Background: Turkey has traditional mold-ripened cheeses that are produced in cellars or caves allowing the growth of filamentous fungi on the cheese surface during ripening.
Objectives: In this study, we aimed to identify the mycobiota of Turkish mold-ripened cheeses and determine the genetic diversity of *Penicillium roqueforti* isolates.
Methods: We isolated 148 filamentous fungi from 61 traditional cheese samples (Konya Kuflu Tulum, Erzurum Kuflu Civil, Divle Cave Tulum, Karaman Tulum, Kars Kuflu Chechil, Sivas-Zara Tulum and Rize-Ardesen Golot). The isolates were molecularly identified using internal transcribed spacer (ITS) region or beta-tubulin (*benA*) gene. Fungal isolates were typed with rep-PCR with GTG5 and RAPD-PCR with M13 primers. The horizontal transfer regions, *Wallaby* and *CheesyTer,* were PCR-screened. The mating type locus (*MAT*) idiomorphs of *P. roqueforti* isolates were determined using PCR. Then, morphological diversity of *P. roqueforti* isolates were investigated using four different media, potato dextrose agar (PDA), yeast extract sucrose agar (YES), malt extract agar (MEA) and oatmeal agar (OA).
Results: The isolates consisted of mostly *Penicillium roqueforti* (81%) in addition to *Penicillium* species such as *P. biforme*, *P. crustosum*, *P. paneum*, *P. nordicum*, *P. brevicompactum*, *P. roseomaculatum*, and *P. solitum*. Other species isolated include *Alternaria alternata*, *Albifimbria verrucaria*, *Cladosporium cladosporioides*, *Cladosporium macrocarpum*, and *Talaromyces kabodanensis.* GTG5 and M13 primers were discriminative at the species level. GTG5 fingerprinting resulted in an identical pattern for all *P. roqueforti* isolates except one, whereas one distinctive band was obtained in RAPD-PCR. Screening for the *Wallaby* and *CheesyTer* regions indicated the presence of these loci in all *P. roqueforti* (n=120) isolates in addition to *P.*

biforme, and *P. solitum*. The mating type distribution of *P. roqueforti* isolates was skewed in favor of *MAT1-2* (95%). Future studies will include the use of microsatellites that might better reflect the genetic diversity of *P. roqueforti* isolates.

Control Number: 2021-A-7969-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP105**

Session Title: **FEMS: Eukaryotic Microbiology and Biotechnology**

Topic 1: FEMS - Eukaryotic microbiology and biotechnology

Keyword 1: sexual reproduction

Keyword 2: strain improvement

Keyword 3: sorghum flour

Abstract Title: **A Novel Medium For The Stimulation Of Sexual Reproduction In Aspergillus Terreus**

Author Block: **A. M. Ahmed El-Imam**[1], P. Dyer[2]; [1]Univ. of Ilorin, Ilorin, Nigeria, [2]Univ. of Nottingham, Nottingham, United Kingdom

Abstract Body:

Background

Aspergillus terreus is an ascomycetous fungus used mainly in the production of itaconic acid, a monomer with vast industrial applications and lovastatin. Sexual reproduction is an important method of strain improvement in industrial microbiology, producing progeny with improved desired traits. However, only a handful of media can adequately result in cleistothecia formation in *A. terreus,* and many of them are not readily available and are expensive to procure.

Objectives

To investigate the potential of a novel medium, sorghum flour agar, to initiate sexual reproduction in *A. terreus.* Determine the most suitable medium in terms of yield of sexual reproduction structures produced on classic and the novel medium

Methods

Genetic relatedness and mating types of twenty four isolates of *Aspergillus terreus* from different environments were investigated by molecular methods. The *ben A* and CF 1/CF 2 primers were used to amplify the β-tubulin and calmodulin regions of the genome, respectively, while the ATEM1F and ATEM1R primers were used to amplify *MAT* genes for the determination of mating types. Three media were compared for their abilities to stimulate the production of sexual reproductive structures, namely Odlum's oatmeal agar (OOA), mixed cereal agar (MCA) and sorghum flour agar (SFA). Strains of opposite mating types were then crossed in all possible combinations using the barrage method on the media and incubated (sealed or unsealed) in duplicates at 32 ^{o}C and 37 ^{o}C for 12 weeks.

Results

The formation of cleistothecia-bearing masses was observed, and the yield on the three media compared. SFA produced significantly higher numbers of cleistothecia than other media under all conditions ($P<0.05$). Temperature was found to important as incubation at 37 ^{o}C resulted in a statistically significant increase in the number of hyphal masses formed. Similarly, conditions of high gaseous

exchange had higher yields while significant variations were seen based on strain types. Intact asci containing ascospores were seen in ruptured cleistothecia. These findings are the first reports of the use of this readily available and inexpensive medium, sorghum flour, in the initiation of sexual reproduction in any microorganism. It opens a vista of opportunities for the improvement of *A. terreus* and "cryptic" species with no defined sexual cycles for better industrial performance.

Control Number: 2021-A-8061-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP105**

Session Title: **FEMS: Eukaryotic Microbiology and Biotechnology**

Topic 1: FEMS - Eukaryotic microbiology and biotechnology

Keyword 1: Cryptococcus neoformans

Keyword 2: iron uptake

Keyword 3: iron utilization

Abstract Title: ***Cryptococcus Neoformans*** **Utilizes Ferritin As An Iron Source**

Author Block: **M. Song**; Chung-ang Univ., Gyeonggi-do, Korea, Republic of

Abstract Body: Ferritin is a cytosolic protein found in almost all organisms, and it stores and releases iron in response to iron deficiency and overload respectively. However, upon infection of the vertebrate host, several pathogenic microbes utilize ferritin as a sole iron source. In this study, we investigated whether the human fungal pathogen *Cryptococcus neoformans* is able to use ferritin as a sole iron source and analyzed the interaction between the fungus and ferritin to identify the underlying mechanism of ferritin utilization in the fungus. We found that *C. neoformans* grew well in the presence of ferritin as a sole iron source in the medium, and our results obtained by fluorescence microscopy showed that the physical interaction between ferritin and a fungal cell is critical. Unlike other pathogenic microbes, *C. neoformans* utilizes ferritin in a protease-independent manner but requires Cfo1 and Cft1, which encode ferrioxidase and iron permease, respectively, for high-affinity iron uptake at the cell membrane. Comprehensive transcriptome analysis is currently being conducted to identify the underlying molecular mechanisms of ferritin utilization in *C. neoformans*, and the results will be presented.

Control Number: 2021-A-8066-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP105**

Session Title: **FEMS: Eukaryotic Microbiology and Biotechnology**

Topic 1: FEMS - Eukaryotic microbiology and biotechnology

Keyword 1: Cryptococcus neoformans

Keyword 2: Superoxide dismutase

Keyword 3:

Abstract Title: **Oxidative Stress Causes Vacuolar Fragmentation In The Human Fungal Pathogen Cryptococcus Neoformans**

Author Block: **D. Kim**; Chung-Ang Univ., Anseong, Korea, Republic of

Abstract Body: Vacuoles are dynamic cellular organelles, and their morphology is altered by various stimuli or stresses. Vacuoles play an important role in the physiology and virulence of many fungal pathogens. A *Cryptococcus neoformans* mutant deficient in vacuolar functions showed significantly reduced expression of virulence factors such as capsule and melanin synthesis and was avirulent in a mouse model of cryptococcosis. In the current study, we found significantly increased vacuolar fragmentation in the *C. neoformans* mutants lacking *SOD1* or *SOD2*, which respectively encode Zn, Cu-superoxide dismutase and Mn-superoxide dismutase, and the *sod2* mutant showed greater vacuole fragmentation. We observed that the vacuoles were highly fragmented when wild-type cells were grown in a medium containing high concentrations of metals, such as iron, copper, and zinc. Moreover, higher temperature and treatment with the antifungal drug fluconazole caused increased vacuolar fragmentation. These conditions also commonly cause an increase in the levels of intracellular reactive oxygen species in the fungus, suggesting that vacuoles are fragmented in response to oxidative stress. Furthermore, our data suggest that vacuolar fragmentation is likely mediated by the target of rapamycin complex 1 and Fab1 in *C. neoformans*.

Control Number: 2021-A-8537-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP105**

Session Title: **FEMS: Eukaryotic Microbiology and Biotechnology**

Topic 1: FEMS - Eukaryotic microbiology and biotechnology

Keyword 1: Anaerobic Fungi

Keyword 2: Neocallimastigomycota

Keyword 3: phylogenetics

Abstract Title: **The Complex Case Of Anaerobic Fungi (Neocallimastigomycota)**

Author Block: **J. Vinzelj**[1], S. Podmirseg[1], K. Fliegerova[2], D. Grilli[3], S. Kvasnová[2], D. Schierová[2], H. Sechovcová[2], J. Mrázek[2], G. Siddi[4], G. Arenas[3], G. Moniello[4]; [1]Univ. of Innsbruck, Innsbruck, Austria, [2]Czech Academy of Sci., Praha, Czech Republic, [3]Univ. Natl. de Cuyo, Mendoza, Argentina, [4]Univ. of Sassari, Sassari, Italy

Abstract Body: **Background** Due to its lignocellulolytic potential the herbivorous gut microbiome is of critical importance for biotechnology. Within the herbivorous gut, anaerobic fungi (class: Neocallimastigomycota) specifically are highly proficient in breaking up recalcitrant lignocellulosic biomass and digesting a variety of complex carbohydrates. Recent years have shown previously unknown anaerobic fungal genera to harbour elevated lignocellulolytic potential which has led to a heightened effort in finding and identifying more genera of this promising clade. The unique nature of anaerobic fungi, however, hampers advancements in harnessing their biotechnological potential. A major hurdle in their phylogenetic classification is their high level of length variance within the ITS regions. A novel bioinformatic approach that classifies amplicon sequence data based on specific sequence variants (termed ASVs) rather than grouping sequences into operational taxonomic units (OTUs) could be of particular interest for their phylogenetic identification.

Objectives In this poster, we would like to present a short overview on this essential but often overlooked group of rumen inhabitants and discuss some of the distinct characteristics that pose problems in current microbial community analyses. Our discussion will be based on data gathered during a study on goats that were shifted from forage-based feed to a high grain diet. **Methods** In this study, the anaerobic fungal community within the rumen was assessed using an ITS2-based amplicon sequencing approach as well as an ITS1-based cloning assay. The amplicon data obtained was further analysed by two different bioinformatic pipelines, one based on the classical OTUs, one on amplicon sequence variants (ASVs).**Results** Overall, the ASV based approach led to slightly higher species richness (Shannon-Wiener index) while the OTU based approach produced a higher number of sequence clusters. As for anaerobic fungi phylogeny in general, the data shows that both, ITS1 and ITS2 marker regions can complement each other, but neither seems to represent the full picture. These results are consistent with previous literature and again point out the need for a more robust phylogenetic marker system.

Control Number:	2021-A-8580-MICROBE
Session Type:	**iPoster**
Session Number:	**FEMSP105**
Session Title:	**FEMS: Eukaryotic Microbiology and Biotechnology**
Topic 1:	FEMS - Eukaryotic microbiology and biotechnology
Keyword 1:	filamentous fungi
Keyword 2:	genomes
Keyword 3:	
Abstract Title:	**Heterologous Protein Expression In Schizophyllum Commune Using Endogenously Expressing Crispr Cas9 For Fungal Synthetic Biology**
Author Block:	**S. Baek**; Korea Univ., Seoul, Korea, Republic of
Abstract Body:	Genome engineering methods based on homologous integration and ectopic integration have been developed in mushrooms including Schizophyllum commune. Although mushrooms are a gold mine of genetic resources for novel enzymes and secondary metabolites, genetic manipulation and genomic engineering are non-trivial because of low efficiency of transformation and editing efficiency without standardization. To develop reliable and reproducible genome engineering tools for fungal synthetic biology, we developed a genetic tool to express various enzymes and proteins in a model mushroom, Schizophyllum commune. We designed endogenously expressing CRISPR CAS9 fused with fluorescent reporter (CAS9-GFP) and guide RNA targeting auxotrophic marker (e.g. URA3) to make the screening process simpler and editing efficiency higher. By using our system containing bioparts (e.g. promoters, introns and terminators) from Schizophyllum commune, the CAS9-GFP was integrated into the genome of two Schizophyllum commune host strains, H4-8 and Δku80. We observed the fluorescence from the mycelium and validated in vitro activity of the CRISPR enzyme in the cell extract. We tested the strain expressing CAS9-GFP to integrate heterologous DNAs into the target site for expression of heterologous proteins including enzymes and antibodies. The system provides a genetic tool for developing a synthetic biology toolbox for genome engineering and facilitates using mushrooms as a molecular chassis.

FEMSP108 FEMS: Health and Food Microbiology

Control Number: 2021-A-7768-MICROBE
Session Type: **iPoster**
Session Number: **FEMSP108**
Session Title: **FEMS: Health and Food Microbiology**
Topic 1: FEMS - Health and food microbiology
Keyword 1: Lactobacillus rhamnosus GG
Keyword 2: conjugation
Keyword 3: probiotic
Abstract Title: **Generation Of Lactose- And Protease-positive Probiotic *Lactobacillus Rhamnosus* GG By Conjugation With *Lactococcus Lactis* NCDO 712**
Author Block: N. Hussain[1], **R. Li**[2], T. M. Takala[2], M. Tariq[1], A. H. Zaidi[1], P. E. J. Saris[2]; [1]Natl. Inst. for Biotechnology and Genetic Engineering, Faisalabad, Pakistan, [2]Univ. of Helsinki, Helsinki, Finland
Abstract Body: **Background:** The benefits of *Lactobacillus rhamnosus* GG (LGG) have been demonstrated by many clinical trials. LGG is added as a probiotic supplement in dairy products. The challenge of using LGG in dairy products is that it can't metabolize the lactose and casein of milk, thus causing its poor growth in milk. Lactose and casein utilization of probiotics or bacteria used in dairy products are two important properties for reducing the symptoms of lactose intolerance and cow's milk protein allergy. The deficiency of LGG could be improved by bacterial conjugation, a non-GMO method. One candidate of the donor in conjugation is the dairy strain *Lactococcus lactis* NCDO 712, as it carries the plasmid pLP712 with the gene encoding the protease for casein degradation as well as the gene for lactose catabolism. **Objectives:** The study aims to use bacterial conjugation to generate a new strain of LGG, which carries the ability of utilizing lactose and casein. **Methods:** Bacterial conjugation was performed by mating *L. lactis* NCDO 712 and LGG in MRS broth. Transconjugants were screened on a selective agar: MRS without glucose and meat extract, and supplemented with 1% lactose, 100 mg/ml vancomycin, and 50 mg/ml bromocresol purple. Obtained colonies were verified by PCR and plasmid isolation to confirm the strain and the presence of plasmid pLP712 in LGG. The identified transconjugant was named as *L. rhamnosus* LAB49. Subsequently, the plasmid stability of conjugated pLP712 was investigated. The final test was to determine the new features of *L. rhamnosus* LAB49, i.e., its protease activity and growth capacity in milk. **Results**: The transconjugants grew on selective agar as yellow colonies, indicating the acid production due to the lactose metabolization, while the wild-type LGG did not change the purple color of bromocresol purple. The strain-specific and plasmid-specific PCR confirmed LAB49 was a derivative of LGG, and it carried the conjugated plasmid. Further, plasmid isolation also visualized the existence of pLP712 in LAB49, as wild-type LGG contained no plasmid. LAB49 was incubated in MRS, and all tested colonies (n= 80) lost their lactose-fermenting ability after 100 generations. The proteolytic activity of LAB49 was analyzed by SDS-PAGE and it showed that β-casein was fully digested in 4 h by LAB49 and NCDO 712 but not at all by LGG. The growth

curve indicated LAB49 grew well in milk, reaching stationary phase in 11 to 12 h after inoculation. These results collectively suggested that, *L. rhamnosus* LAB49, an upgraded food-grade and non-GMO derivative of LGG has been generated.

Control Number: 2021-A-7798-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: Bacteriophage Endolysin

Keyword 2: Antibacterial Activity

Keyword 3: Antibiofilm Activity

Abstract Title: **A Novel Bacteriophage Endolysin LysSTG2 With High Antibacterial And Antibiofilm Activity Against*Salmonella*Typhimurium And*Pseudomonas* On Various Food And Food Contact Surfaces**

Author Block: **Y. Zhang**, Hung-Hsin Huang, Hoang Minh Duc, Yoshimitsu Masuda, Ken-ichi Honjoh, Takahisa Miyamoto; Kyushu Univ., Fukuoka, Japan

Abstract Body: *Salmonella* spp. is one of the major causes of foodborne illnesses, among which *Salmonella* Typhimurium is a major serovar frequently associated with human infection. *Pseudomonas aeruginosa*, an opportunistic pathogen, is one of the most commonly isolated multidrug-resistant (MDR) bacteria in clinical samples. In addition, biofilm formation of *P. aeruginosa* is another form of resistance mechanism that makes it tolerant to chemicals with antibacterial activity. As a novel solution for non-thermal decontamination of foodborne pathogens, endolysins, a class of lytic enzymes produced by bacteriophages during the lytic cycle, are promising candidates.In this study, LysSTG2, an endolysin from *Salmonella*-lytic bacteriophage STG2 belonging to the Peptidase_M15 superfamily was expressed as a recombinant protein. The recombinant LysSTG2 showed strong antimicrobial activity against chloroform-treated *S.* Typhimurium cells after the incubation at 4 to 50 °C for 30 min, at pH ranging from 7.0 to 11.0 and in the presence of NaCl from 0 to 300 mmol/L. It also showed lytic activity against all the 14 tested Gram-negative strains treated with chloroform, including *Salmonella*, *E. coli* and *Pseudomonas aeruginosa*, but not against the Gram-positive bacteria tested. In addition, the LysSTG2 reduced viability of *S.* Typhimurium planktonic cells by 1.2 log after 1-h treatment. Sequential treatment of slightly acidic hypochlorous water containing 40 mg/L available chlorine and LysSTG2 (100 μg/mL) was effective on *S.* Typhimurium cells in biofilm, reducing more than 99% of viable counts.LysSTG2 was further used to control *Pseudomonas aeruginosa* and *P. putida*, which showed high sensitivity to LysSTG2, in various foods, and the biofilms formed on the surface of polystyrene resin and stainless steel. In bottled water, LysSTG2 combined with EDTA reduced the viable counts of *P. aeruginosa* and *P. putida* by 2.2 log and to lower than the limit of detection, respectively. The addition of 1 mg/mL LysSTG2 alone reduced the viable counts of *P. aeruginosa* and *P. putida* by about 1 log in chicken and salmon samples contaminated with these bacteria. A strong effect of LysSTG2 on viable counts of *P. aeruginosa* and *P. putida* in biofilms was observed on the polystyrene resin and stainless steel surfaces, reducing 99% of viable counts in biofilm after 2-h

incubation at 37 °C. These results suggest the endolysin LysSTG2 seems to be a promising biocontrol agent in various foods and food processing environments.

Control Number: 2021-A-7892-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: Pseudomonas spp.

Keyword 2: carbapenem resistance

Keyword 3: efflux pumps

Abstract Title: **High Prevalence Of Efflux Pumps And Resistance To Carbapenems In *Pseudomonas Spp.*From The Pork Production Chain: A Risk Of Transmission To Humans**

Author Block: **A. Botelho**[1], M. Camoez[1], O. Bouchami[1], H. Fernandes[2], M. Fraqueza[2], M. Miragaia[1]; [1]Lab. of Bacterial Evolution and Molecular Epidemiology, MOSTMICRO, ITQB NOVA, Oeiras, Portugal, [2]CIISA, Faculty of Vet. Med., Univ. of Lisbon, Lisbon, Portugal

Abstract Body:

Background: Carbapenems are used as a last resort antibiotic but the emergence of carbapenem-resistant gram-negative bacteria is presently one of the main public health threats. Although livestock production has been linked to the emergence of resistant bacteria, the role of the food production chain in the transmission of carbapenem resistance to the clinical setting is elusive. In this study, we explored the meat production chain as a reservoir of carbapenem-resistant *Pseudomonas spp*. (CRP) for transmission to humans.

Objectives: Determine if the pork production chain constitutes a risk for human colonization/infection with CRP. Identify the carbapenem-resistance mechanisms present in CRP from the pig meat production chain. Determine whether CRP from the pork production chain are a reservoir of resistance determinants to the clinical setting.

Methods: Live pigs, slaughterhouse workers and equipment surfaces, raw and cooked meat, and the hands of human consumers before and after meat manipulation were sampled in Portugal (2019). Samples were cultured in a selective media with meropenem (4 mg/L) to screen for CRP and CFUs counts were determined. Carbapenem resistance was confirmed for a selection of isolates (n=105/420) by the Kirby-Bauer method. The whole genome of representative isolates (n=26) was sequenced and their resistome determined by CARD database.

Results: CRP were quantified in the pork production chain ($2.3x10^2$ CFU/hand to $8.4x10^5$ CFU/cm^2), with the highest concentrations obtained from sliced raw meat and equipment surfaces. Hands of operators and consumers after meat manipulation had up to $2.7x10^5$ and $5.4x10^3$ CFU/hand of CRP, respectively. Overall, 54.44% of the samples were contaminated with CRP, of which raw meat had the highest frequency. The representative isolates tested were either resistant (56%, n=59/105) or had intermediary resistance to carbapenems (44%, n=46/105). *Pseudomonas spp.* carried six different potential carbapenem resistance mechanisms - efflux pumps (MexAB-OprM, MexMN-OprM, MexPQ-OpmE, MexXY-OprM) and carbapenemases (SFH-1, PDC-1). The most common mechanisms of carbapenem resistance were efflux pumps, which prevalence varied between 23% (MexMN-OprM, MexXY-OprM) and 100% (MexAB-

OprM). All isolates carried at least one efflux pump and could accumulate up to four different putative carbapenem resistance mechanisms. Contact with the pork production chain and meat handling can constitute a risk for human colonization with CRP, if proper hygiene measures are not applied.

Control Number: 2021-A-7933-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: Campylobacter jejuni phages

Keyword 2: Campylobacter jejuni

Keyword 3: EDTA

Abstract Title: **Genetic Characterization Of *Campylobacter Jejuni* Phages And Biocontrol Of *C. Jejuni* Using The Phages And EDTA**

Author Block: **S. Z. Lwin**, Miku Hirono, Takayuki Nasu, Yoshimitsu Masuda, Ken-ichi Honjoh, Takahisa Miyamoto; Kyushu Univ., Fukuoka, Japan

Abstract Body:

Campylobacter jejuni is reported to be one of the leading causes of human food-borne gastroenteritis worldwide. The most important of vehicles for infection are raw and undercooked poultry meats. To decrease its contamination on poultry meat, bacteriophage is an effective alternative to antibiotics. The effects of phages can be enhanced by using Ethylenediaminetetraacetic acid (EDTA), a food additive with antimicrobial activity. This study investigated the characterization of the *C. jejuni* specific phages and the combined effects of phage and EDTA on *C. jejuni*.Among 26 *C. jejuni* phages isolated and purified in our laboratory, phage PC10, PC11 and PC22 were used in this study. They have broad host range against *C. jejuni* isolates and showed the enormous stability under the temperature ranging from 4 to 60°C and pH ranging from 3 to 9. TEM images of PC10 revealed the phage belong to *Myoviridae* family. The genomic DNA of PC10 and PC22 were extracted using viral nucleic acid kit with some modifications. The whole genome was sequenced and annotated. Phage PC10 possesses a dsDNA with 136,144 bp consisting of 31.8% G+C contents and 217 ORFs. Phage PC22 is also dsDNA with 163,392 bp containing 30.2% G+C contents and 174 ORFs.

Time course of the effects of these phages and/or EDTA on viability of *C. jejuni* was investigated at MOIs of 10 and 1000 and 42 and 4 °C, respectively. At 42°C, viable counts of *C. jejuni* reduced or did not increase at 12 h of incubation in the presence of the single phages or the cocktail of the 3 phages at MOI of 10. Control cells without phage and EDTA increased the viable counts from 4.2 log CFU/ml to 8.2 log CFU/ml after the incubation for 24 h. In the presence of 0.67mM EDTA, the growth of *C. jejune* was retarded, but the viable count reached to the same level of that of the control after 48 h. However, in the presence of 0.67mM EDTA, the phage cocktail decreased the viable counts by 1 log and inhibited the regrowth of the phage resistant population after a 48-h incubation. At 4°C, Phage PC10 reduced the viable count of *C. jejuni* by 3 log after the incubation for 48 h in BHI broth. In contrast to the results at 42 °C, EDTA affected the lytic activity of the phages. Viable counts largely decreased in the presence of PC10, compared to the count in the presence of both phage cocktail and EDTA. According to the findings, *C.jejuni* phage PC10 seems to be a potential antibacterial agent to

reduce *C.jejuni* contamination under chilled temperature, 4°C. Moreover, it was found that the combination of phage cocktail and EDTA successfully control the regrowth of bacteria at 42°C.

Control Number: 2021-A-8011-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: myricetin

Keyword 2: phage suceptibility

Keyword 3: gene expression

Abstract Title: **Effect Of Myricetin On The Phage Susceptibility And Gene Expression Of Escherichia Coli**

Author Block: **P. H. H. Kyaw**, Tomoka Murayama, Shota Tanaka, Koshiro Futada, Yoshimitsu Masuda, Ken-ichi Honjoh, Takahisa Miyamoto; Faculty of Agriculture, Fukuoka, Japan

Abstract Body: In recent years, there has been various studies on flavonoids because of their safety and effectiveness against numerous pathogenic bacteria. To control food-borne pathogens, phage-based approaches are a promising to decrease pathogen contamination in various foods. However, the emergence of phage-resistant bacterial population hinders the expansion of usage of phages as bio control agents. Therefore, our previous study focused on genes involved in phage resistance and susceptibility of bacteria and identified that *ΔdnaK* deletion strain of *Escherichia coli* (*E. coli*) markedly increased the susceptibility to phage. However, it is still needed to find out the methods to control the phage resistance bacterial populations. Since, myricetin is well-known for its antimicrobial activity and a specific inhibitor of the function of DnaK, the present study subjected to investigate the effect of myricetin on phage susceptibility and gene expression level of *E. coli. E. coli* BW25113 was treated with myricetin (Myr) (final concentration of 500 µmol/L) at 37°C for 0, 3, 6, 12 and 24 h and, phage S127BCL3 cultivated in our laboratory was added to the Myr treated cells. The viable cell counts were determined by plating method using Tryptic Soy Agar. Moreover, real time qPCR analysis was used to evaluate the effect of Myr on the transcription level of *dnaK*. The results show that the viable counts of *E. coli* treated with 500 µmol/L Myr for 6 h were decreased by 1 log immediately after the addition of the phage compared to that of control without myricetin. Therefore, *E. coli* was treated with Myr for 6 h (final concentration of 200, 500 and 1000 µmol/L), and phage was added at 6 h. The viable counts of *E. coli* treated with 500 µmol/L Myr at 10 and12 h were about 1.5 and 1.0 log CFU/ml lower than those of the untreated. Furthermore, the real time qPCR analysis revealed that the transcription level of *dnaK* in the *E. coli* treated with 500 µmol/L Myr was lowered by -3.88 log2 fold compared with that of the untreated. Based on our findings, it could be suggested that 6 h-treatment of *E. coli* with myricetin, not only increased the susceptibility of *E. coli* to phage but also retarded the regrowth of the phage resistant population of *E. coli.* In addition, myricetin treated cells of *E. coli* could inhibit the transcription level of *dnaK*. Therefore, our studies on improving bacterial phage

susceptibility and controlling resistant bacterial population by naturally occurring substances are expected to contribute to the expansion of effective use of phages.

Control Number: 2021-A-8052-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: Giardia lamblia

Keyword 2: bivalves

Keyword 3: oysters

Abstract Title: ***Giardia Lamblia*** **In Commercial Oysters (*Crassostrea Virginica*) And Mussels (*Mytilis Edulis*) From The Bronx, New York City**

Author Block: **G. Mayer**; Manhattan Coll., Riverdale, NY

Abstract Body: *Giardia lamblia (G. lamblia)* is a human intestinal parasite that causes gastroenteritis. It is transmitted through the fecal-oral route by mode of contaminated water. Bivalves are filter feeders that have been previously shown to shown to sequester a number of microbes including protozoan parasites such as *Cryptosporidium parvum* and *G. lamblia*. We have previously shown prevalence of *G. lamblia* in non-commercial bivalves, including oysters and mussels collected from various beach sites in the Bronx, NY. Oysters are very important economically and are commonly consumed raw. Commercial oysters (*Crassostrea virginica*) were examined for the presence of *G. lamblia* using molecular techniques. A total of 30 oyster and 72 blue mussels (*Mytilis edulis*) specimens were purchased from a popular fish store located in the Bronx in the fall of 2019. Each specimen was dissected to isolate the following tissues: digestive gland, adductor muscle, mantle, gills, and hemolymph. DNA was extracted from each tissue. Nested-PCR was performed using primers that target the β-giardin gene. *G. lamblia* DNA was detected in 20/30 of the oyster specimens with a resulting prevalence of 6.6%. On the other hand, *G. lamblia* DNA was detected in 9/72 blue mussels (12/5%). In the oysters, the mantle and the adductor muscle were the only tissue in which *G. lamblia* DNA was detected with a prevalence of 3.4%, respectively. In blue mussels, *G. lamblia* DNA was observed in the mantle, the gills, the digestive glands, and the foot at a prevalence of 5.5%, 5.5%, and 1.4%, respectively. These data indicate that commercial bivalves are potential sources of *G. lamblia* infection.

Control Number:	2021-A-8092-MICROBE
Session Type:	**iPoster**
Session Number:	**FEMSP108**
Session Title:	**FEMS: Health and Food Microbiology**
Topic 1:	FEMS - Health and food microbiology
Keyword 1:	fermentation
Keyword 2:	Enterobacteriaceae
Keyword 3:	Lactobacillus
Abstract Title:	**Growth Of Gamma (ɣ)-proteobacteria, Mostly *Enterobacteriaceae*, And *Lactobacillaceae* In Cucumber Juice And Fermenting Cucumbers**
Author Block:	M. A. Rothwell[1], K. Y. Zhai[1], C. G. Pagan-Medina[2], **I. M. Perez-Diaz**[2]; [1]NC State Univ., Raleigh, NC, [2]USDA-Agricultural Res. Service, Raleigh, NC
Abstract Body:	**Background:** While the abundance of specific α-proteobacteria species varies among vegetable type, several harbor *Enterobacteriaceae* and *Pseudomonadaceae* that benefit the plant system. It is documented that these bacteria die off early in vegetable fermentations, thus it is assumed that they do not contribute to the quality of finished products. **Objectives:** This study explored the viability of α-proteobacteria in vegetable fermentations, which are characterized by an extremely acidic pH. The ability of several α-proteobacteria, particularly *Enterobacteriaceae* indigenous to cucumber, to grow in a cucumber juice model system (CJM) and in the fermentation of the fruit was evaluated. The growth of the α-proteobacteria was compared to that of the lactobacilli that prevail in vegetable fermentations, such as *Lactiplantibacillus plantarum*, *Lactiplantibacillus pentosus* and *Levilactocbacillus brevis*. **Methods:** Growth of ɣ-proteobacteria and lactobacilli was characterized in a Cucumber Juice Medium (CJM) and cucumber fermentations. Cucumber fermentations were inoculated to 4 Log CFU/mL with *Pantoea agglomerans*, a ɣ-proteobacteria that reached the highest cell density in CJM. Metabolite concentrations in CJM and fermentations were measured using High Performance Liquid Chromatography. **Results:** The ɣ-proteobacteria and lactobacilli proliferated in CJM prepared with juice expressed from size 1B, 2A/B and 3A/B cucumbers with an average μ_{max} of 0.3895 ± 0.0929 and T_d of 1.8845 ± 0.4645 as determined by optical density at λ_{630nm}. A significant difference was found between the ɣ-proteobacteria μ_{max} and T_d relative to *Lactiplantibacillus pentosus* and *Levilactobacillus brevis* but not *Lactiplantibacillus plantarum* 3.2.8. A group of five ɣ-proteobacteria were unique in maintaining the pH of the CJM after incubation and in producing acetic acid but not lactic acid or ethanol. Inoculation level insignificantly altered the μ_{max} and T_d but impacted growth curve trends and the length of lag and stationary phases for the lactobacilli. Supplementation of CJM with a cover

brine typical of low salt cucumber fermentation inhibited the ɣ-proteobacteria, unlike that of the lactobacilli. *P. agglomerans* was unable to proliferate in cucumber fermentations at a pH of 6.0 ± 0.1 and the population of *Enterobacteriaceae* was outcompeted by the lactobacilli within the first 36 h of the bioconversion. It is concluded that the cover brine and indigenous microbiota hinder the growth of ɣ-proteobacteria in cucumber fermentations.

Control Number: 2021-A-8174-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: Hanseniaspora

Keyword 2: co-fermentation with Saccharomyces cerevisiae

Keyword 3: beer bioflavouring

Abstract Title: **Use Of Non-conventional Yeasts To Enrich The Aroma Profile Of Beer: Hanseniaspora Opuntiae And H. Guilliermondii As Case Study**

Author Block: **N. B. Melo**[1], M. Palma[1], M. Pinto Rocha[1], A. Ferreira[2], M. Rosário Bronze[2], I. Sá-Correia[1]; [1]Inst. Superior Técnico, Univ.e de Lisboa, Lisboa, Portugal, [2]Inst. de Biologia Experimental e Tecnológica, Univ.e Nova de Lisboa, Oeiras, Portugal

Abstract Body: **Background:** *Saccharomyces* yeasts have several characteristics advantageous to brewing, like the efficient production of and tolerance to ethanol, the production of desirable aromas, and the absence of toxins. This justifies why most commercialised beers are produced using species of the *Saccharomyces* genus, such as *S. cerevisiae* (ales) and *S. pastorianus* (lagers). However, limiting microbial diversity during fermentation reduces the sensorial complexity of beer and eliminates subtle aromatic notes. This has motivated brewers to explore non-*Saccharomyces* species for bioflavouring. Mixed starters, combining the fermentative power of *Saccharomyces* and the distinct aroma production of non-*Saccharomyces*, are a promising strategy to obtain a beverage with improved organoleptic profile meeting the demands of a growing number of consumers. **Objective:** To identify non-*Saccharomyces* yeast species with potential for beer bioflavouring, and characterise the beverage produced by the most promising ones. **Methods:** Yeast strains were isolated from grape must and beer wort and identified by molecular methods. A preliminary screening was performed to select the most promising isolates for brewing based on a sensory analysis. Single- and mixed-culture fermentations of *S. cerevisiae* and the selected yeasts were carried out. At the end of each fermentation, pH, residual sugar concentration, ethanol content, and volatile composition were determined, and an organoleptic evaluation performed. **Results:** Preliminary screening led to the selection of *Hanseniaspora opuntiae* IST408 and *H. guilliermondii* IST315 as producers of interesting aromas, despite their inability to consume most sugars available in the beer wort and production of low ethanol concentrations. Each *Hanseniaspora* species was inoculated with *S. cerevisiae* US-05 to obtain a fully attenuated beer with a distinct aroma profile. *Hanseniaspora* strains did not alter residual sugars or ethanol content of the final beverage resulting from *S cerevisiae* fermentation but did indeed shape its volatile composition, decreasing the concentration of ethyl esters while increasing acetate esters. Specifically, *H. guilliermondii* caused an up to 8.2-fold increase in phenylethyl acetate (‘rose’, ‘honey’ aroma) in the final beverage. This work demonstrates, for the first time, the impact

of *Hanseniaspora* species on the volatile composition of beer and reinforces the general notion that non-*Saccharomyces* yeasts can be harnessed to produce beers with distinct organoleptic profiles.

Control Number: 2021-A-8180-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: poultry

Keyword 2: drinking water

Keyword 3: sanitation

Abstract Title: **Bacteria Residues In Poultry Drinking Water Pipelines After The Waterline Sanitation Treatments**

Author Block: **A. Mustedanagic**[1], E. Singer[2], M. Wagner[3], B. Stessl[3]; [1]FFoQSI - Feed and Food Quality Safety and Innovation GmbH, Vienna, Austria, [2]HYGIENICUM GmbH Inst. of Food Safety and Hygiene, Graz, Austria, [3]Inst. of Food Safety, Food Technology and Vet. Publ. Hlth., Vienna, Austria

Abstract Body: Microbial quality of poultry drinking water has an important effect on broiler health and overall performance. The aim of the study was performing comprehensive microbiological survey of poultry drinking water quality from 22 poultry farms in Austria. The water samples were collected before and after the waterline sanitation and analyzed by culture-dependent methods. Most abundant bacteria in water samples were isolated and identified on taxonomic level by 16 S rDNA sequencing. The waterlines were sanitized chemically with 4 ppm chlorine dioxide and during one sanitation application additional mechanical waterline cleaning was performed. In 17% chemically and 47% chemically and mechanically sanitized water samples the aerobic mesophilic count (AMC) was less than 4 log colony forming units (CFU) per milliliter. Furthermore, 32% of bacteria isolates after the chemical and 28% of isolates after the additional mechanical waterline sanitation were opportunistic pathogens. Most prevalently isolated opportunistic bacteria were *Pseudomonas aeruginosa*, followed by *Stenotrophomonas maltophilia*. There was a frequent isolation of *Citrobacter murliniae* after chemical and *Pseudomonas veronii* after combined chemical and mechanical sanitation. *Campylobacter jejuni* was isolated from one water sample that was collected before the waterline sanitation. The waterline sanitation with chlorine dioxide did not result in significant decrease of the bacterial load in the waterlines, however an improvement of waterline sanitation was observed upon combination of chlorine dioxide disinfection with mechanical cleaning of the waterlines. In conclusion, the high abundance of bacteria with biofilm forming capacity is an indicative of high biofilm presence in poultry drinking water system. The identified bacteria represent potential source of waterborne pathogen transmission and risk for animal and human health. Furthermore, the absence of proper hygiene management at poultry farms can lead to increased antibiotic use and elevated presence of antibiotic resistant bacteria in the waterlines.

Control Number: 2021-A-8285-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: Probiotics

Keyword 2: Oral health

Keyword 3: Antimicrobial effect

Abstract Title: **Prevention Effects Of Dental Caries By Antibacterial Effect Of *Lactobacillus Brevis* Strains Against *Streptococcus Mutans* Kcom 1054**

Author Block: **H. Jang**, J. Kim, N-K. Lee, H-D. Paik; Konkuk Univ., Seoul, Korea, Republic of

Abstract Body: **Background:** Probiotics are defined as live microorganism that give beneficial effects to host when administered in appropriate amounts. Especially, *Lactobacillus* sp. have been world-wide used as probiotics for a long time, and as food additives for improvement of oral health. **Objective:** The main objective of the present study was to assess the prevention effects of dental caries through the antimicrobial effect of *Lactobacillus brevis* stains isolated from Korean fermented foods against *Streptococcus mutans* KCOM 1054. **Methods and Results:** To identify the dental caries effects of *L. brevis* strains, minimum inhibitory concentration (MIC), antimicrobial activity, auto-aggregation, cell surface hydrophobicity, exopolysaccharides (EPS) production, biofilm formation, and morphological changes were evaluated. Among the *L. brevis* strains, *L. brevis* KU15153 was found to be the most effective overall in all experiments. *L. brevis* KU15153 showed the lowest MIC (12.5%), exhibited the highest antimicrobial activity against *S. mutans* KCOM 1054 (28.67±4.16 mm). Moreover, auto-aggregation (38.32%), cell surface hydrophobicity (27.08%), and EPS production rate (58.52%) of *S. mutans* KCOM 1054 slightly decreased on treatment with *L. brevis* KU15153. In addition, crystal violet stanning and scanning electron microscopy (SEM) showed the inhibition of biofilm formation by *L. brevis* KU15153 in comparison to that observed in the negative control (untreated *S. mutans* KCOM 1054). Therefore, the *L. brevis* KU15153 might be used as a potential probiotic for maintaining oral health in diverse fields.

Control Number: 2021-A-8292-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: hydroponic ginseng
Keyword 2: neuroprotective effect
Keyword 3: antioxidative activitiy

Abstract Title: **Antioxidant And Neuroprotective Effects Of Hydroponic Ginseng Fermented By *Lactococcus Lactis* KC24**

Author Block: **Y. Chung**, J. Park, K-T. Kim, H-D. Paik; Konkuk Univ., Seoul, Korea, Republic of

Abstract Body: **Background**: *Panax ginseng* is traditionally known as a functional pharmaceutical plant in northeast Asia. Many compounds in ginseng have been proven to have bio-functional effects for human health including antioxidant and neuroprotective effects. **Objectives**: The purpose of this study was to ferment 2-year-hydroponic ginseng(HG) and evaluate its antioxidant activity and neuroprotective effect compared to commercial 6-year-soil-cultivated ginseng(SG). **Methods**: Extracts of both 2-year-hydroponic ginseng(HG) and 6-year-soil-cultivated ginseng(SG) were fermented by *Lactococcus lactis* KC24 for 12h at 37℃. Antioxidant activities were assayed by DPPH(2,2-diphenyl-1-picrylhydrazy) radical scavenging assay, ABTS(2,2'-azino-di-(3-ethylbenzthiazoline sulfonic acid)) radical scavenging assay and reducing power assay. Neuroprotective effects were determined by identifying the survivability of oxidative stressed SH-SY5Y cells. **Results**: As a result, the antioxidant activities of fermented HG were estimated to be 23±15%, 18±15%, 56±33% higher than fermented SG in DPPH, ABTS, and reducing power assay, respectively. In addition, it appeared that neuroprotective effect of HG was approximately 69% greater than that of SG. **Conclusion**: This study suggests that hydroponic ginseng could be applied practically in functional food for nervous disorder patient and fermentation could improve its bio-functionality forward.

Control Number: 2021-A-8299-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: Lactobacillus brevis

Keyword 2: Probiotics

Keyword 3: Anti-inflammation

Abstract Title: **Protective Effects Of *Lactobacillus Brevis* Ku15152 Against *Staphylococcus Aureus* Lipoteichoic Acid-induced Intestinal Inflammation**

Author Block: **W-J. Kim**, J-H. Hyun, N-K. Lee, H-D. Paik; Konkuk Univ., Seoul, Korea, Republic of

Abstract Body: **Background:** Probiotics has been reported to regulate immune system, which can provide prophylactic benefits against various diseases. Lipoteichoic acid (LTA), one of cell wall components of Gram-positive bacteria, can induce inflammation. **Objectives**: This study was aimed to evaluate the probiotic characterization of *Lactobacillus brevis* KU15152 isolated from Chonggak kimchi and whether the strains could alleviate *Staphylococcus aureus* LTA (aLTA)-induced intestinal inflammation. **Methods and Results**: *Lactobacillus rhamnosus* GG (LGG) was used for comparative analysis. *L. brevis* KU15152 showed a high survival rate under artificial gastric conditions. The survival rates of the strain against gastric environment and bile salt are 99.36±0.81% and 108.24±3.74%, respectively. In addition, adhesion ability of the strain to HT-29 cells is 12.92±2.61%. This strain did not produce harmful enzymes such as β-glucuronidase. Protective effects of *L. brevis* KU15152 against aLTA-induced inflammation was investigated in HT-29 cells. Treatment with *L. brevis* KU15152 alleviated the production of interleukin-8 without significant cytotoxicity. It was found that *L. brevis* KU15152 inhibited the activation of ERK and Akt signaling pathways. **Conclusion**: These results indicated that *L. brevis* KU15152 possesses probiotic characteristics and anti-inflammatory effect by inhibiting the ERK and Akt signaling pathways in intestinal epithelial cells. Thus, *L. brevis* KU15152 might be used for development of therapeutic and prophylactic products for aLTA-induced intestinal damage.

Control Number: 2021-A-8304-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: Salmonella

Keyword 2: biofilm

Keyword 3: virulence

Abstract Title: **Effect Of Ph And Salinity On *Salmonella* Spp Ability To Form Biofilm**

Author Block: **S. Petrin**[1], M. Mancin[1], C. Losasso[1], J. E. Olsen[2], L. Barco[1]; [1]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy, [2]Univ. of Copenhagen, Frederiksberg C, Denmark

Abstract Body: *Salmonella* is among the major causes of bacterial infections in Europe but only a few serovars (*S.* Enteritidis, *S*. Typhimurium and the monophasic variant of *S*. Typhimurium) are responsible for the majority of human infections. The severity of *Salmonella* infections results from combination of genotypic and phenotypic features, and environmental factors. Biofilms are defined as complex microbial communities enclosed in an extracellular polymeric matrix attached to a surface, and are considered as an environmental adaptation strategy. They provide protection against different environmental challenges, including antimicrobial agents and biocides, and it has been shown that bacteria in biofilms present a markedly different physiology, often associated with greater virulence. Objectives In order to establish whether different conditions of pH and salinity could have an influence on *Salmonella* ability to form biofilm, 88 different strains, belonging to 15 serovars, among which the three most frequent serovars responsible for human infections were included. and isolated from animal, food and human sources were tested. Methods A pure colony of each strain was inoculated in different experimental conditions (Tryptic soy broth (TSB); TSB 4% NaCl pH 4.5; TSB 10% NaCl pH 4.5; TSB 4% NaCl pH 7; TSB 10% NaCl pH 7). Biofilms were detected after 24 h at 37°C, using crystal violet staining. The quantification was based on the difference between the optical density measurements (λ=570 nm) of the strains and negative controls (uninoculated wells). A linear mixed effect model was applied to compare results from the different experimental conditions. Results Among the tested serovars, *S*. Dublin showed the greatest ability to form biofilm even at pH 4.5 that inhibits the ability to form biofilm in the other tested serovars. *S*. Senftenberg and the monophasic variant of *S*. Typhimurium showed the highest biofilm in TSB 10% NaCl pH 7 (Figure 1). In general, we observed a high influence of pH on the ability to form biofilm, with most of the tested strains not being able to produce biofilm at pH=4.5. In contrast, salinity had only a limited influence on biofilm production. Serovars that cause the highest number of human infections produced only small amounts of biofilm in the tested conditions. It would be interesting to focus on other serovars, such as *S.* Senftenberg

and *S.* Dublin that showed a strong ability to produce biofilm in stressful conditions. *S*. Dublin is a host-specific serovar and persists in cattle herds but it also in rare cases cause highly mortal human infections.

Control Number: 2021-A-8308-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: Mycoplasma

Keyword 2: rapid tests

Keyword 3:

Abstract Title: **A Decision Equivalence Comparability Study Between Compendial And QPCR Test Methods For Detection Of Mycoplasma**

Author Block: **C. Iversen**; Merck BioReliance UK, Glasgow, United Kingdom

Abstract Body: **Background** Mycoplasma detection is part of a comprehensive program required to demonstrate safety of biopharmaceutical products. The compendial culture-based method takes 28 days, whereas qPCR methods can be performed within 1 day. A full validation must be completed for alternative rapid tests to be used for GMP purposes, including a comparability study to demonstrate equivalence of the rapid alternative method to the existing compendial method. **Objectives** The objective was to perform a Decision Equivalence comparability study for a rapid qPCR test for the detection of mycoplasma in biopharmaceutical materials against the current harmonized compendial culture based method. **Methods** The compendial method comprises direct agar inoculation, broth sub-culture, and indicator cell methods of detection compliant to the requirements described in the EP, USP, JP, and FDA PTC 1993. The qPCR uses primers that are specific to a comprehensive range of mycoplasmas. To perform the comparability study a representative sample type (C8166 T cells) were inoculated with mid-log stocks of 7 strains of mycoplasmas. Inoculation was performed at 4 contamination levels with 6 replicates per level. Each replicate was divided and tested be qPCR and each part of the compendial method. **Results** A detected/not detected result was generated from each of the assays. A Chi-squared analysis for Relative Accuracy/Equivalence was used to measures the degree of correspondence between the results obtained. The Relative Limit of Detection (RLOD) was determined for the combined dataset. All strains were detected at 10 CFU/mL by qPCR and by at least one part of the compendial method. This comparability study demonstrated that both the compendial and qPCR assay systems meet the levels of detection and equivalent performance required by regulators for effective mycoplasma screening of biological material.

Control Number: 2021-A-8312-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: Lactobacillus brevis

Keyword 2: Streptococcus mutans

Keyword 3: antibiofilm

Abstract Title: **Anti-biofilm Effect Of The Cell-free Supernatant Of *Lactobacillus Brevis* Kccm 202399 Against *Streptococcus Mutans* Kctc5458**

Author Block: **J. Kim**[1], N. Lee[2], H-D. Paik[2], H. Jang[1]; [1]Konkuk Univ., seoul, Korea, Republic of, [2]Konkuk Univ., Seoul, Korea, Republic of

Abstract Body: **Background:** *Streptococcus mutans* is one of the common bacteria that forms dental biofilm and cause dental caries. Especially, biofilm forming *S. mutans* is more resistance to antimicrobial agent than planktonic cells. *Lactobacillus* strains have been promoted as probiotic agents against *S. mutans*, though the inhibitory effect of *Lactobacillu*s strains on caries has not been accurately identified. Several oral care products are used for oral hygiene. But the use of several antibacterial agent containing oral care products may disrupt the natural microbial ecology of the mouth. Oral care probiotics have been commercialized as substitutes for the conventional treatment for oral diseases, including halitosis, dental caries, gingivitis, and periodontitis. **Objective:** The aim of this study determined the anti-biofilm effect of cell-free supernatant (CFS) of *L. brevis* strains. **Methods:** To study anti-biofilm mechanism, antimicrobial activities, cell surface properties (auto-aggregation and cell surface hydrophobicity), exopolysaccharides (EPS) production, biofilm inhibition and eradication assay was investigated. In addition, the anti-biofilm effects of CFS against *S. mutans* KCTC 5458 were also confirmed through scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM). **Result:** *L. brevis* KCCM 202399 showed the highest anti-biofilm effect on *S. mutans* KCTC 5458. The minimum inhibition concentration of *L. brevis* KCCM 202399 was 25% dilution of CFS against *S. mutans* KCTC 5458. *L. brevis* KCCM 202399 CFS was change cell surface characteristic. Compare with control treatment, CFS inhibited the bacterial adhesion of *S. mutans* KCTC 5458 by decreasing auto-aggregation, cell surface hydrophobicity and EPS production. The CFS inhibited the biofilm formation of *S. mutans* KCTC 5458 by 35.28-72.33%. Mature biofilm of *S. mutans* KCTC 5458 also eradicated by 24.37-56.44%. Anti-biofilm effect of CFS were evaluated by SEM and CLSM compared with control treatment.

Control Number:	2021-A-8316-MICROBE
Session Type:	**iPoster**
Session Number:	**FEMSP108**
Session Title:	**FEMS: Health and Food Microbiology**
Topic 1:	FEMS - Health and food microbiology
Keyword 1:	Lactobacillus
Keyword 2:	bile salts
Keyword 3:	pH
Abstract Title:	**Properties Of Different *Lactobacillus* Strains As Probiotic Bacteria**
Author Block:	**A. Zawistowska-Rojek**, A. Kociszewska, S. Tyski; Natl. Med.s Inst., Warsaw, Poland

Abstract Body:

Background. *Lactobacillus* strains belong to the probiotic bacteria and are important components of gastrointestinal microbiota. *Lactobacillus* strains can be active ingredients of dietary supplements, functional foods or medicinal products. The survival during gastrointestinal transit is the most important feature of these bacteria. They have to be resistant to the low pH in the stomach and to bile salts released into the intestine. Probiotic strains of *Lactobacillus* should also have aggregation ability, which enable them to form a barrier, impeding adherence of pathogenic strains to the gastrointestinal tract mucosa. **Objectives.** The aim of this study was to investigate the ability of *Lactobacillus* strains to grow in an acidic environment and in bile salt presence, enabling survival in the digestive tract. Moreover, the auto-aggregation property of these strains, as process impeding pathogen adherence, was also investigated.**Methods.** The 38 *Lactobacillus* strains belonging to *L. rhamnosus, L. paracasei, L. plantarum* and *L. acidophilus* species, isolated from the probiotic products (dietary supplements and yoghurts) and from clinical material, were tested. Resistance to low pH of all strains was examined in MRS broth of pH values 2.0, 3.0 and 4.0. The survival of bacteria in low pH medium was determined from 10^7 CFU/ml concentration, by the plate count method on MRS agar after 1 and 2 h of incubation. The effect of bile salts on bacterial growth was indicated by an increase in absorbance in cultures growing on MRS medium and on MRS medium with 0,3% bile after 6 h of incubation.For auto-aggregation test of *Lactobacillus* strains, the difference in absorbance at the beginning of experience and after 24 h of growth was determined.**Results.** Most of the tested strains were characterized by good survival in an environment of pH 3.0 and 4.0, after 2 h of incubation. In a medium with pH 2.0 after 2 h, the growth of *L. rhamnosus*, *L. paracasei* and *L. plantarum* strains was less than 10^1 CFU/ml, while *L. acidophilus* growth was from none up to 10^5 CFU/ml, depending on the strain.The bile tolerance test showed

that all tested strains tolerated well the environment with the addition of 0,3% bile salt. The growth inhibition coefficient was in the range between 0.05- 0.40. Auto-aggregation after 24 h incubation was in the range of 37-89%. The highest value was observed for *L. acidophilus* strains (50-87%). The tested *Lactobacillus* strains were able to survive in low pH, bile salt environment, and also showed auto-aggregation ability. All investigated properties are strain-dependent.

Control Number: 2021-A-8351-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: Dietary Fiber

Keyword 2: Systems Biology

Keyword 3: Metagenomics

Abstract Title: **Global, Distinctive And Personal Changes In Molecular And Microbial Profiles Induced By Specific Fibers**

Author Block: **S. M. Lancaster**, B. Lee-McMullen, M. Snyder; Stanford Univ., Palo Alto, CA

Abstract Body: Dietary fibers act through the gut microbiome and improve cardiovascular health, metabolic disorders and cancer prevention. They are typically studied as whole plants and not as individual purified fibers. To better understand the health benefits of dietary fiber we investigated the systemic effects of two popular purified fiber supplements, arabinoxylan (AX) and long-chain inulin (LCI), and a defined mixture of five fibers. We found multi-omic signatures of metabolomics, lipidomics, proteomics, metagenomics, a cytokine panel and clinical measurements on healthy and insulin resistant participants. We found that each fiber was associated with fiber- and often dose-dependent biochemical and microbial responses. Notably, AX consumption, but not LCI, was associated with a significant reduction in LDL and increase in secondary bile acids, which we hypothesize is contributing to cholesterol reduction. LCI is associated with an increase in the abundance of Bifidobacterium - often assumed as a healthy bacterium. However, at the highest dose (30g/day) of LCI there was increased inflammation and elevation in the liver enzyme alanine aminotransferase - an effect we found not associated with cholestasis. Overall we found that a) individuals have specific responses to fibers, b) different fibers cause distinct, specific systemic and metabolic effects that are associated with changes in the microbiome, and c) individual fibers may confer a stronger biological impact than mixed fibers. This study yields new discoveries about the effects of fiber supplementation, it provides insights into mechanisms behind fiber-induced cholesterol reduction, it shows the association of individual, purified fibers with the microbiome, and it demonstrates how dietary fiber can be used to intelligently drive host physiology.

Control Number: 2021-A-8372-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: Klebsiella pneumoniae

Keyword 2: UTI

Keyword 3: food-associated human infection

Abstract Title: **Close Genetic Relationship Between *Klebsiella Pneumoniae* From Urinary Tract Infections (utis) And Retail Meat Recovered In Spain During 2020**

Author Block: I. García-Meniño, L. Lestón, R. Fernández-Pedrero, P. Lumbreras, J. Fernández, A. Käsbohrer, **J. A. Hammerl**, A. Mora; German Federal Inst. for Risk Assessment, Berlin, Germany

Abstract Body:

Background *Klebsiella pneumoniae* is recognized as a major cause of nosocomial infections and one of the predominant bacteria associated with urinary tract infections (UTIs) in both community and health care settings, worldwide. Besides their clinical impact, there is a gap of knowledge on the impact of retail meat products in the spread of globally emerging *K. pneumoniae* high-risk clones.

Objetives This study aims the genetic and genome-based comparison of *K. pneumoniae* isolates recovered from human infections and retail meat products of the same geographical area in Spain under the "One Health" approach.

Methods In 2020, 64 clinical *K. pneumoniae* isolates were consecutively recovered from human UTIs (n=52) and retail meat products (turkey meat, n=8; chicken meat, n=3 and pork meat, n=1) in the northwest of Spain. *In depth* characterization including whole-genome sequencing (WGS) was performed for all isolates. The phylogenetic relationship of the *K. pneumoniae* was analysed using XbaI-macrorestriction (XbaI-PFGE) profiling as well as MLST and SNP analysis of the core genomic regions.

Results The comparison of the XbaI-PFGE profiles revealed a high diversity within the collection. A total of seven phylogenetic clusters were detected. Four of them contained both meat- and human-associated isolates, which appeared scattered throughout the PFGE dendrogram. It is of note that two turkey meat isolates were assigned to the recently emerging high-risk clones ST307 and ST147, respectively. Both of them exhibited high-identity XbaI-macrorestriction profiles (>85%) with two human isolates. Three further sequence types previously detected in clinical cases (ST111, ST983 and ST219) were also identified among the food strains. Finally, SNP-based phylogenetic analyses confirmed the relationship between meat- and human-associated isolates. This "One Health" approach indicates a high genetic identity between meat *K. pneumoniae* isolates of meat and human clinic (UTI) origin in the same geographical region. In view of our results, poultry meat products would represent a yet underestimated source of human clinically important *K. pneumoniae* clones. To prevent a further spread of high-risk *K. pneumoniae* clones in the future, a better understanding of the zoonotic impact of these bacteria and novel public health surveillance strategies are needed.

Control Number: 2021-A-8394-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: Salmonella

Keyword 2: food-borne pathogens

Keyword 3:

Abstract Title: **Relationship Between Growth Fitness, Virulence, And Resistance To Food-Processing Related Stresses In Non-typhoidal Salmonellae**

Author Block: **S. Guillén**, M. Marcén, E. Fau, P. Mañas, G. Cebrián; Univ. de Zaragoza/Inst. Agroalimentario de Aragón-IA2, Zaragoza, Spain

Abstract Body: **Background:** The capacity of *Salmonella* to resist and adapt to harsh conditions is one of the major features that have made this microorganism such a relevant health hazard. Despite its relevance, the impact of these resistance responses on other aspects of *Salmonella* physiology, such as virulence and growth fitness, is still not fully understood. **Objectives:** The objective of this study was to determine the growth fitness, virulence, and other phenotypic characteristics of 23 *Salmonella* strains, and to compare them with their previously determined stress resistance parameters to several environmental stresses and non-thermal food preservation technologies (1). **Methods:** 23 strains belonging to 15 serovars of *Salmonella enterica* were selected. Growth fitness characterization were carried out in three different media. Virulence capacity was determined by CaCo-2 cell assay. And biofilm-forming ability and antimicrobial resistance were determined. **Results:** Significant differences in growth fitness, virulence, and biofilm-forming ability were found among the strains studied. Nevertheless, whereas less than 3-fold change, determined in the same medium, was observed among the lowest and the highest growth rate, the percentage of cells capable of adhering to Caco-2 cells varied by nearly 15-fold. Moreover, the percentage of cells capable of invading Caco-2 cells varied more than 100-fold between the different strains. Similarly, the ability of *Salmonella* strains to form biofilms varied widely, more than 30-fold, and the antibiotic MICs varied up to 512-fold, depending on the antibiotic. Results indicate that those strains with the highest cell adhesion ability were not always the most invasive ones and suggest that, in general terms, the higher stress resistance of some strains/serovars did not imply a fitness cost in the media studied. No association between stress resistance and biofilm formation ability (except for acid stress) or antibiotic resistance (with minor exceptions) was found. Our data also suggest that, in *Salmonella*, resistance to acid stress would be associated with virulence, since a positive correlation of that feature with adhesion and a negative correlation with invasion was found. This study contributes to a better understand *Salmonella* physiology and the relationship between bacterial stress resistance, growth fitness, and virulence. It

also provides new insight on the intra-specific variability of a series of phenotypic characteristics of *Salmonella* that are relevant from the food safety perspective.

Control Number: 2021-A-8409-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: anti-biofilm

Keyword 2: multi-species pathogens

Keyword 3: ε-polylysine

Abstract Title: **ε-polylysine Inhibited Biofilm Formations And Viabilities Of Food Pathogens Found On Chicken Meats**

Author Block: **D-U. Lee**, N-K. Lee, H-D. Paik, Y. Park, C. Kang; Konkuk Univ., Seoul, Korea, Republic of

Abstract Body:

Background *Salmonella* Enteritidis, *Listeria monocytogenes* and *Escherichia coli* were lethal foodborne pathogens found on chicken meats, occurring symptoms of diarrhea, abdominal cramping and septicaemia. These pathogens produce toxins and biofilms for protection against external attacks and threaten a safety of chicken food industry. ε-polylysine (PL) is a homopolypeptide composing of approximately 25-30 L-lysine residues having powerful antibacterial effect against gram-positive and gram-negative. **Objectives** This study aimed to determine the antibacterial and antibiofilm effect of PL against foodborne pathogens, *S.* Enteritidis KCCM 12021*, L. monocytogenes* H7962 serotype 4, *E. coli* O157:H4 FRIK 125 and *E. coli* ATCC 259222, in chicken imitative model. **Methods** Antibacterial effect was detected by minimum inhibitory concentrations (MIC), minimum bacterial concentrations (MBC) and a number of live cell. Antibiofilm effect was detected by biofilm inhibition and degradation effect using crystal violet assay, as previously described on Song et al (2020) [1]. To check biofilm inhibition effect Co-aggregation, hydrophobicity and exopolysaccharide (EPS) production was detected to understand the properties of initial stage on biofilm formation [1]. To figure out interactions between microorganisms, not only mono-species but also multi-species bacterial cultures were used in seven groups: G1=*S.* Enteritidis KCCM 12021, G2= *L. monocytogenes* H7962 serotype 4, G3=*E. coli* O157:H4 FRIK 125*+E. coli* ATCC 259222, G4=G1+G2, G5=G1+G3, G6=G2+G3, G7=G1+G2+G3. A chicken juice (CJ), a supernatant of grinded chicken meats, replaced the Tryptic Soy Broth (TSB) to mimic chicken model. **Results** MIC against G2 was 0.031mg/mL of PL and those against other groups were 1mg/mL. PL decreased the live cell viability time-dependently. Biofilm Inhibition effects against G1-G7 on TSB at MIC were 29.79%, 50.27%, 27.21%, 23.58%, 30.46%, 27.84% and 35.83%, respectively. In addition, those on CJ were 29.79%, 44.99%, 48.44%, 47.48%, 30.46%, 54.95% and 35.83%. All of biofilm degradation effects at MIC were below 67.28% on TSB and below 54.95% on CJ. Co-aggregation and hydrophobicity were getting lower only in G2 and exopolysaccharide (EPS) production activity was weakened only in G1 at 1/2 × MIC. Overall, multi-species groups (G4-G7) tended to follow the properties of dominant mono-species group. These results demonstrated PL inhibited biofilm formations and viabilities of food pathogens considering interactions between co-cultured strains.

Control Number: 2021-A-8417-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: colistin

Keyword 2: mcr-4

Keyword 3: plasmids

Abstract Title: ***Mcr*-4 Carrying *Escherichia Coli* Isolates Of Europe Exhibit A High Genetic Diversity But A Highly Conserved Plasmid Type Encoding The Colistin Resistance**

Author Block: I. García-Meniño, A. Oliveira, C. Almeida, A. Käsbohrer, A. Mora, **J. A. Hammerl**; German Federal Inst. for Risk Assessment, Berlin, Germany

Abstract Body: **Background** Colistin represents an important antimicrobial for the veterinary and human sectors. Besides its outstanding antimicrobial use in gastrointestinal infections in animals, it is currently a last-line treatment option for human infections caused by multidrug-resistant Gram-negative bacteria. Successively after the description of the first mobile colistin resistance (*mcr*-1) element in *Escherichia coli*, the identification of other genes (*mcr*-2 to *mcr*-10) and variants has forced the understanding of the colistin resistance and dissemination mechanisms in Enterobacterales. **Objetives** For comparative analysis, a collection of Spanish (n=28), German (n=14) and Portuguese (n=9) *E. coli* isolates of porcine origin exhibiting the mobile colistin resistance determinant *mcr*-4 were subjected to phenotypic and genotypic *in depth* characterization. **Methods** The isolates were investigated for their antimicrobial susceptibility, macrorestriction profiles (XbaI-PFGE), plasmid patterns (S1-PFGE) and plasmid transmission (*in vitro* filter mating studies). Short-read whole-genome sequencing (WGS) data were used for *in silico*-based typing of the genomes. **Results** Overall, the investigated isolates of the three countries differed substantially in their macrorestriction profiles.. While the Portuguese and Spanish isolates exhibited a closer phylogenetic identity, in correlation with their geographic origin, German isolates showed high heterogeneity. Similar results were observed from S1-PFGE analysis. However, all isolates showed a low size *mcr*-4 carrying plasmid (range10 to 25 kb). *In vitro* transmission to the sodium azide-resistant *E. coli* J53 strain was confirmed in at least 50% of the Spanish and Portuguese isolates. Interestingly, most of the transconjugants harbored two plasmids of which only one carried the mobilizable *mcr*-4 plasmid, while the second would probably act as a helper for the transmission. While the *mcr*-4 plasmids seem to be based on a highly conserved ColE10 plasmid backbone, the majority of their host are highly heterogeneous. Our results indicate a close relationship of the individual *mcr*-4 carrying plasmids of Portugal, Spain and Germany. Thus, we hypothesize that dissemination of the conserved plasmid-type is due to a common ancestor. However, the impact of this gene is currently unknown, since no comprehensive information on *mcr* determinants in colistin-resistant isolates from human infections exists.

Control Number: 2021-A-8419-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: UV-C

Keyword 2: Stainless Steel Surface

Keyword 3:

Abstract Title: **Effect Of UV-C On *Escherichia Coli*, *Staphylococcus Aureus, Salmonella Typhimurium*and Sars-Cov-2 Virus Surrogate (MS2 Bacteriophage) Inoculated Onto Stainless Steel Surface**

Author Block: **A. Ortali**[1], J. Wright[2], B. Onarinde[3]; [1]Univ. of Lincoln - NCFM, Holbeach, United Kingdom, [2]Lincat Limited, Lincoln, United Kingdom, [3]Univ. of Lincoln - NCFM, Spalding, United Kingdom

Abstract Body: Stainless steel is commonly used in the food, beverage and medical industry due to ease of cleaning and sanitising. It is an ideal material for food processing, catering and medical equipment or devices because of its inability to impact colour, odour and flavour to products and high corrosion resistance. However, stainless steel is susceptible to bacterial adhesion which can lead to cross contamination and spread of spoilage and or pathogenic microorganisms. Chemical disinfectants have been used in industries to address the problem associated with microbial adhesion on stainless steel. However, research has shown that bacterial cells attached to surfaces can withstand these disinfectants due to the development of extracellular polymers that act as a protective cover against these chemicals. Ultraviolet (UV) light decontamination technique has been explored for decontaminating water, food and surfaces and has been shown to provide rapid and effect inactivation of microorganism through a physical process. The aim of this study was to investigate the efficiency of a UV appliance on selected microorganisms. Freshly prepared inoculum of *Escherichia coli*, *Staphylococcus aureus* (NCTC 10656), *Salmonella* Typhimurium (NCTC 13348) and MS2 bacteriophage (ATCC® 15597B1™) surrogate of SARS-CoV-2 virus (Coronavirus) were individually spread on a square shaped stainless steel coupons (100 x 100 x 1 mm) and allowed to dry in a safety cabinet overnight. After drying each stainless-steel coupon was subjected to UV treatment (UV dose ranging between 25.5 mJ/cm^2 and 31.4 *mJ/cm*^{2}). Microbial population before and after UV treatment was enumerated using both selective and non-selective media for each microorganism. The results show that the exposure of stainless-steel surface inoculated with *E. coli*, *S. aureus*, *S. Typhimurium* and MS2 bacteriophage at 30 seconds UV treatment resulted in a reduction of 99 % of *E. coli* (dose of 25.5 mJ/cm^2), 99.9 % of *S. aureus* (dose of 26.2 mJ/cm^2) and 90 % of *S*. Typhimurium (dose of 28.0 mJ/cm^2). The reduction of 99.9% of MS2 bacteriophage was observed with 30 seconds UV treatment (dose of 31.4 mJ/cm^2). Average log reduction recorded using counts on selective media were 2.2, 3.4 and 1.8 Log CFU/coupon for *E. coli*, *S. aureus* and *S*. Typhimurium respectively. For MS2 phage a 3.0 Log PFU/coupon was

recorded. In conclusion the study reveal that 30 sec of UV treatment is effective for decontaminating stainless steel surface from *E. coli*, *S. aureus*, *S*. Typhimurium and SARS-CoV-2 Virus Surrogate.

Control Number: 2021-A-8427-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: Listeria monocytogenes

Keyword 2: Cantaloupe

Keyword 3:

Abstract Title: **Growth Potential Of *Listeria* Monocytogenes On Cantaloupe (*Cucumis Melo*) Pulp Evaluation Under A Dynamic Time-temperature Profile**

Author Block: **A. Ortali**[1], B. Onarinde[2]; [1]Univ. of Lincoln - NCFM, Holbeach, United Kingdom, [2]Univ. of Lincoln - NCFM, Spalding, United Kingdom

Abstract Body: **Background:** Although ready-to-eat (RTE) fruits and vegetables are a healthy and convenience food choice, they have in most recent years been associated with several foodborne outbreaks. Several bacteria pathogens have been implicated in such outbreaks however *Listeria monocytogenes* is reported to have a higher case-fatality rate compared to *Salmonella* and *Escherichia coli*. A recent case that was reported involving *Listeria* was relating to consumption of contaminated cantaloupe which led to the death of two people. *Listeria* is ubiquitous in the environment, hence contamination of fresh produce such as melons could occur in field where the outer rind of melons, especially the irregular rough rind of cantaloupe, aids the adhesion of soil and microorganisms. Also, storage period and temperature are important factors influencing the growth and survival of *L. monocytogenes* in foods such as RTE fruits. While temperature is not always guaranteed during cold chain, growth potential (the difference between the final and initial counts) (δ) could be an instrument to evaluate the storage conditions that can be most suitable for controlling level of *L. monocytogenes* in fruits. The aim of this study was to evaluate the influence temperature has on the Growth Potential (δ) of *L. monocytogenes* during a simulation of real cold chain scenario. **Method:** *L. monocytogenes* was prepared by inoculating *L. monocytogenes* culture in in freshly prepared TSB (Tryptic Soy Broth), incubated overnight at 37°C in agitation, followed by centrifugation and washing with 0.1% of peptone. Cantaloupe was cut into cubes of 10g and inoculated with 10 µl of *L. monocytogenes* inoculum. **Results:** The initial level of *L. monocytogenes* was 5.7 Log CFU/g. Two time-temperature scenarios (4°C for 24 h then 8°C or 12°C for 48h) were assessed and the trial was repeated three times. The average δ after 24h at 4°C resulted 0, 0.9 after only 24h at 8°C and 1 after 48h. The δ after 24h at 12°C resulted 1.6 and 1.7 after 48h. Growth potential δ of >0.5 Log means a food cannot support the growth of *Listeria*. **Conclusion:** The results show that the temperature has a fundamental impact on δ. Indeed, in only 24h of abuse of temperature the δ increased by 1 point changing the denomination of the product from non-supporting to supporting the growth of *Listeria*. The temperatures applied in the trial are commonly detected in the real life, especially in the final stages of cold chain (retail and household).

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Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: corn

Keyword 2: contamination

Keyword 3: aflatoxin

Abstract Title: **Aflatoxin Corn Contamination In Serbia And Aflatoxigenic Potential Of *Aspergillus Flavus* Strains**

Author Block: M. Grahovac[1], **D. Budakov**[1], M. Loc[1], J. Grahovac[2], V. Vlajkov[2], D. Milic[1], T. Novakovic[1]; [1]Univ. of Novi Sad, Faculty of Agriculture, Novi Sad, Serbia, [2]Univ. of Novi Sad, Faculty of Technology, Novi Sad, Serbia

Abstract Body: *Aspergillus flavus* is a corn pathogen that does not cause significant damage to plants and yield, but is much more important due to its potential for aflatoxin production which causes heavy contamination of corn. In the year 2020 in Serbia, corn sampling was performed at two phases. First sampling phase was for corn harvested in 2019 at 18 localities, and the second phase was after the 2020 harvest, for corn harvested in the same year at 10 localities. Samples were analysed for the presence of aflatoxins, while samples from 2019 harvest were also used for *A. flavus* isolations. Aflatoxin contamination was analyzed using ELISA and HPLC methods. Isolations and identification of *A. flavus* were perfomed by standard phytopathological techniques. Subsequently, 10 selected monosporial *A. flavus* strains were analysed for aflatoxin production capacity by CAPs analysis (four multiplex PCR reactions). Capacity for mycotoxin production of the isolates was futher tested by artificial inoculation of corn grains and their analysis for the presence of aflatoxins by HPLC. The results showed that in 2019 and 2020 in Serbia, corn was not contaminated by aflatoxins. Contamination of all samples for total aflatoxins and aflatoxin B1 was below detectable limit, with an exception of one sample where presence of toxins was detected (0.002 µg/kg) and was below permitted level. CAPs analysis of 10 *A. flavus* strains revealed that 9 strains do not have significant deletions of genes for aflatoxin synthesis, while one strain has significant deletions that may influence its aflatoxigenic properties. Analysis of corn grains artificially inoculated with the strains revealed that in 7 of 10 tested strains, genes expressed their activity, resulting in heavy contamination of corn (321.8 – 2,147 µg/kg total aflatoxins, 102.7 – 1,354.4 µg/kg aflatoxin B1). Two strains withouth significant gene deletions did not synthethize aflatoxin in artificial inoculation test, as well as the strain whose genetic profile suggested its atoxigenic nature. The obtained results suggest that highly aflatoxigenic population of *A. flavus* in Serbian corn is present. However, expression of its toxigenic potential is heavily dependent on environmental conditions, which in 2019 and 2020 did not favor corn infection. Nonetheless, *A. flavus* has to be monitored and controlled, due to the fact that in years when favorable conditions

occur, toxigenic isolates may show their full potential resulting in heavy corn contaminations and unbearable economic losses Serbia already experienced in 2012-2013.

Control Number: 2021-A-8475-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: Biocontrol

Keyword 2: Patulin

Keyword 3: Stone Fruit

Abstract Title: **Control Of *Penicillium Expansum* By An Antagonistic Yeast In Stone Fruit**

Author Block: P. Tejero, **A. Rodríguez**, A. Hernández, C. Hidalgo, R. Casquete, M. Ana, A. Martín; Univ. of Extremadura, Badajoz, Spain

Abstract Body: Background: *Penicillium expansum* is one of the most important postharvest pathogenic moulds infecting stone fruits including peach (*Prunus persica*) and nectarine (*Prunus persica var. nucipersica*). This mould species can provoke fruit decay and causing postharvest quality, safety, and shelf-life problems of the fruit. In addition, *P. expansum* may produce patulin, a toxic secondary fungal metabolite, when the environmental and nutritional conditions are propitious. Patulin is a mycotoxin that primarily damages vital organs. This mycotoxin can also contaminate stone fruit-derived products such as juices and jams. For this reason, it is necessary the implementation of new and effective strategies to avoid the occurrence of patulin in stone fruits. Mycotoxin contamination is traditionally minimised by the application of synthetic fungicides; however, the use of these compounds presents several drawbacks. Biocontrol is one of the most promising alternatives to synthetic fungicides from a food safety perspective. Objective: The aim of this study was to evaluate the effectiveness of the yeast strain *Pichia kudriavzevii*, PK18 producer of volatile organic compounds to control the growth of the *P. expansum* strain PE-CECT2280 in wounded peaches and nectarines packaged in transparent polyethylene and stored at 25ºC for up to 6 days. Methods and results: The incidence and severity of mould disease, patulin production, and the expression of the *idh* biosynthetic gene, were tested every 2 days. A control batch consisting of fruits inoculated only with PE-CECT2280 was also checked. Results showed that although the presence of the yeast did not affect the incidence in peach, it provoked a decrease of this in nectarine. Regarding the severity caused by PE-CECT2280, it was decreased in both stone fruits tested. About mycotoxin production, patulin synthesised by PE-CECT2280 was diminished almost 100% in the presence of the yeast at day 4 of incubation in peach while this was stimulated in nectarine. The *idh* gene expression correlated with the production of mycotoxin in peach and nectarine, so the *idh* gene expression may be a good indicator of patulin contamination in these fruits. Findings suggest that *P. kudriavzevii* PK18 is an effective approach to control *P. expansum* and patulin production in peach at ambient temperature storage. The *idh* gene expression may be used as an indicator to detect the presence of patulin in stone fruit.

Control Number: 2021-A-8481-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: Yersinia

Keyword 2: pig

Keyword 3: food-borne pathogen

Abstract Title: **Prevalence Of *Yersinia* Species In Tonsils Of Slaughtered Pigs In Latvia**

Author Block: **I. Meistere**, L. Alksne, S. Gradovska, O. Valciņa, M. Terentjeva; Inst. of Food Safety, Animal Helath and Environment BIOR, Riga, Latvia

Abstract Body: *Yersinia enterocolitica* and *Y. pseudotuberculosis* are foodborne pathogens which may cause yersiniosis – foodborne infection characterized with gastrointestinal disorders and secondary sequelae as arthritis and *erythema nodosum*. Pigs are expected to be the principal carriers of pathogens and slaughter of *Y. enterocolitica* or *Y. pseudotuberculosis*-positive pigs may facilitate the spread of the pathogen into food chain. Other *Yersinia* species are considered to be non-pathogenic and widespread in general environment. The aim of the present study was to detect the prevalence of *Yersinia* species in pigs at slaughter. A total of 100 pig tonsils samples were collected from five slaughterhouse located in Latvia from December, 2020 to March, 2021. Samples were investigated in accordance to ISO 10273:2017, negative samples were cold enriched for 2 weeks. From one to three presumptive colonies resembling *Yersinia* species were confirmed with matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). *Y. enterocolitica* pathogenicity was confirmed with qPCR for *ail* and *ystA/YstB* genes.*Yersinia* spp. were isolated from 45 out of 100 samples (45%). Altogether seven *Yersinia* species were isolated of which *Y. enterocolitica* and *Y. pseudotuberculosis* were pathogenic while *Y. kristensenii*, *Y. frederiksenii*, *Y. intermedia* and *Y. massiliensis* were non-pathogenic. The highest prevalence was reported for *Y. enterocolitica* (38%) followed by *Y. intermedia* (4%), *Y. kristensenii* (3%), *Y. pseudotuberculosis* (1%), *Y. frederiksenii* (1%) and *Y. massiliensis* (1%). The prevalence of *Y. enterocolitica* varied from 0 to 100% within the examined herd. In 33 out of 38 *Y. enterocolitica*-positive samples represented by 51 different isolates *ail* gene was confirmed with Ct from 17.31 to 24.05. Findings of the present study show the high prevalence of pathogenic Yersiniae in pig at slaughter and confirm that pigs are important source of human pathogenic *Y. enterocolitica*.

Control Number: 2021-A-8494-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: pork

Keyword 2: ail gene

Keyword 3: foodborne pathogen

Abstract Title: **Prevalence Of *Yersinia Enterocolitica* In Retail Meats In Latvia**

Author Block: **I. Meistere**, T. Kiseljova, S. Gradovska, L. Alksne, O. Valciņa, M. Terentjeva; Inst. of Food Safety, Animal Hlth.and Environment BIOR, Riga, Latvia

Abstract Body: *Yersinia enterocolitica* is an important foodborne pathogen which may cause yersiniosis in humans. Pigs and pork are expected to be significant source of pathogen for consumer. Characterization of pathogenicity of *Y. enterocolitica* is important since non-pathogenic *Y. enterocolitica* may contain virulence genes and were implicated in clinical cases. The aim of the present study was to detect the prevalence and virulence of *Y. enterocolitica* in retail meats in Latvia.A total of 171 pork and 150 beef samples were collected between 2015 to 2021 from retail outlets in Latvia. Samples included retail cuts, offal and minced meats. Samples were cultured according to ISO 10273:2017. Presumptive *Y. enterocolitica* isolates were confirmed with matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). Pathogenicity of *Y. enterocolitica* was confirmed with qPCR by targeting of *ail* and *YstA/YstB* virulence genes.The overall prevalence of *Y. enterocolitica* in meat was 28% (90/321). For beef, the prevalence of Y. enterocolitica was 20% (30/150) while in pork - 40% (69/171). In beef, all *Y. enterocolitica* isolates were *ail* negative. In pork, ail-gene was detected in 5 out 69 Y. enterocolitica-positive samples (7%) and the pathogen was confirmed in pork cuts, minced meat and pig tongue sample. Additionally all Y.enterocolotica isolates were characterized by presence of *YstA/YstB* virulence genes.Despite the low overall prevalence, the evidence of the presence of ail-positive *Y. enterocolitica* in pork at retail confirms the importance of pork in transmission of pathogenic *Y. enterocolitica* to consumers.

Control Number: 2021-A-8507-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: Microbiota

Keyword 2: Endocrine Disruptors

Keyword 3: Culturing

Abstract Title: **Microbiome Culturing As Strategy For The Isolation Of New Generation Probiotic (NGP) Taxa Repairing Effect Of Microbiota Disrupting Chemicals (MDC)**

Author Block: Á. Ruiz-Moreno, **A. López-Moreno**, K. Cerk, A. Torres-Sánchez, J. Pardo, P. Ortiz, M. Aguilera; Univ. of Granada, Granada, Spain

Abstract Body:

Currently, the exposure to endocrine disruptors can lead to microbiome dysbiosis. These dysbiosis can be associated with metabolic diseases, such as type II diabetes, obesity, and other endocrine disorders. Imbalances in the intestinal microbiota can be prevented or intervened with the administration of beneficial microbes and probiotics, helping to regulate the physiological hormonal axis. The search for microbiological differences between obese and the non-obese microbiota taxa groups allows us to identify potential probiotics and even detoxifying microorganisms, which could be used as NGP. However, this is followed by isolation and characterization of potential probiotics and so far, all bacteria in the microbiota cannot be cultured in vitro yet. Microbiome sample culturing through testing multiple media and conditions allows to obtain new microbial resources. This work focuses on directed culturing searching for different microorganism culture techniques to increase the catalogue of bacteria isolated from the human gut microbiota, more specifically, bacteria tolerant to different concentrations of bisphenol A (BPA). For this, different conditions and culture mediums were used for optimizing the uncultured bacterial growth. The samples were spread in the different media and cultivated in anaerobic conditions. At the same time, a specific treatment was carried out to favour the isolation of spore-forming bacteria. For this, the samples were homogenized in 70% ethanol and under aerobic conditions and treated with a bile acids solution for the metabolic activation of the spores. Then, the samples were processed and analysed as described above. The 16s rRNA from all the isolated colonies were analysed. A total of 32 microbiome samples from obese and non-obese children followed the managed culturing approach that allowed us to obtain 105 BPA tolerant and potentially biodegrading microorganism. The main cultivated BPA tolerant species are the following taxa component groups: strictly anaerobic sporulated/non-sporulated: *Clostridium* sp.*,* Clostridia *Blautia* sp.; sporulated anaerobic facultative: *Bacillus* sp.*, Lysinibacillus fusiformis; Lactobacillaceae* species*: Latilactobacillus sakei, Lactobacillus casei, Lactobacillus paracasei; Enterobacteriaceae* species*: Enterococcus faecalis, Enterococcus faecium; Actinomycetales* species*: Microbacterium* sp.*,*

Micrococcus sp*., Kocuria* sp*.* In addition, it had been successfully cultivated a catalogue of previously considered *uncultured* microorganisms*.*

Control Number: 2021-A-8513-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: Sourdough

Keyword 2: Metagenomics

Keyword 3: spontaneous fermentation

Abstract Title: **Dynamics Of Microbial Ecology During *Tritordeum* Sourdough Preparation**

Author Block: **K. Arora**; Free Univ. of Bolzano, Bolzano, Italy

Abstract Body: Microbial ecology during *Tritordeum* (*Triticum durum* x *Hordeum chilense*) sourdough preparation was described by 16S and 26S rDNA genes sequencing. Viable plate counts of presumptive lactic acid bacteria, the ratio between lactic acid bacteria and yeasts, the rate of acidification, biochemical (pH, total titratable acidity, sugars and organic acids) features, the number of operational taxonomic units (OTUs), and diversity indices all together demonstrated the maturity of the sourdough during 10 days of propagation. The new cereal *Tritordeum* flour was mainly contaminated by genera (*Acinetobacter, Pantoea, Pseudomonas, Comamonas, Enterobacter, Erwinia*, and *Sphingomonas*) belonging to the phylum Proteobacteria or Bacteroidetes (genus *Chryseobacterium*). Their relative abundances after 5 days of propagation was almost completely inhibited. Although members of the phylum Firmicutes were present at very low or intermediate relative abundances in the flour, they became dominant soon after 1 day of propagation. Lactic acid bacteria were almost exclusively representative of the Firmicutes by this time. The succession of LAB and yeast species during sourdough propagation was also investigated by RAPD-PCR analysis and genotypic identification using 16S and 26S rDNA sequencing techniques. *Weissella confusa* already dominant in *Tritordeum* flour and stably persisted, though it was later (from day 5 onwards) flanked by facultative heterofermentative LAB (e.g. *Lactiplantibacillus plantarum*). Other subdominant species were detectable throughout propagation. Yeast diversity seemed to be consistent throughout the sourdough propagation with *Saccharomyces cerevisiae* as the dominant population. Notwithstanding variations due to environmental and technology determinants, the results of this study represent a clear example of how the microbial ecology evolves during sourdough preparation. Besides, the autochthonous LAB and yeast species can be used as potential starters for fermented *Tritordeum* based baked products.

Control Number: 2021-A-8528-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: Campylobacter jejuni

Keyword 2: Campylobacter coli

Keyword 3: Broiler

Abstract Title: **Campylobacter Genotypes Present At Austrian Broiler Farm Level Indicate Global Character**

Author Block: **B. Stessl**[1], A. Mustedanagic[1], S. Dobryzynska[1], M. Matt[2], E. Singer[3], M. Wagner[1]; [1]Univ. of Vet. Med. Vienna, Wien, Austria, [2]AGES, Innsbruck, Austria, [3]SIMA Consulting GmbH, Aigen im Ennstal, Austria

Abstract Body: Within the project “Austrian Competence Centre for Feed and Food Quality, Safety and Innovation FFoQSI”, a collaboration with the poultry industry and a private diagnostic laboratory was initialized. This study aimed to analyze the diversity of thermophilic *Campylobacter* genotypes present at broiler farm level and to identify genotypes potentially shared between different flocks. During 2017-2020 chicken caecal content, feces, environmental samples at farm level were investigated chronologically. Representative isolates of *C. jejuni* and *C. coli* were confirmed by a species-specific PCR and characterized by pulsed-field gel electrophoresis (PFGE). *Campylobacter* isolates were submitted to multi-locus sequence typing (MLST) for genotype comparison to previous studies and estimation of *Campylobacter* global spread or local clonality (Schallegger et al. 2016). A total of 382 *Campylobacter* isolates were confirmed by PCR method (n=316 *C. jejuni*; n=66 *C. coli*). The *Campylobacter* spp. isolates were assigned to abattoirs (A-E), associated broiler farms (A1-E4; n=22) and districts. The following *C. jejuni* and *C. coli* genotypes were detectable in caecal samples at several sampling times: ST-267, ST-354, ST-400, ST-2066, ST-446 and ST-828. *C. jejuni* and *C. coli* genotypes isolated in this study are present in the PubMLST database in varying numbers and give an indication of their global occurrence: *C. jejuni* ST-267 and ST-354 are adapted to multiple habitats (human-animal-environment interface), while *C. coli* ST-828 clearly indicates the pig niche. *C. jejuni* ST-400 and ST-446 are associated to both human and poultry. Two new *C. jejuni* sequence types have been detected and seem to occur only locally. Antimicrobial resistance genes were detected in the isolates to ciprofloxacin, ampicillin and tetracycline. In addition, all *C. coli* isolates belonging to the ST-828 complex had a multidrug efflux pump, which enables the ejection of various biocidal substances, antibiotics and bile salts from the cell. Since a co-selection of antibiotics and biocides is reported for particularly environmentally adapted genotypes, the isolates should be tested subsequently to gain insight into the potential adaptability of individual genotypes. The study will give an insight into the genetic diversity of thermophilic *Campylobacter*

species in different Austria districts in order to help the poultry industry to track down the pathways of contamination, so that control measures can be implemented.

Control Number: 2021-A-8529-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: Bacillus subtilis

Keyword 2: Campylobacter jejuni

Keyword 3: antagonism

Abstract Title: **Interaction Of The Potential Probiotic *Bacillus Subtilis* Ps-216 And The Intestinal Pathogen *Campylobacter Jejuni***

Author Block: **K. Simunovic**, A. Erega, P. Stefanic, A. Klancnik, S. Smole Mozina, I. Mandic Mulec; Biotechnical Faculty, Univ. of Ljubljana, Ljubljana, Slovenia

Abstract Body: *Campylobacter jejuni* is one of the most common causes of bacterial gastroenteritis worldwide, with an increasing prevalence since 2005, and represents a major public health and economic problem. With its increasing antimicrobial resistance, there is a growing need for alternative options for the management of this pathogen. Here, we test the hypothesis that *Bacillus subtilis* has a potential to control the growth of *C. jejuni*. Under microaerobic conditions (either at 37^{o}C or 42^{o}C), where *C. jejuni* growth is favored, *B. subtilis* strains indeed showed a strong inhibitory effect on *C. jejuni*. We tested fifteen natural isolates of *B. subtilis* and they all inhibited the growth of this pathogen. We also tested fifteen *C. jejuni* strains (human, slaughterhouse, and water) with *B. subtilis* in co-culture. *C. jejuni* strains from human feces were the most susceptible with a reduction of 4.2 log CFU/mL after 72 h, compared with the control. In contrast, *C. jejuni* water strains were least susceptible (ΔlogCFU/mL=2.8). Next, we evaluated the growth of *C. jejuni* in co-cultures with different ratios (10:1, 100:1, 10,000:1, and 1:10) of *C. jejuni* and *B. subtilis*. *B. subtilis* showed a strong inhibitory effect at all ratios except for the co-culture with an initial abundance of *C. jejuni* 10,000-fold higher than *B. subtilis* (average inhibition only ΔlogCFU/mL=1.5). However, under aerobic conditions toxic to *C. jejuni*, *B. subtilis* was less efficient, and at 20^{o}C, *B. subtilis* facilitated *C. jejuni* persistence even after 48 h. To further test if the growth inhibition was due to *B. subtilis* competition or antagonism, we exposed *C. jejuni* to *B. subtilis* spent medium. *C. jejuni* growth in spent medium was reduced by only 1-2 logCFU/mL after 24 h of incubation, compared with the control. The low rate of inhibition in spent medium suggests that the observed inhibitory effects of *B. subtilis* are not solely due to the antibiotic production, and that *B. subtilis* may require the presence of active *C. jejuni* in the co-culture to reach maximal inhibitory potential. We conclude that in environments favoring the growth of *C. jejuni, B. subtilis* is a strong competitor of this pathogen, efficiently controlling its numbers even when the initial abundance of *C. jejuni* greatly exceeds that of *B. subtilis*.

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Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: Acetobacter

Keyword 2: antibiotic resistance

Keyword 3:

Abstract Title: **Do Vinegar Producing Bacteria Pose Potential Health Risk?**

Author Block: E. Cepec, **J. Trcek**; Univ. of Maribor, Faculty of Natural Sci. and Mathematics, Maribor, Slovenia

Abstract Body: The preference of consumers towards eating healthy food has changed trends in vinegar producing companies. These are oriented towards production of organic vinegar from fruits that go through very restricted treatment in orchards, but also to vinegar that is not filtered at the end of the technological process and thus contains bacteria. This trend at the food market raises questions for the manufacturer and the consumer, such as: are acetic acid bacteria resistant to antibiotics, especially those that are clinically relevant, and whether these properties can be transmitted to pathogenic bacteria. Therefore, in this study, resistance of acetic acid bacteria to the following antibiotics was studied: ampicillin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, and trimethoprim. In this pilot study, 20 reference strains of acetic acid bacteria and 14 isolates from apple cider vinegar were analysed. The novel vinegar isolates were identified to the level of species using an established method based on 16S-23S ITS sequence analysis. All species were from genera *Komagataeibacter* and *Acetobacter*. Since the method for testing antibiotic resistance of acetic acid bacteria is not standardized, we used growth media with different carbon sources and pH-value to assess resistance by disc diffusion method. Using medium RAE we identified 5 strains resistant to all tested antibiotics, on medium GY 2 strains, and on medium MA none. Although a percentage of antibiotic resistant strains to each tested antibiotic differed among media used for the analysis, the resistance to trimethoprim, erythromycin and ciprofloxacin was always over 58%. Besides, over 38% of strains were resistant to chloramphenicol. Next, we looked for genes that potentially encode proteins for antibiotic resistance (as defined in CARD database) in genomic sequences of the reference strains. The most common function was a pump to transport antibiotics from the cell. These genes may be in contact with other bacteria in gastrointestinal tract transferred to human pathogens. Since this may cause a risk to human health, a potential of this scenario has to be studied into more details.

Control Number: 2021-A-8584-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: Prebiotic

Keyword 2: Cross-feeding

Keyword 3: Inulin

Abstract Title: **Analysis Of Cross-feeding Interactions During Prebiotic Utilization Reveals Inulin-degrading *Lactobacillus Casei* M38 Supporting The Growth Of *Bacteroides Ovatus*.**

Author Block: **M. A. Vega**, D. Garrido; Pontificia Univ. Católica de Chile, Santiago, Chile

Abstract Body:

Prebiotics are popular food ingredients aimed to increase the viability and permanence of beneficial microorganisms in the gut. This is accomplished by selectively promoting their growth and their metabolism, providing a health benefit for the consumer. Inulin is a prebiotic polysaccharide of the fructan type (polymer of fructose chains with a degree of polymerization up to 30), linked by β-2,1 bonds and found in vegetables, is usually fermented by strains of *Lactobacillus* and *Bifidobacterium*. In this study, we found an efficient degrader of inulin using a panel of 16 strains of bacteria, including isolated from fecal samples from Chilean.

Lactobacillus casei M38 showed the most vigorous growth using inulin. Analysis performed by thin-layer chromatography (TLC) qualitatively shows full consumption of inulin after 48 hours and a reduction in the polysaccharide size after 24 hours. This ability to degrade this prebiotic was supported by genomic information on transporters (ABC-type transporters and phosphotransferase systems (PTS)) and GH32 glycolytic enzymes found in its annotated genome. These enzymes act specifically on the β-2,1 bonds of inulin, producing fructose and fructooligosaccharides. The specificity of M38 for inulin could not be observed when it was grown in xylan and resistant starch, because it could not grow on these substrates.

Cross-feeding analysis in unidirectional assays using the supernatant of the M38 strain grown in inulin, showed that the release of less complex oligosaccharides helped the growth of *Bacteroides ovatus* HM222. This *Bacteroides* strain was not able to grow using inulin as the sole carbon source. Studies have shown metabolic interactions in the presence of fructooligosaccharides or inulin, but most of the primary degraders are bifidobacteria. Finally, bacterial growth studies in the utilization of xylan and resistant starch were mostly restricted to *Bacteroides* strains such as *B. ovatus* HM222.

As conclusion, inulin was the most fermentable fiber among the isolates included in this study. *L. casei* M38 could be a potential probiotic acting as a feeder of essential bacteria in a healthy microbiome that would positively affect the host's health.

Control Number:	2021-A-8625-MICROBE
Session Type:	**iPoster**
Session Number:	**FEMSP108**
Session Title:	**FEMS: Health and Food Microbiology**
Topic 1:	FEMS - Health and food microbiology
Keyword 1:	Kombucha
Keyword 2:	beverages
Keyword 3:	Low-alcohol
Abstract Title:	**Exploring Applicability Of A Kombucha Scoby For The Low-alcohol Beverage Production Using Alternative Substrates**
Author Block:	**F. Magalhães**[1], E. Sohlberg[1], P. Jouhten[1], B. Gibson[2], R. Juvonen[1]; [1]VTT Technical Res. Cwntre of Finland Ltd, Espoo, Finland, [2]Technical Univ. of Berlin, Berlin, Germany
Abstract Body:	In recent years, there has been an increased interest in naturally fermented foods fueled by a growing appreciation of the role of fermentation in maintaining health and wellness. Kombucha in particular, has captured the attention of consumers due to being low in alcohol, and having pleasant taste and aroma and potential health benefits. Kombucha is produced by fermenting tea with a Symbiotic Culture Of Bacteria and Yeast (SCOBY). However, a SCOBY could potentially ferment also other plant substrates into appreciated products, or act as a source of good fermentative microbes. To test this hypothesis, a kombucha SCOBY was used to inoculate a low-gravity malt extract and bilberry juice. The cultures were then repeatedly backslopped to allow the microbial community to adjust to the new environment and changes to the microbial community and chemical composition were monitored. Seven different microbial species were identified in the stabilised SCOBY community when maintained in tea, including yeast, lactic acid bacteria (LAB) and acetic acid bacteria (AAB). Analysis of the microbial community showed that the bacterial proportion was predominantly composed of cellulose-producing *Komagataeibacter* species with low relative abundance of lactobacilli. Yeasts included members of *Dekkera*, *Starmerella* and *Zygosaccharomyces* genus in approximately equal proportions. Compared to the species composition in tea, the abundances of AAB relative to LAB remained unchanged in malt extract and *D. anomala* was enriched in both substrates. Fermented bilberry juice had a low alcohol content (<0.7% ABV) and pH (2.9) similar to a traditional kombucha, whereas fermented malt extract contained only traces of ethanol and had higher pH value (3.5). The SCOBY community was efficient at reducing aldehydes from the malt extract while producing pleasant fruity esters. However, there were also noticeable concentrations of disagreeable compounds that imparted a smoky/earthy aroma to the final product. In the bilberry juice the high quantities of terpenes and ketones were largely reduced by the fermentations. On the other hand, the kombucha community led to an increased production of ethyl esters

(fruity and floral aromas). This study shows that there is potential to use the kombucha microbial community to ferment alternative substrates. Further understanding of the roles of each species in the community will facilitate the design of rationalized starter cultures and novel fermentation systems for consistent production of naturally fermented foods and beverages.

Control Number: 2021-A-8694-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: high pressure processing

Keyword 2: Enterococcus sp.

Keyword 3: virulence, antibiotic resistance

Abstract Title: **High Pressure Processing Effect On Virulence Factors And Tetracycline-resistance Among *Enterococcus* Sp. Isolated From Food**

Author Block: **U. Zarzecka**, A. Zadernowska, W. Chajęcka-Wierzchowska; Univ. of Warmia and Mazury in Olsztyn, Olsztyn, Poland

Abstract Body: Background: High pressure processing (HPP) treatment is a promising nonthermal food preservation method that inactivates foodborne pathogens and spoilage microorganisms. Food preservation by nonthermal methods has been used for enzyme inactivation to keep the natural characteristics of foods. HPP enables to reduce the count of spoilage-causing microflora while keeping the sensory and nutritional quality of the treated product. It can be used in the food industry alone or simultaneously with some other conventional techniques. So far, the vast majority of studies on the effects of HPP on microorganisms have focused on pathogens, while opportunistic or non-pathogenic microorganisms have been ignored. Current studies on the response of *Enterococcus* to stress caused by HPP are focused on the changes in the metabolism, growth and cells. It is also suggested that HPP can influence bacterial gene expression. The influence of HPP on the expression of genes encoding virulence factors and antibiotic resistance is currently very poorly understood. Objectives: The aim of the study was to determine the effect of sublethal high pressure value on the expression of selected virulence factors (gelatinase, biofilm formation, slime production and hemolytic activity) and virulence-related genes (*gel*E, *asa*1, *esp* and *cyl*L) as well as tetracycline resistance and expression of tetracycline resistance *tet*M gene among *Enterococcus* sp. isolates from food of animal origin. Methods: First the analysis was carried out phenotypically and then genotypically, using the Real-Time PCR technique. Pressures value selected for the analysis was 500 MPa. Results: Obtained results indicate that the sublethal HPP causes changes the expression of virulence-related genes as well as tetracycline-resistance genes in the tested strains. These results might suggest the need for further research to understand the mechanisms linking the stress response with the expression of virulence factors and antibiotic resistance among *Enterococcus* sp.

Control Number: 2021-A-8796-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: Campylobacter

Keyword 2: backyard poultry

Keyword 3: antibiotic resistance

Abstract Title: **Prevalence And Characterization Of *Campylobacter* Isolated From Broilers Reared Under Different Production Systems**

Author Block: **P. Teixeira**, N. Ferreira; Univ.e Católica Portuguesa, Porto, Portugal

Abstract Body: Background: *Campylobacter* spp. is the major cause of bacterial gastroenteritis in developed countries. Broiler chickens are considered the biggest reservoir of *Campylobacter*. Objectives:This study aimed to characterize *Campylobacter* isolates from commercial broilers, free range and backyard chickens and estimate the prevalence of *Campylobacter* in the different production systems. Methods: Detection and quantification of *Campylobacter* spp. in chicken skin was performed following the ISO 10272-2:2017. *Campylobacter* species were determined by multiplex PCR. Genotyping of isolates was performed by PFGE . Antimicrobial susceptibility tests were performed by the disk diffusion method using Mueller-Hinton agar supplemented with 5% defibrinated horse blood and 20 mg/L of β-NAD. Susceptibility to ampicillin (10 ug), amoxicillin/clavulanic acid (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), nalidixic acid (30 µg), tetracycline (30 µg), erythromycin (15 µg), imipenem (10 ug) and meropenem (10 ug) were studied. Results: An overall prevalence of 90.3% of *Campylobacter* was observed. All samples from commercial broiler and free-range chickens were positive while only 72.7% of backyard chicken samples were positive for *Campylobacter*. Levels of contamination were on average 3.0E+03 CFU/g, 4.4E+02 CFU/g and 4.2E+04 CFU/g in commercial broiler, free range and backyard chicken, respectively. *Campylobacter jejuni* and *Campylobacter coli* were the only species detected. From the isolates studied (n = 87), 16 genetic profiles were determined by PFGE. A multiplicity of antibiotic resistant phenotypes was detected, mostly to nalidixic acid (99%), ciprofloxacin (99%), tetracycline (94%), ampicillin (90%) and erythromycin (22%), corresponding to 9 resistance profiles. 86% of the isolates were resistant to two or more antibiotics of different families.Prevalence of *Campylobacter* was lower for the backyard poultry samples but this type of production presented the highest level of contamination. Commercial broiler and backyard chicken presented levels of contamination above the limit set by the European legislation for process hygiene criteria (1.0E+03 CFU/g). Macrolides and fluoroquinolones are the first line of antibiotics to treat severe *Campylobacter* infections. The high rate of antibiotic resistance, mainly ciprofloxacin, and the high amount of multi-resistant strains detected is a serious concern of public health that requires interventions at multidisciplinary levels.

Control Number: 2021-A-8809-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP108**

Session Title: **FEMS: Health and Food Microbiology**

Topic 1: FEMS - Health and food microbiology

Keyword 1: Foodborne pathogens

Keyword 2: Cross-contamination

Keyword 3: Consumer mishandling

Abstract Title: **From Chicken To Salad: Table Salt As An Unexpected Vehicle Of *Campylobacter Spp*., *Salmonella Spp*. *An*D *Listeria Monocytogenes* Cross-contamination**

Author Block: **P. Teixeira**, Â. Alves, N. Ferreira, V. Ferreira; Univ.e Católica Portuguesa, Porto, Portugal

Abstract Body: **Background:**The incidence of illness transmitted through consumption of contaminated food is a major public health problem worldwide. Consumer mishandling of foods at home plays a significant role in the occurrence of foodborne diseases, specifically by episodes of cross-contamination between contaminated raw food and surfaces or ready-to-eat (RTE) food. **Objectives:**In this study, the transfer of three foodborne pathogens, i.e., *Campylobacter* spp., *Salmonella* spp. and *L. monocytogenes*, from artificially contaminated chicken meat to RTE salads via table salt during simulated domestic handling practices was investigated. In addition, we assessed the survival of these foodborne pathogens on artificially inoculated table salt. **Methods:**Chicken meat samples were inoculated with a mixed cocktail of *Campylobacter* spp. (n=8), *Salmonella* spp. (n=5) or *L. monocytogenes* (n=7) to achieve initial contamination levels ranging from 10^1 to 10^6 CFU/g. Cross-contamination was tested via unwashed hands that touched table salt after handling the artificially contaminated chicken, followed by lettuce seasoning. For the survival experiment, table salt was inoculated with a cocktail of each pathogen (ca. 10^6 CFU/g) and stored at room temperature. Bacterial numbers were determined following the International Standards (ISO). Three independent transfer experiments were carried out. **Results:***Salmonella* spp. and *L. monocytogenes* were detected in cross-contaminated lettuce samples at all contamination levels tested, whereas *Campylobacter* was detected when the chicken was contaminated with levels above 10^2 CFU/g. *Salmonella* spp. and *L. monocytogenes* were able to survive in table salt for at least 4 months; *Campylobacter* survived up to 4 hours. These findings reveal a novel indirect cross-contamination route of foodborne pathogens in domestic settings, and a putative contamination source to RTE foods that are seasoned with salt.

FEMSP112 FEMS: Infection Biology and Pathogens

Control Number:	2021-A-7757-MICROBE
Session Type:	**iPoster**
Session Number:	**FEMSP112**
Session Title:	**FEMS: Infection Biology and Pathogens**
Topic 1:	FEMS - Infection biology and pathogens
Keyword 1:	Smoking
Keyword 2:	Tobacco
Keyword 3:	Microbial Flora
Abstract Title:	**EFFECT OF TOBACCO SMOKING ON ORAL MICROBIAL FLORA AND THE RELATIONSHIP WITH ORAL HEALTH CARE IN KHARTOUM STATE 2020**
Author Block:	**N. B. Ahmed**; Omdurman Islamic Univ. Faculty of Med. Lab. Sci., Khartoum, Sudan
Abstract Body:	Cigarette smoking is a public health problem. Reduces the equal population of normal florain the oral cavity resulting in an increase in pathogenic microbes. It causes oral cancer,periodontitis, tooth discolouration, bad breath, tooth decay, and other health effects. Thestudy was designed to determine the changes that tobacco smoking causes on the microbialcharacteristics and oral health conditions of cigarette smokers.This study was conducted in the Microbiology Laboratory in the LaboratoryAdministrationbetween December and April 2020.In our study70 subjects from 40 tobacco smokers and 30 non-smokers were enrolled in thestudy, and oral swabs were collected from the subject's oral cavity using sterile swabs undersepsis A methods, and samples were subjected to microscopy and culture. Organisms wereidentified using standard microbiological techniques, and higher microbial rates [30] 75%were recovered from the oral cavity in smokers compared to non-smokers, [4] 13%. Theeffect of tobacco smoke was statistically significant on smokers of oral flora, andStaphylococcus aureus was the most prevalent isolated bacterium [33%] followed byMicrococcus spp [20%], Streptococcus pyogenes [12.5%], and Enterococcus [12.5%], whileStreptococcus Candida [50%] and Candida [50%] are the most common microorganismsamong non-smokers.Smokers had a varied microbial colonization compared to non-smokers. Smoking may havealtered the acquisition of bacteria and colonization of the oral mucosa in favor of thedominant pathogens, so the campaign against smoking should be intensified as this mayhelp improve oral health conditions in smokers.Keywords :Tobacco smoking, microbial flora, oral health

Control Number:	2021-A-7765-MICROBE
Session Type:	**iPoster**
Session Number:	**FEMSP112**
Session Title:	**FEMS: Infection Biology and Pathogens**
Topic 1:	FEMS - Infection biology and pathogens
Keyword 1:	Avian-Pathogenic Escherichia coli; APEC
Keyword 2:	virulence-associated genes; VAG
Keyword 3:	Broilers
Abstract Title:	**High Prevalence Of Virulence Associated Genes Among Avian *Escherichia Coli* Isolated From Broilers**
Author Block:	**A. Goudarztalejerdi**, A. Mohammadzadeh, S. Varmaziar Najafi, F. Nargesi; Dept. of Pathobiology, Faculty of Vet. Sci., Bu-Ali Sina Univ., Hamedan, Iran, Islamic Republic of
Abstract Body:	**ackground:** Avian colibacillosis is responsible for mortality, and economic loss in the poultry industry, worldwide. Avian Pathogenic *Escherichia coli* (APEC) is the main causative agent of colibacillosis. The pathogenicity of APEC is associated with virulence factors encoded by virulence-associated genes (VAG). APEC virulence factors are involved in colonization, adhesion, iron acquisition, invasion, toxin production, and survival of *E. coli* against host defenses.**Objectives:** Considering the importance of poultry industry in Iran, and the important role of *E. coli* in avian colibacillosis in the broiler farms, the objective of this study was to investigates the presence of new types of VAGs in APEC and Avian Faecal *Escherichia coli* (AFEC) isolates from broilers with suspected colibacillosis and healthy broilers, respectively.**Methods**: In the present study, 100 APEC isolates from colibacillosis-suspected broilers and 100 AFEC isolates from healthy broilers in Iran were examined for the presence of 10 virulence-associated genes: *fimC* (adhesion), *iutA*, *chuA*, *sitA* (iron acquisition system), *iss* (increased serum survival), *cvaA/B*, *hylA*, *stx1*, *stx2* (toxins), and *yjaA* (stress response protein). The multiplex PCR approaches were used to assay VAGs presence in APEC and AFEC isolates.**Results:** Among APEC strains, the predominant VAG was *iutA* (97%), followed by *fimC* (87%), *iss* (84%), *sitA* (82%), *chuA* (79%), *cvaA/B* (54%), *hylA* (42%), *yjaA* (41%), *stx2* (6%) and, *stx1* (4%), whereas in AFEC strains, the prevalent VAG was *fimC* (95%), followed by *iss* (93%), *sitA*(87%), *iutA* (79%), *chuA* (77%)**,** *yjaA* (52%), *cvaA/B* (27%), *hylA* (12%), *stx2* (2%) and, *stx1* (0%). The present study results indicates that prevalence of *iutA*, *hylA*, and *cvaA/B* VAGs in APEC strains significantly higher than AFEC strains.Therfore these VAGs could be use as

candidates for predicting the pathogenicity of avian *E. coli* strains and these genes could be used for future vaccine design against avian colibacillosis.

Control Number: 2021-A-7872-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP112**

Session Title: **FEMS: Infection Biology and Pathogens**

Topic 1: FEMS - Infection biology and pathogens

Keyword 1: American foulbrood

Keyword 2: detection

Keyword 3: hive debris

Abstract Title: **American Foulbrood In Honeybees: Qpcr-based Detection And Quantification Of *Paenibacillus Larvae*Spores In Hive Debris**

Author Block: **U. Zajc**, L. Žvokelj, B. Papić, M. Pislak Ocepek, M. Ocepek, D. Kušar; UL Vet. Faculty, Natl. Vet. Inst., Ljubljana, Slovenia

Abstract Body: **Background: American foulbrood (AFB) is a highly contagious and devastating bacterial disease of honeybees, caused by a Gram-positive bacterium *Paenibacillus larvae*. Spores of *P. larvae* infect the honeybee larvae through contaminated food. Reliable detection and quantification of *P. larvae* spores in hive debris would help to implement improved control measures to reduce the spread of AFB. Cultivation of *P. larvae* from hive debris is challenging and unreliable due to the use of toxic solvents and inconsistent spore germination. Several conventional (PCR) and SYBR-based real-time PCR (qPCR) assays have been developed for the detection and/or quantification of *P. larvae*, but a more specific, sensitive and reproducible TaqMan-based qPCR assay was not available. Objectives: To this aim, we constructed a TaqMan qPCR assay targeting a one-copy metalloproteinase gene of *P. larvae* for reliable detection and quantification of *P. larvae* spores in hive debris. Methods: After assay construction, we extracted the total DNA from AFB-positive hive debris in three biological replicates using a commercial extraction kit and prepared a 5-fold DNA dilution series. Each sample was quantified in three technical qPCR replicates. To enable absolute quantification of spores in hive debris, qPCR was calibrated using digital PCR (dPCR), overcoming the limitations of plate counting. Results: After validation and calibration of the constructed *P. larvae*-specific qPCR assay, the observed limit of quantification (LOQ) was 707 and the limit of detection (LOD) 188 spores of *P. larvae* per millilitre of hive debris. The developed TaqMan-based qPCR assay used here for the quantification of *P. larvae* spores in hive debris represents an important contribution to the early diagnosis of AFB.**

Control Number: 2021-A-7875-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP112**

Session Title: **FEMS: Infection Biology and Pathogens**

Topic 1: FEMS - Infection biology and pathogens

Keyword 1: Streptococcus pyogenes

Keyword 2: Membrane

Keyword 3: Virulence

Abstract Title: **FabT, A Transcriptional Regulator Involved In *Streptococcus Pyogenes* Virulence**

Author Block: **C. Lambert**[1], K. Gloux[2], A. Gruss[3], A. Solgadi[4], F. Guillonneau[5], C. Broussard[6], B. Saint-Pierre[7], C. Poyart[8], A. Fouet[9]; [1]Université de Paris, Paris, France, [2]INRAE, Jouy-en-Josas, France, [3]INRAE, Jouy en Josas, France, [4]SAMM facility, Université Paris-Saclay, Chatenay Malabry, France, [5]3P5-proteom'IC facility, Université de Paris, Paris, France, [6]3P5-proteom'IC facility, CNRS, Paris, France, [7]GENOM'IC facility, APHP, Paris, France, [8]APHP, Paris, France, [9]CNRS, Paris, France

Abstract Body: The strictly human pathogen *Streptococcus pyogenes*, also known as Group A Streptococcus (GAS), is a Gram-positive bacterium responsible for non-invasive and invasive infections and post streptococcal sequelae, leading altogether to 517,000 deaths yearly. A recent study has shown that *fabT* GAS mutant strains display an attenuated virulence, particularly in a non-human primate of necrotizing fasciitis (Eraso *et al.* 2016). FabT is the transcriptional repressor of the Fatty Acid Synthesis pathway (FASII) in numerous streptococci. Fatty acids are main constituents of membrane phospholipids and are essential for maintaining membrane homeostasis. Streptococci incorporate exogenous fatty acids in their membranes (Brinster *et al.* 2009), and long chain fatty acids coupled to an acyl carrier protein are FabT corepressors. The causes of the virulence defect of the GAS *fabT* mutant strains have not been investigated in depth. We hypothesized that overproduction of membrane fatty acids in the *fabT* mutants also has consequences on cell wall composition. These changes could impair adaptation and explain attenuated virulence of the *fabT* variants. We characterized the role of FabT as a transcriptional repressor by establishing the transcriptome profiles of wild-type and *fabT* mutant strains after growth in the presence and absence of exogenous fatty acids. In parallel, changes in GAS membrane and cell-wall compositions (fatty acids, lipids, proteins) were analyzed by appropriate "omic" approaches. Our data suggest that changes in surface composition due to *fabT* mutations also impact the capacity of GAS to adapt to its environment. This is currently under investigation. Our data establish a first link between *fabT* mutations and the virulence defect of these strains.

Control Number: 2021-A-7939-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP112**

Session Title: **FEMS: Infection Biology and Pathogens**

Topic 1: FEMS - Infection biology and pathogens

Keyword 1: Staphylococcus aureus

Keyword 2: virulence factors

Keyword 3: community-acquired infections

Abstract Title: **Evaluation Of Virulence Potential Of Methicillin-sensitive And Methicillin-resistant *Staphylococcus Aureus* Isolates From A German Refugee Cohort**

Author Block: **I. Creutz**[1], T. Busche[1], F. Layer[2], H. Bednarz[1], J. Kalinowski[1], K. Niehaus[1]; [1]Bielefeld Univ., Bielefeld, Germany, [2]Robert Koch Inst., Wernigerode, Germany

Abstract Body: Background: Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) seem to be highly transmissible, often infect otherwise healthy humans and frequently occur in hospital outbreaks. Objectives: Healthy refugees, living in accommodations in Germany were screened for nasal carriage of *S. aureus*. The isolates were investigated regarding resistance and virulence.Methods: *S. aureus* was cultivated from nasal swabs, identified, and *spa*-typed. The isolate´s resistance and virulence were analyzed phenotypically and by whole genome data analysis.Results: 5.6% (9/161) of the refugees are carriers of *S. aureus*. 2.5% (4/161) are MRSA carriers. Among the refugees, *spa*-types t021, t084, t304, t991 and t4983 were detected, as well as the new *spa*-types t18794 and t18795. t304 and t991 are assumed to be local *spa*-types from the middle east. The isolates are less resistant and marginal biofilm formers. Each isolate has a remarkable set of virulence genes, although genes, encoding for proteins strongly associated with invasive *S. aureus* infections, like Panton-Valentine leukocidin were not detected.Conclusion: The detection of strains from the middle east, supports the assumption that strains co-travel with the refugees and persist despite a transition of the host's living conditions. Whole genome data analysis does not permit to finally evaluate a germ's virulence. Nevertheless, an impression of the virulence potential of the strains, regarding skills in colonization, resistance, immune evasion, and host cell damaging can be pictured.

Control Number: 2021-A-7950-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP112**

Session Title: **FEMS: Infection Biology and Pathogens**

Topic 1: FEMS - Infection biology and pathogens

Keyword 1: Leishmania donovani

Keyword 2: Superenhancer

Keyword 3: miR146a

Abstract Title: **Super Enhancer Drive Mir146a-5p Transcription And Twitches M2 Polarization During *Leishmania Donovani* Infection**

Author Block: **S. Das**; CSIR-Indian Inst. of Chemical Biology, KOLKATA, India

Abstract Body: The outcome of *Leishmania donovani* infection depends upon the dynamic interchanges between M1 and M2 macrophages. Information of the involvement of microRNAs (miRNAs) and epigenetic modifiers in regulating macrophage plasticity during *L. donovani* infection is still elusive. Differential expression analysis of polarization-regulating miRNAs, revealed significant enrichment of miR146a-5p during *L. donovani* infection. A sustained enrichment of miR146a-5p was observed in both infected bone marrow derived macrophages (BMDMs) and BALB/c mice organs. We found involvement of miR146a-5p in phagocytosis and survivability of parasites. Moreover, miR146a-5pgot enriched in interleukin 4- stimulated BMDMs, indicating its possible involvement in M2 polarization. Upon transfecting BMDMs with miRVANA anti-146a oligos, M2 markers (CCR7, YM-1, FIZZ-1, arginase-1, IL10 and IL4) and transcription factors (p-STAT6 and c/EBPβ) got depleted with concomitant augmentation of M1-polarizing transcription factors (p-STAT1, AP1 and IRF1), miR146a target genes (TRAF6 and IRAK1), M1 cytokines (IL12 and TNFα), iNOS, nitric oxide, and nuclear translocation of phospho p-65 subunit. Neutralization of intracellular mature miR146a-5p pool in infected BALB/c mice lower organ parasite burden and expressions of M2 markers and IL10 with enrichment of M1 markers like iNOS and IL12. Additionally, we explored the novel role of super enhancer (SE), a cis-acting regulatory component, to enrich miR146a-5p expression during infection. Enhanced expression and nuclear retention of SE components like BET bromodomain 4 (BRD4) and p300 were found in infected BMDMs. Upon silencing BRD4, expressions of miR146a-5p and M2 markers were down regulated and TRAF6, IRAK1 and *i*NOS levels increased. STRING V.11 based predication and immune precipitation confirmed the strong interaction amongst BRD4, p300 and RNA pol II (RpbI). Chromatin immune precipitation studies suggested the recruitment of BRD4 at the enhancer loci of miR146a-5p gene during infection. Altogether, our findings revealed a novel role of BRD4/p300-depdendent super-enhancer in regulating miR146a expression during *L. donovani* infection which in turn mediates M2 polarization and immunesuppression.

Control Number: 2021-A-7967-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP112**

Session Title: **FEMS: Infection Biology and Pathogens**

Topic 1: FEMS - Infection biology and pathogens

Keyword 1: Plasma technology

Keyword 2: pathogenic prokaryote

Keyword 3: fungal dermatophytes

Abstract Title: **Plasma-based Water Treatment Ability Against Pathogen Microorganism**

Author Block: **M. Rasouli**, M. Ghoranneviss; Tehran Univ. of Med. Sci., Tehran, Iran, Islamic Republic of

Abstract Body: *Pseudomonas aeruginosa* and *Staphylococcus aureus* are opportunistic human pathogen with resistance to various antimicrobial agents, leading to high mortality and morbidity worldwide. *T. mentagrophytes*, *M.canis*, and *C.albicans* as the most common cosmopolitan fungus dermatophyte, causing ringworm and onychomycosis. Arthrospores of dermatophytes are highly resistant and were able to survive in the environment for several years, providing a long-term source of infection. The ongoing COVID-19 pandemic emphasizes the importance of fighting microorganisms. Therefore, a new strategy must be designed to combat microorganisms. To this end, we evaluate the potential efficacy of arc discharge and pulsed spark discharge on inactivation of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Microsporum canis*, *Trichophyton mentagrophytes*, and *Candida albicans*. Our results show that the electrical discharge plasma systems are effective in the inactivation of pathogenic microorganisms. The inactivation of the considered strains was greatly affected by the type of microorganisms. Higher viability losses of the pathogenic strains were observed in bacterial strains than fungal strains. Moreover, in the case of fungal strains, the population of *C.albicans* was decreased the most, followed by *Trichophyton mentagrophyte* and the population of *Microsporum canis* was decreased the least. Besides, the arc discharge system was compared with the pulsed spark discharge system. It can be obtained from the results that the pulsed spark discharge treatment successfully enhanced the reduction of the pathogenic cells more than arc discharge treatment. More efficiency of pulsed spark discharge is due to the generation of discharge streamers on the water surface. The SEM analyses showed that electrical discharge plasma had serious damage to pathogenic eukaryotes and prokaryotes microorganisms. Also, changes in pH values and temperature values were measured. pulsed spark discharge treated Samples have more changes in pH value while arc discharge treated samples have more temperature changes.

Control Number: 2021-A-8165-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP112**

Session Title: **FEMS: Infection Biology and Pathogens**

Topic 1: FEMS - Infection biology and pathogens

Keyword 1: Mumps

Keyword 2: Vaccine potential

Keyword 3: Viral population

Abstract Title: **Altering Diversity Of Mumps Virus Populations**

Author Block: **M. Jurkovic**, J. Ivančić Jelečki, A. Slović, D. Forčić, T. Košutić Gulija, R. Jug, M. Jagušić; Univ. of Zagreb, Ctr. for research and knowledge transfer in biotechnology, Zagreb, Croatia

Abstract Body: **Background**Mumps virus belongs to RNA family of viruses. It causes respiratory infection that is usually characterized with parotid gland swelling. Encephalitis or meningitis occurs in 1 - 10% of mumps cases due to highly neurotropic nature of this virus. Vaccines currently in use pose two distinct issues - some of them are not sufficiently attenuated while others are overly weakened or do not protect against currently circulating strains. Rational design of vaccines presents ways to develop them in more efficient manner. Altering viral population diversity, a main reason of severe virulence of RNA viruses, has been described as a rational approach to design attenuated RNA viruses. **Objectives**The aim of this study was to isolate a variant containing polymerase of changed fidelity (lower or higher) from mumps virus population by passaging virus in vitro in the presence of mutagen. This was expected to result in increased or decreased population diversity and potentially attenuated phenotype. **Methods** A wild-type mumps virus was passaged over the course of 21 passages in A549 cells in the presence or absence of ribavirin. Concentration of ribavirin that does not effect cell viability or proliferation was chosen based on MTT assay. RNA was isolated from cell culture supernatants of control and ribavirin-treated samples from 6 different passages as well as from parental virus. It was used for amplification and preparation of library for next-generation sequencing (NGS) which was performed on Illumina Miniseq platform. **Results**Analysis of the sequencing data showed accumulation of ribavirin specific mutations in treated viruses. Diversity in ribavirin treated viral populations was approximately 2 - 4 fold higher than in control or parental virus depending on passage. Despite this high rate of mutations, virus was still able to replicate to high levels in the presence of ribavirin. We hypothesize that obtained viral population is resistant to ribavirin treatment which points to very high adaptive capability of mumps virus population. Our next step is to determine if there is a specific viral variant present in this population that is resistant to ribavirin and what are biological characteristics of obtained viral population. The findings of this study emphasize the importance of interaction of different viral variants in the same population as well as observing viral population as a single evolution unit.

Control Number: 2021-A-8199-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP112**

Session Title: **FEMS: Infection Biology and Pathogens**

Topic 1: FEMS - Infection biology and pathogens

Keyword 1: cytomegalovirus

Keyword 2: B-cell malignancies

Keyword 3: oncoprotection

Abstract Title: **Human Cytomegalovirus Infection May Proffer A Degree Of Oncoprotection Against B-cell Neoplasia: A Single-institution Experience**

Author Block: **M. Jankovic**[1], A. Knezevic[1], M. Todorovic[1], I. Djunic[1], B. Mihaljevic[1], I. Soldatovic[1], N. Mikovic[2], V. Stoiljkovic[2], T. Jovanovic[1]; [1]Med. Faculty Univ. of Belgrade, Belgrade, Serbia, [2]Inst. of Virology, Vaccines, and Sera "Torlak", Belgrade, Serbia

Abstract Body: *Background* The possible oncogenic effect of cytomegalovirus (CMV) and prominent role played by B- lymphocytes in CMV infection have recently renewed scientific interest in B-cell disorders. While a molecular panoply that may well lead to bona fide tumorigenesis has been clearly evidenced with CMV, conversely, an oncoprotective quality of the pathogen was described in other works as well. Although CMV was evaluated as a possible etiological agent of hematological malignancies, no research reports on the interplay between the pathogen and B-cell neoplasms in this context. In the face of clinical diversity, a composite cohort of patients with B-cell malignancies would provide a uniform empirical context to explore tumoral activity of CMV. Thus far, the true role this virus plays in the pathogenesis of malign blood disorders remains to be elucidated. *Objectives* Our aim was to explore the possible oncogenic property of CMV and report on the significance of latent CMV infection in exclusively B-cell leukemia/lymphoma. *Methods* Patients with strictly B-cell derived leukemia/lymphoma (N=83, median age, 49.4) were compared to control cohort without malignant background (N=259, median age, 41.8 years). CMV-specific antibody classes were screened directly on serum specimens. The IBM® SPSS Statistics 26 software was used to infer statistical results. *Results* The age/gender adjusting on the patient and control cohorts revealed a significantly larger CMV-seropositivity in control subjects (98.7%) than in patients (90.4%) (p=0.035). Most IgG positives were among patients with non-Hodgkin lymphoma (94.3%), followed by B-cell chronic lymphocytic leukemia (88.9%), and Hodgkin's disease (76.5%). *Conclusion* We try to draw attention to a possible antilymphoma effect conferred by endogenous CMV infection against risk of *de novo* B-cell dyscrasia. Immunocompetent hosts to chronically latent CMV seem to enjoy a somewhat better defense against cancer of B-cell origin relative to CMV seronegative individuals. This appears to also hold for different races and ethnicities in a number of countries and communities the world over supporting our single-centre evidence.

Control Number:	2021-A-8214-MICROBE
Session Type:	**iPoster**
Session Number:	**FEMSP112**
Session Title:	**FEMS: Infection Biology and Pathogens**
Topic 1:	FEMS - Infection biology and pathogens
Keyword 1:	honey bee
Keyword 2:	microbiome
Keyword 3:	
Abstract Title:	**Gut Microbiome Response To Protective Treatments Against *Nosema* Sp. Infection In The Honey Bee (*Apis Mellifera*)**
Author Block:	**L. Tran**[1], I. Medici de Mattos[2], R. Ortega Polo[3], M. Cunningham[1], E. Simko[2], M. M. Guarna[1]; [1]Agriculture and Agri-Food Canada, Beaverlodge, AB, Canada, [2]Univ. of Saskatchewan, Saskatoon, SK, Canada, [3]Agriculture and Agri-Food Canada, Lethbridge, AB, Canada
Abstract Body:	Background: Honey bees (*Apis mellifera*) are essential pollinators of various crops with high economic value and their pollination services can be compromised by disease. Nosemosis is an infection of the honey bee midgut with the microsporidium *Nosema* sp. which is found among managed colonies across the globe. This can affect the honey bee gut microbiome, a community of microorganisms known to contribute to bee health and immunity. Objective: To examine the effect of different commercial protective treatments on the honey bee gut microbiome infected with *Nosema* sp.. Methods: The honey bee gut microbiome of winter bees maintained in cages was analyzed using 16S rRNA amplicon sequencing to determine the microbial composition at three time points: pre-treatment (TP1), post-treatment (TP2), and post-infection with *Nosema* sp. (TP3). Amplicon sequence variants (ASVs) were identified at each time point using the bioinformatics platform QIIME2 to determine the microbial composition of the gut. Results: Gut microbiome genera with high representation included *Lactobacillus* spp., *Gilliamella* spp., *Snodgrasella* spp., as well as *Bartonella* spp. and *Commensalibacter* spp.. Interestingly, ASVs of the family Enterobacteriaceae were also detected. Beta-diversity analysis revealed a shift in the relative abundance of the microbial community over time while in cages, with a significant decrease in the proportion of *Lactobacillus* spp. and an increase in Enterobacteriaceae, particularly at TP3. At this post-infection time point, the *Nosema*-infected group had a different microbiome profile than the negative control group, indicating that *Nosema* sp. infection caused dysbiosis. In particular, the relative abundance of Enterobacteriaceae was significantly increased in infected bees compared to

non-infected bees. Treatment with oxytetracycline, an antibiotic used to treat bee brood diseases, and the feeding stimulant Honey B Healthy, appeared to have a protective effect and resulted in a microbiome profile with increased similarity to the profile of non-infected bees. In summary, our findings show that *Nosema* sp. infection in caged honey bees results in bacterial dysbiosis. Certain treatments, however, can contribute a protective effect and help maintain the natural composition of the honey bee gut microbiome.

Control Number: 2021-A-8246-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP112**

Session Title: **FEMS: Infection Biology and Pathogens**

Topic 1: FEMS - Infection biology and pathogens

Keyword 1: Flavobacterium psychrophilum

Keyword 2: fish skin bacteria

Keyword 3: rainbow trout

Abstract Title: **Skin Bacteria Of Rainbow Trout Antagonistic To The Fish Pathogen Flavobacterium Psychrophilum**

Author Block: **M. Takeuchi**[1], E. Fujiwara-Nagata[2], T. Katayama[3], H. Suetake[4]; [1]Natl. Inst. of Advanced Industrial Sci. and Technology, Ikeda, Japan, [2]KINDAI Univ., Nara, Japan, [3]Natl. Inst. of Advanced Industrial Sci. and Technology, Tsukuba, Japan, [4]Fukui Prefectural Univ., Obama, Japan

Abstract Body:

Background: Rainbow trout fry syndrome (RTFS) and bacterial coldwater disease (BCWD) is a globally distributed freshwater fish disease caused by *Flavobacterium psychrophilum*. In spite of its importance, an effective vaccine is not still available. Manipulation of the microbiome of skin, which is a primary infection gate for pathogens, could be a novel countermeasure. For example, increasing the abundance of specific antagonistic bacteria against pathogens in fish skin might be effective to prevent fish disease.

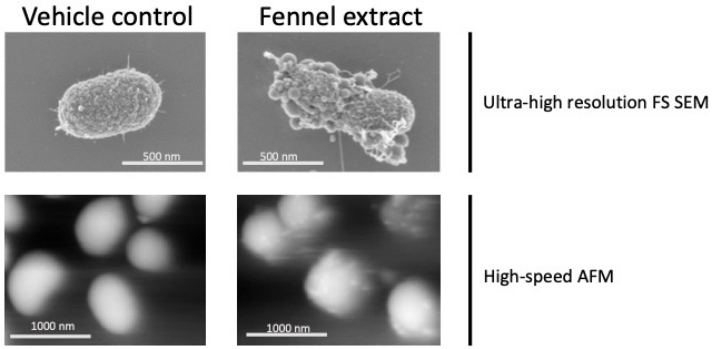
Objectives: Our objectives were to obtain insight into the skin microbiome of the rainbow trout (*Oncorhynchus mykiss*) and searched for skin bacteria antagonistic to *F. psychrophilum*.

Methods: We combined cultivation using multiple culture media with 16S rRNA gene amplicon sequencing.

Results: We obtained 174 isolates spanning 18 genera. Among them, *Bosea* sp. OX14 and *Flavobacterium* sp. GL7 respectively inhibited the growth of *F. psychrophilum* KU190628-78 and NCIMB 1947 ^{T}, and produced antagonistic compounds of < 3 kDa in size. Sequences related to our isolates comprised 4.95% of skin microbial communities, and those related to strains OX14 and GL7 respectively comprised 1.60% and 0.17% of the skin microbiome. Comparisons with previously published microbiome data detected sequences related to strains OX14 and GL7 in skin of other rainbow trout and Atlantic salmon.

Control Number:	2021-A-8270-MICROBE
Session Type:	**iPoster**
Session Number:	**FEMSP112**
Session Title:	**FEMS: Infection Biology and Pathogens**
Topic 1:	FEMS - Infection biology and pathogens
Keyword 1:	Porphyromonas gingivalis
Keyword 2:	outer membrane proteins
Keyword 3:	
Abstract Title:	**Dual Inhibitory Effects Of Fennel Seeds Against Porphyromonas Gingivalis**
Author Block:	N. Yoshino[1], T. Ikeda[2], **R. Nakao**[3]; [1]S&B Foods Inc., Tokyo, Japan, [2]Sojo Univ., Kumamoto, Japan, [3]Natl. Inst. of Infectious Diseases, Tokyo, Japan
Abstract Body:	**Background:** *Porphyromonas gingivalis* is a black-pigmented Gram-negative anaerobe that resides in periodontal pockets. This microorganism is regarded as an etiological agent of chronic periodontitis, as it disrupts integrity of periodontal immunity by releasing a diverse repertoire of virulence factors such as gingipains and lipopolysaccharides. Fennel, a common herb in indian food, has been used as a traditional medicine since ancient times. **Objectives:** We aim to investigate antimicrobial activity of fennel against *P. gingivalis*, and also to identify the antimicrobial compound in fennel. **Methods and Results:** When 92 samples extracted from 23 raw plant by four different solvents were subjected to *P. gingivalis* growth inhibition assay, n-hexane-extracted fennel (HEF) showed the strongest inhibitory activity among them. HEF showed bactericidal effect on *P. gingivalis* within 5 min after the treatment. Spatiotemporal imaging of bacterial surface revealed HEF induced formation of nanotube-like membrane vesicles or cell lysis at low (8 μg/mL) or high (more than 50 μg/mL) concentration of HEF, respectively. The molecular profiling of subcellular fractions in *P. gingivalis* demonstrated that HEF deprived the outer membrane of RagA and RagB, which are two major outer membrane proteins essential for nutrient acquisition in *P. gingivalis*, by means of releasing membrane vesicles carrying the cargo proteins. In addition, HEF could protect oral squamous epithelial cells from cell detachment induced by gingipains' proteolytic activity. Furthermore, we could isolate and identify petroselinic acid from fennel seeds as the major compound responsible for dual inhibitory activities against *P. gingivalis*, i.e., as both bactericide and protease inhibitor. With the mechanistic insights into antimicrobial activities of HEF, we suggest the clinical applicability for preventive and therapeutic measures against chronic

periodontitis.



Morphological observation of *P. gingivalis* ATCC 33277 treated with Fennel seeds extracts
Bacterial cells treated with fennel seed extracts (Fennel extract) or 1% DMSO (the vehicle control) were visualized by ultra-high resolution field-emission scanning microscopy (Ultra-high resolution FS SEM, S-5200, Hitachi High-Tech.)(Top columns) and and high-speed atomic force microscopy (high-speed HS-AFM, BIXAM, Olympus) (bottom columns).

Control Number: 2021-A-8339-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP112**

Session Title: **FEMS: Infection Biology and Pathogens**

Topic 1: FEMS - Infection biology and pathogens

Keyword 1: staphylococcus

Keyword 2: membrane potential

Keyword 3: bacteraemia

Abstract Title: ***S. Aureus*Serum Response And Toxicity Require Optimal Membrane Potential Mediated By *Mpsb***

Author Block: E. J. Douglas, **S. Duggan**, T. Brignoli, R. Massey; Univ. of Bristol, Bristol, United Kingdom

Abstract Body: *S. aureus* is a major human pathogen and critical concern in the context of anti-microbial resistance. With an increasing incidence of methicillin resistant strains and attempts to develop a vaccine failing, it is imperative for microbiologists to investigate the contribution of individual factors to this bacterium's pathogenicity with a view to identifying new targets for interference.The ability to resist serum and produce toxins are distinct processes by which *S. aureus* portrays its virulence and survives in the human host. We previously reported that *mpsB* contributes to patient mortality during bacteraemia and this current work investigates the mechanisms unpinning this finding. By comparing a wild type and *mpsB* mutant in a suite of phenotypic assays, we found inactivation of *mpsB* lead to increased resistance to human serum and host factors HNP-1 and LL37. Using CCCP, an inhibitor of membrane potential, we demonstrated the serum resistance phenotype is dependent on altered membrane potential of the *mpsB* mutant cells. We further observed that *mpsB* mutants were less cytolytic to monocytes. To determine if this is caused by reduced agr expression, we measured agr using an RNAIII::GFP reporter and failed to detect agr in the *mpsB* mutant. To verify if altered membrane potential prevented agr activity, we measured RNAIII::GFP in wild type cells in the presence of CCCP, and found reduced membrane potential caused a reduction in agr activity. Taken together, these data suggest that *mpsB* contributes to patient mortality in bacteraemia via its control of membrane potential and ensuing effects on resistance to host factors and toxin production.

Control Number: 2021-A-8347-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP112**

Session Title: **FEMS: Infection Biology and Pathogens**

Topic 1: FEMS - Infection biology and pathogens

Keyword 1: interactions

Keyword 2: gram-negative bacteria

Keyword 3:

Abstract Title: ***Campylobacter Jejuni*-derived Adp-heptose Drives The Inflammatory Response In Human Intestinal Epithelial Cells**

Author Block: **J. Cui**; Univ. Med. Ctr. Utrecht, Utrecht, Netherlands

Abstract Body:

***Campylobacter jejuni*-derived ADP-heptose drives the inflammatory response in human intestinal epithelial cells**

AbstractThe Gram-negative bacterium *Campylobacter jejuni* is a major cause of foodborne disease in humans. After infection, *C. jejuni* rapidly colonizes the mucus layer of the small and large intestine and induces a potent pro-inflammatory response characterized by the production of a large repertoire of cytokines, chemokines, and innate effector molecules, resulting in (bloody) diarrhea. The virulence mechanisms by which *C. jejuni* causes this intestinal response are still largely unknown. Here we show that *C. jejuni* releases a potent pro-inflammatory compound into its environment, which activates an NF-kB-mediated pro-inflammatory response including the induction of CXCL8, CXCL2, TNFAIP2 and PTGS2. This response was dependent on a functional ALPK1 receptor and independent of Toll-like Receptor and Nod-like Receptor signaling. Chemical characterization, inactivation of the heptose-biosynthesis pathway by the deletion of the hldE gene and in vitro engineering identified the released factor as the LOS-intermediate ADP-heptose. *C. jejuni* infection of intestinal cells showed a potent inflammatory response solely after the bacterial release of ADP-heptose without the need of a type III or type IV injection machinery. Our results classify ADP-heptose as a major virulence factor of *C. jejuni* that may play an important role during *Campylobacter* infection in humans.

Control Number: 2021-A-8352-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP112**

Session Title: **FEMS: Infection Biology and Pathogens**

Topic 1: FEMS - Infection biology and pathogens

Keyword 1: Staphylococcus aureus

Keyword 2: peptidoglycan hydrolase

Keyword 3: cell wall binding domain

Abstract Title: **Differential Binding Of Human And Murine IgGs To Catalytic And Cell Wall Binding Domains Of *Staphylococcus Aureus*Peptidoglycan Hydrolases**

Author Block: **M. Wang**[1], S. van den Berg[2], Y. Mora Hernández[1], A. Hinke Visse[1], E. Vera Murguia[1], D. G. A. M. Koedijk[1], C. Bellink[1], H. Bruggen[1], I. Bakker-Woudenberg[2], G. Buist[3], J. van Dijl[3]; [1]Univ. of groningen, Groningen, Netherlands, [2]Erasmus Univ. Med. Ctr., Rotterdam, Netherlands, [3]Univ. of Groningen, Groningen, Netherlands

Abstract Body: *Staphylococcus aureus* is an opportunistic pathogen causing high morbidity and mortality. Since multi-drug resistant *S. aureus* lineages are nowadays omnipresent, alternative tools for preventive or therapeutic interventions, like immunotherapy, are urgently needed. However, there are currently no vaccines against *S. aureus*. Surface-exposed and secreted proteins are regarded as potential targets for immunization against *S. aureus* infections. Yet, many potential staphylococcal antigens of this category do not elicit protective immune responses. To obtain a better understanding of this problem, we compared the binding of serum IgGs from healthy human volunteers, highly *S. aureus*-colonized patients with the genetic blistering disease epidermolysis bullosa (EB), or immunized mice to the purified *S. aureus* peptidoglycan hydrolases Sle1, Aly and LytM and their different domains. The results show that the most abundant serum IgGs from humans and immunized mice target the cell wall-binding domain of Sle1, and the catalytic domains of Aly and LytM. Interestingly, in a murine infection model, these particular IgGs were not protective against *S. aureus* bacteremia. In contrast, relatively less abundant IgGs against the catalytic domain of Sle1 and the N-terminal domains of Aly and LytM were almost exclusively detected in sera from EB patients and healthy volunteers. These latter IgGs may contribute to the protection against staphylococcal infections, as previous studies suggest that serum IgGs protect EB patients against severe *S. aureus* infection. Together, these observations focus attention on the use of particular protein domains for vaccination to direct potentially protective immune responses towards the most promising epitopes within staphylococcal antigens.

Control Number: 2021-A-8491-MICROBE
Session Type: **iPoster**
Session Number: **FEMSP112**
Session Title: **FEMS: Infection Biology and Pathogens**
Topic 1: FEMS - Infection biology and pathogens
Keyword 1: Coxiella
Keyword 2: symbiosis
Keyword 3: genomics
Abstract Title: **Genomic Mechanisms Driving Evolution Of *Coxiella* Genus Along The Parasitism-mutualism Continuum**

Author Block: **D. Santos-Garcia**[1], O. Morel[1], H. Henri[1], A. El Filali[1], M. Buysse[2], V. Noël[2], K. McCoy[2], Y. Gottlieb[3], L. Klasson[4], L. Zenner[1], O. Duron[2], F. Vavre[1]; [1]CNRS - Univ. Lyon 1, VILLEURBANNE Cedex, France, [2]Université de Montpellier - Inst. pour la Recherche et le Développement (UR 224), Montpellier, France, [3]Koret Sch. of Vet. Med., The Hebrew Univ. of Jerusalem, Rehovot, Israel, [4]Uppsala Univ., Uppsala, Sweden

Abstract Body: **Background***: Coxiella burnetii* is a parasitic intracellular bacterium initially isolated from a tick. It causes Q fever, a zoonotic disease in humans, and represents a notable problem for livestock production. *C. burnetii* presents a biphasic cycle with both active and resistance forms. The latter can persist for long periods in the environment and is the primary infective form. Contrary to other intracellular pathogens, *C. burnetii* replicates inside the host cells phagolysosomes, which presents an acidic pH. Interestingly, several *Coxiella*-like endosymbionts (*Coxiella*-LEs) phylogenetically related to *C. burnetii* have been recently described as nutritional mutualists of ticks.
Objectives: *C. burnetii* and *Coxiella*-LEs relationship have led to the question of the evolutionary origin of *C. burnetii:* Did it evolved from a mutualistic symbiont by gaining different virulence factors or was the ancestor a parasite?
Methods: To answer this question, we conducted a comparative genomic and phylogenetic analysis using two newly obtained *Coxiella*-LE from ticks and 40 additional Coxiellaceae genomes from five genera: *Aquicella*, *Berkiella*, *Rickettsiella*, *Diplorickettsia*, and *Coxiella* (including several environmental representatives).
Results: All *Coxiella* representatives were found to be able to produce B vitamins and co-factors, while this was rarely possible in the other examined Coxiellaceae. The Dot/Imc T4 Secretion System (Dot/Imc SS) is essential for *C. burnetii* pathogenicity since it is used to hijack the host's phagolysosome to allow its replication. This secretion system is generally present among all Coxiellaceae. In *C. burnetii,* the Dot/Imc SS is part of a pathogenic island that has been lost or inactivated in Coxiella-LEs. Hence, the ancestor of *C.*

burnetii and *Coxiella*-LE probably was a parasitic organism able to produce vitamins and cofactors. Interestingly, *C. burnetii* lineage has acquired laterally a Na^+/H^+ Mrp (Multiple resistance and pH) antiporter which is involved in alkali resistance and sporulation in other bacteria. We propose that this Mrp antiporter may have a role during the environmental phase of *C. burnetii* (resistance form).

Control Number: 2021-A-8524-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP112**

Session Title: **FEMS: Infection Biology and Pathogens**

Topic 1: FEMS - Infection biology and pathogens

Keyword 1: Mobile genetic elements

Keyword 2: Pseudomonas aeruginosa

Keyword 3: genomics

Abstract Title: **Mapping The Chromosomal And Extrachromosomal Mobile Genetic Element Repertoire Of *Pseudomonas Aeruginosa* Colonizing Cystic Fibrosis Airways.**

Author Block: **I. Kyrkou**[1], J. A. Bartell[2], H. Krogh Johansen[2], S. Molin[1]; [1]Novo Nordisk Fndn. Ctr. for Biosustainability (DTU Biosustain), Kgs. Lyngby, Denmark, [2]RigsHosp.et, Copenhagen, Denmark

Abstract Body: The core genome of the opportunistic pathogen *Pseudomonas aeruginosa* was recently estimated to make up only 1% of its large and diverse pan-genome (Freschi et al., 2019). The accessory genome of each strain is estimated to constitute up to 20% of the genomic content, and it comprises an abundance of mobile genetic elements which are thought to contribute beneficial traits to their host strains. According to Freschi et al, 5% of the genetic modules (clusters of genes) that map to plasmids are associated with antimicrobial resistance, while 12% of the modules incorporated both antimicrobial resistance and/or virulence genes. However, the contribution of the accessory genome to the fitness of the ubiquitous *P. aeruginosa* in different environments has not been clarified systematically. This ongoing study sets out to investigate the role of plasmids and prophages in the in-patient fitness of clinical *P. aeruginosa* isolates. We have assembled a collection of initial clinical airway isolates of *P. aeruginosa* from a large cohort of 72 young cystic fibrosis patients with a growing dataset on whether these strains persist or rapidly disappear from their airway habitat after first isolation. Follow-up isolates from a sub-cohort of 12 patients with a high-resolution history of persistent infection are also being analysed. We are currently employing gravity-column based gDNA extractions and whole-genome sequencing using Nanopore MinION technology to identify extrachromosomal prophages and plasmids. To trace potentially intact chromosome-integrated prophages we scan assembled genomes using a machine-learning based tool (Prophage Hunter). We find that 30% of the sequenced isolates contain extrachromosomal mobile genetic elements. Many of the elements detected are mega-plasmids, which have recently been implicated in the spread of multidrug resistance in *P. aeruginosa*. Further, overall nucleotide comparisons of an extrachromosomal element with a high copy number point to a plasmidial prophage. With regards to chromosomal mobile genetic elements, we detect an average of three intact prophages that persist throughout the patient's infection history, indicating that they may play a role in strain fitness and long-term survival in human airways.

Control Number: 2021-A-8543-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP112**

Session Title: **FEMS: Infection Biology and Pathogens**

Topic 1: FEMS - Infection biology and pathogens

Keyword 1: Salmonella

Keyword 2: Dictyostelium discoideum

Keyword 3: T3SS Effector Proteins

Abstract Title: **Contribution Of T3ss Effectors Gtge And Sopd2 To The Intracellular Survival Of *Salmonella* Typhimurium In *Dictyostelium Discoideum***

Author Block: **F. Amaya Inzunza**, A. Sabag, S. A. Álvarez, C. A. Santiviago; Univ. de Chile, Santiago, Chile

Abstract Body:

After invading a host cell, *Salmonella* survives and replicates intracellularly in a specialized membranous compartment, known as the *Salmonella*-containing vacuole (SCV). To establish and maintain the SCV, *Salmonella* translocates effector proteins that subvert the vesicular trafficking in the host cell by means of two “type III secretion systems” (T3SS) encoded in pathogenicity islands SPI-1 and SPI-2. Many of these effectors target small GTPases, as Rab32. Among other functions, Rab32 is part of a host defense pathway that restricts the intracellular growth of bacterial pathogens such as *Listeria*, *Mycobacterium* and *Salmonella*. Effectors GtgE and SopD2 of *Salmonella* Typhimurium acts cooperatively to cleave Rab32, preventing its recruitment to the SCV and allowing the intracellular survival of this pathogen in macrophages. During its life cycle, *Salmonella* interacts with amoebae and other predatory protozoa in the environment. The ability of *Salmonella* to survive within amoebae, including the social amoeba *Dictyostelium discoideum*, has been reported. However, the virulence factors and molecular mechanisms involved in the interaction of *Salmonella* with these organisms have not been fully characterized. Our group reported that *S*. Typhimurium requires T3SS-1 and T3SS-2 to survive intracellularly in *D*. *discoideum*. In addition, we have shown that this pathogen resides intracellularly in a SCV-like compartment in this amoeba. In this work, we studied the role played by T3SS effectors GtgE and SopD2 during *D*. *discoideum* infection by *S*. Typhimurium. To this end, we generated Δ*gtgE*, Δ*sopD2* and Δ*gtgE* Δ*sopD2* mutants of *S*. Typhimurium and evaluated their intracellular survival in *D*. *discoideum* by competitive infection assays. Of note, all mutants displayed increased internalization and reduced intracellular survival in *D*. *discoideum* when compared to the wild type at different times of infection. The genome of *D. discoideum* encodes four putative Rab32 proteins. Our sequence-based analyses confirmed that all four Rab32 proteins of *D*. *discoideum* share a high sequence similarity with Rab32 proteins from human, mice, and green monkey. Furthermore, the Rab32 proteins of *D*. *discoideum* retain the sequence motif experimentally defined as the GtgE cleavage site. Overall, our results indicate that effectors GtgE and SopD2 contribute to the

intracellular survival of *S*. Typhimurium in *D*. *discoideum*, and suggest that all Rab32 proteins encoded in the genome of the amoeba are potential targets for proteolytic cleavage by GtgE.

Control Number: 2021-A-8548-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP112**

Session Title: **FEMS: Infection Biology and Pathogens**

Topic 1: FEMS - Infection biology and pathogens

Keyword 1: Scedosporium

Keyword 2: Cystic fibrosis

Keyword 3: Antigen

Abstract Title: **Immunoproteomics-based Analysis Reveals Candidate Antigens For The Diagnosis Of *Scedosporium* In Cystic Fibrosis Patients**

Author Block: **L. Martin Souto**[1], M. Areitio[1], L. Aparicio-Fernandez[1], E. Santos-Fernandez[1], I. Buldain[1], A. Antoran[1], M. Martin-Gomez[2], A. Rementeria[1], F. Hernando[1], A. Ramirez-Garcia[1]; [1]Univ. of the Basque Country (UPV/EHU), LEIOA, Spain, [2]Vall D'Hebron Hosp., Sant Cugat del Valles, Spain

Abstract Body: **Background:** *Scedosporium* species are emergent fungal pathogens ranking second among filamentous fungi causing chronic colonization of the airways of cystic fibrosis (CF) patients. While detection methods are currently available for the most common fungal colonizer of CF airways, *Aspergillus fumigatus*, the detection of *Scedosporium* spp. relies upon low sensitivity and specificity non-standardized culture procedures. The presence of these fungi may lead to chronic inflammation or even to life-threatening invasive disease in cases of immunodeficiency. Therefore, a delayed diagnosis along with the high virulence of these pathogens contribute to treatment failure and increased morbimortality. **Objective:** To contribute to the finding of new diagnostic strategies, this work aims to characterize relevant antigens for diagnosis of *Scedosporium* spp. using CF patients' sera. **Methods:** The proteome of *Scedosporium boydii* was resolved by two-dimensional electrophoresis (2-DE), and the immunoreactive proteins were detected by immunoblotting with pooled sera from CF patients. Serum samples used in this study were classified into three groups: patients with positive sputum cultures for *Scedosporium* spp. (S+), for *Aspergillus* spp. (A+), and patients with negative fungal cultures acting as control group (S-/A-). By comparing the immunomes obtained with the three groups of sera, *Scedosporium*-specific antigens were detected and identified by LC-MS/MS analysis. Finally, a bioinformatic analysis of the protein sequences was performed in order to predict molecular and biological functions. **Results:** The immunoproteomic study of *S. boydii* revealed nine immunoreactive spots specifically recognized by sera from CF patients colonized with *Scedosporium* spp., which corresponded to six different proteins. The identified antigens showed molecular weights (Mr) and isoelectric points (pI) ranging from 28 kDa to 62 kDa and from 5.2 to 7.2, respectively. Moreover, the bioinformatic analysis disclosed the biological processes in which the identified proteins are involved. Mainly, they contribute to metabolic processes such as translation, oxidation-reduction steps, gluconeogenesis or proteolysis; and cell organization and

biogenesis.In this sense, the characterization of these molecules sheds light to the discovery of new antigenic markers with promising diagnostic utility to detect serologically *Scedosporium* in CF patients.

Control Number: 2021-A-8549-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP112**

Session Title: **FEMS: Infection Biology and Pathogens**

Topic 1: FEMS - Infection biology and pathogens

Keyword 1: Pectobacterium sp.

Keyword 2: Outer membrane vesicles

Keyword 3:

Abstract Title: **Identification And Functional Characterization Of *Pectobacterium Spp*. Outer Membrane Vesicles**

Author Block: **S. Maphosa**, L. N. Moleleki; Univ. of Pretoria, Pretoria, South Africa

Abstract Body:

Background

Membrane Vesicles (MVs) are evolutionarily conserved spherical entities released by bacteria. They are sophisticated secretion systems that augment other secretion systems burdened with protein secretion. Hence, it is no surprise that they contain diversified cargo. Biological functions ascribed to MVs include roles in bacterial fitness under stress or host-pathogen interaction. Therefore, it is imperative to understand the significance of MVs in the survival and pathogenesis of phytobacteria. Based on the cargo and functional diversity of MVs, we postulate that this system has a fitness role in microbial communities and delivers effector proteins for host infection and modulation of defenses. To test this hypothesis, we used Pectobacterium brasiliense (*Pcb*) 1692, an important soft rot and blackleg pathogen of potatoes.

Objectives

1.To determine whether *Pcb* 1692 produces MVs

2.To identify and functionally annotate MV cargo

Methods

Firstly, to establish whether *Pcb* 1692 produces MVs, *Pcb* 1692 cultured to exponential and stationary phase in complex media was analyzed using transmission electron microscopy (TEM) and scanning electron microscopy (SEM). MVs were isolated by ultracentrifugation of cell-free supernatants. The supernatants and isolated MVs were analyzed using nanoparticle tracking analysis (NTA) to reveal nanoparticle concentration and size distribution. TEM was used to analyze negatively stained isolated MVs. MV protein cargo was identified from three biological replicates using mass spectrometry and Mascot. Sequence analysis tools annotated the identified proteins functionally.

Results

SEM and TEM images of *Pcb* 1692 revealed outer membrane vesicle (OMV)-like nanoparticles attached to the cells. NTA recorded nanoparticles ranging between 100-300 nm. TEM showed two types of MVs: OMVs and outer, inner membrane vesicles (OIMVs). A total of 129 proteins present in at least two of three biological replicates were considered actual MV cargo and consequently for downstream sequence analysis. Thirty-one virulence factors, including T3SS substrates, were annotated. Virulence factors included nine carbohydrate-active enzymes. Eight antibiotic resistance agents were also identified. Furthermore, 9 type six secreted effectors were also identified as OMV cargo. According to the functional annotations, *Pcb* 1692 MVs have potential roles in stress response, virulence, and competition.

Future objectives are to validate the proteomics results through MV virulence, competition, and stress response assays.

Control Number: 2021-A-8600-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP112**

Session Title: **FEMS: Infection Biology and Pathogens**

Topic 1: FEMS - Infection biology and pathogens

Keyword 1: Phage satellite

Keyword 2: Phage packaging

Keyword 3: Bacterial evolution

Abstract Title: **Investigating A Novel Phage Packaging Interference Mechanism By A Subfamily Of Staphylococcal Phage Satellites**

Author Block: **A. D. Ojiogu**; Univ. of Glasgow, Glasgow, United Kingdom

Abstract Body: *Staphylococcus aureus* pathogenicity islands (SaPIs) are phage satellites that exploit the life cycle of their 'helper' phages. SaPIs protect hosts against complete phage predation. Most SaPIs are packaged using helper phage machinery through a headful (*pac*) packaging mechanism. SaPIs interfere with *pac* phage reproduction through variety of strategies, including the redirection of phage capsid assembly to form small capsids which can accommodate the smaller SaPI genome. This process typically depends on the expression of the SaPI-encoded *cpm*A and *cpm*B genes encoded in the operon 1 of the SaPI genome. However, another SaPI subfamily, which includes SaPI1028, can remodel one of the helper phage capsids into a small capsid without encoding *cpm*AB homologs. Hence, the basis for this interference remains to be deciphered. Using techniques, such as DNA cloning, Southern blotting and cryo-electron microscopy, I have identified and characterized a novel mechanism by which SaPIs manipulate the helper phage capsid. This process depends on a new SaPI-encoded gene, *rcp* (redirecting capsid packaging) which encodes a protein involved in the remodelling of the phage capsid into small capsid to package SaPI genome. As the protein sequences of Rcp and CpmAB are unrelated, this strategy represents a fascinating example of convergent evolution. This result, moreover, indicates that the production of SaPI-sized particles is a widespread strategy of phage interference conserved during SaPI evolution.

Control Number: 2021-A-8612-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP112**

Session Title: **FEMS: Infection Biology and Pathogens**

Topic 1: FEMS - Infection biology and pathogens

Keyword 1: human pathogenicity

Keyword 2: Vibrio vulnificus

Keyword 3: serum resistance

Abstract Title: **Rapid Identification Of The Human Virulent *Vibrio Vulnificus*Isolates**

Author Block: **E. Sanjuan**, A. Segui, C. Amaro; Univ. de Valencia, Burjassot, Spain

Abstract Body: *Vibrio vulnificus* is a gram-negative aquatic bacterium found in warm and tropical brackish water that produces infections in humans and fish. This bacterium is found in estuarine and marine environments throughout the world, present in waters, sediments, plankton, mollusks, crustaceans, and finfish. This bacterium is a highly invasive pathogen in both humans and fish. Case reports of *V. vulnificus* human infection have been described in several countries throughout the world. The most significant form of *V. vulnificus* disease in humans is primary septicaemia, which normally follows ingestion of raw or undercooked seafood, mainly oysters and occurs in persons with certain underlying and chronic diseases. Some bacterial factors have been observed to be important for virulence in humans and/or eels, such as the capsule, LPS, or siderophores. However, these determinants are found in all the isolates of the species, regardless of the origin (clinical or environmental) or the genetic lineage of the strain. Despite this, only a few strains of the diverse population of *V. vulnificus* are associated with the disease. To this day, it does not exist a reliable test to predict pathogenicity for humans. Thus, in the absence of definitive information, on the contrary, it is assumed that all strains are equally virulent. Here, we present a protocol based on human serum growth that allows the classification of the *V. vulnificus* isolates into three categories that allow the discrimination between the avirulent, the strains that might produce infection in humans with predisposing conditions, and the extremely virulent that could produce the infection in all humans.

Control Number: 2021-A-8698-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP112**

Session Title: **FEMS: Infection Biology and Pathogens**

Topic 1: FEMS - Infection biology and pathogens

Keyword 1: Staphylococcus aureus

Keyword 2: teichoic acids

Keyword 3: toxicity

Abstract Title: **Wall Teichoic Acid: Holding The Balance Between Toxicity And Fitness**

Author Block: **T. Brignoli**, R. Massey; Univ. of Bristol, Bristol, United Kingdom

Abstract Body: *Staphylococcus aureus* is a major human pathogen, able to express a plethora of virulence factors, including several cytolytic toxins. A genome wide association study carried out on a collection of ST239 clinical isolates identified a single nucleotide polymorphism (SNP) associated with an increase in the cytolytic capacity (toxicity) of the staphylococcal strains. The SNP is in the intergenic region between the *tarK* and *tarF* genes, which are part of the wall teichoic acid (WTA) biosynthetic pathway. WTAs are important polymers bound to the peptidoglycan which are involved in several cell functions, ranging from cell division to host interactions. The isolates carrying the new allele produce more WTA than the isolates with the most common allele. Using a molecular genetic approach, we show that lower WTA production is associated with lower toxin secretion. On the other hand, strains that produce less WTA can grow to higher cell densities. We hypothesise that an imbalance in WTA intermediates determines changes in the activity of the PhoPR two component system, which has an impact on *S. aureus* metabolism. The emergence of different alleles controlling *tarF* expression seems to reflect different strategies across *S. aureus* clinical isolates, with a trade-off between toxicity and the ability to grow in a low nutrient environment.

Control Number: 2021-A-8761-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP112**

Session Title: **FEMS: Infection Biology and Pathogens**

Topic 1: FEMS - Infection biology and pathogens

Keyword 1: Bordetella pertussis

Keyword 2: pertactin-ACT fusion protein

Keyword 3: vaccine

Abstract Title: **Fusion Proteins Of Modified Pertactin And The Receptor Binding Site Of Adenylate Cyclase Toxin Induce Protective Immunity Against Bordetella Pertussis**

Author Block: **C. Espinosa Vinals**, L. Bumba, J. Masin, J. Holubova, O. Stanek, L. Brazdilova, P. Sebo; Inst. of Microbiol., Czech Academy of Sci., Prague, Czech Republic

Abstract Body: ***Introduction.*** *Bordetella pertussis* is still a concern for public health worldwide. The current acellular vaccines protect the individuals against disease, but do not avoid colonization and transmission. Hence, the improvement of current formulations and the development of new generation of acellular pertussis vaccines are required. Adenylate cyclase toxin (ACT) from *Bordetella pertussi*s is a promising candidate for this endeavor as it is related to the hijack of the immunity in the early stages of *Bordetella pertussis* infection. Using the current knowledge regarding the mechanism of action of the toxin and the structural approaches, we obtained a deletion protein that induces neutralizing antibodies. We fused this construct to another pertussis virulence factor, pertactin, which is also related to the early stages of colonization and represents one of the adhesins of the pathogen. ***Material and methods.*** The folding kinetics of the fusion constructs were studied by circular dichroism, fluorimetry and nano differential light scattering. Identity and antigenicity were assessed by Western blot and inhibition ELISA respectively. Mice were immunized with alum-containing formulations and challenged with the pathogen. ***Results.*** Both moieties were recognized by specific antibodies elicited against the parental proteins. The fusion proteins outcompete the parental proteins for binding to specific antibodies. The constructs induced high levels of specific antibodies against ACT and native pertactin. The induced immunity protected the mice against colonization and reduced the bacterial load in lungs. The induced antibodies neutralized the ACT and induced opsophagocytic killing of the bacterium. ***Conclusion.*** The generated fusion proteins constitute promising components to be included in acellular formulations of pertussis vaccines as they induced neutralizing and opsonizing antibodies, which tackle at once the adhesion of the bacterial cells and the toxic effect of ACT on phagocytes.

FEMSP115 FEMS: Infectious Diseases (Bacteria)

Control Number: 2021-A-7676-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP115**

Session Title: **FEMS: Infectious Diseases (Bacteria)**

Topic 1: FEMS - Infectious diseases

Keyword 1: Salmonella serogroup c1

Keyword 2: bacterial pathogenesis

Keyword 3:

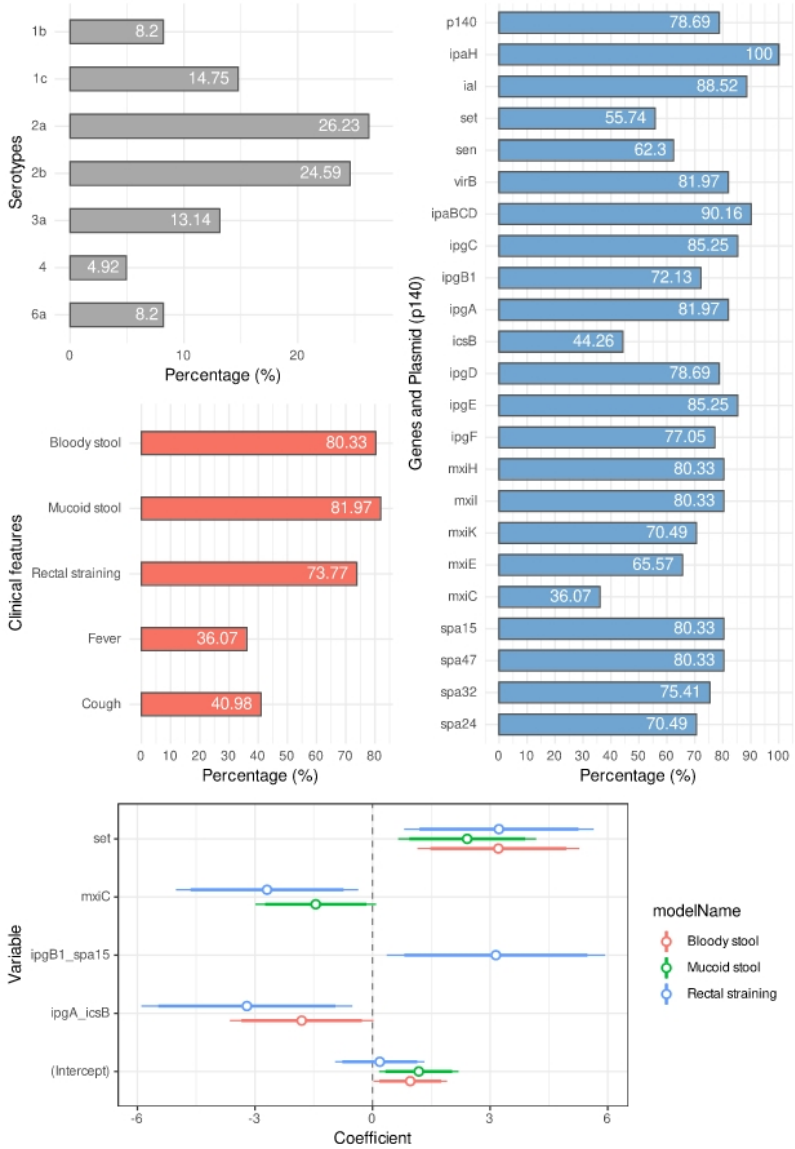
Abstract Title: **Pathogenesis Of *Salmonella* Serogroup C_1 Serovars**

Author Block: **S. S. Natta**, S. Nasrin, K. T. Sears, S. M. Tennant; Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract Body: **Background**: Non-typhoidal *Salmonella* (NTS) are facultative, Gram-negative bacterial pathogens that are the leading cause of foodborne infections globally. NTS are responsible for causing gastroenteritis in healthy individuals and invasive disease in infants, the elderly, and HIV-infected patients. *Salmonella* serogroups B (e.g., serovar Typhimurium) and D (e.g., serovar Enteritidis) are recognized as the most common causes of NTS infections. However, serogroups C_1 and C_2-C_3 are also responsible for a large burden of disease. In particular, some serogroup C_1 strains (e.g., Choleraesuis) have a high predilection for causing invasive disease in humans. We hypothesize that *Salmonella* serogroup C_1 strains possess different pathogenic characteristics than strains of other serogroups. The objective of this study was to investigate the pathogenesis of *Salmonella* serogroup C_1 serovars and to determine how they differ from serovars of other serogroups.**Methods:** Gentamicin killing assays were performed to measure bacterial uptake into J774 murine macrophage cells, uptake and intracellular replication in murine RAW 264.7 macrophage cells, and invasion of HEp-2 epithelial cells. Motility tests were also performed by stabbing bacteria onto the surface of semi-solid medium containing 1% tryptone, 0.5% NaCl, and 0.35% agar and measuring the zone of motility.**Results:** We tested a sample of NTS isolates from serogroups B (n=1), D (n=1), C_1 (n=6) and C_2-C_3 (n=4) using J774 cells. We observed a statistically significant difference in uptake of serogroup C_1 isolates by J774 cells than strains of other serogroups (p=0.010). There was no statistically significant difference in intracellular survival within RAW 264.7 cells over 20 hours between *S*. Typhimurium and *S*. Choleraesuis (p=0.327). Furthermore, we tested NTS isolates from serogroups B (n=1), D (n=1), C_1 (n=3) and C_2-C_3 (n=2) for invasion using HEp-2 cells. We found that there was no statistically significant difference in invasion between *Salmonella* serogroup C_1 serovars to serovars of other serogroups (p=0.6820). Lastly, we tested NTS isolates from serogroups B (n=10), D (n=10), C_1 (n=14), and C_2-C_3 (n=10) using a motility assay. We found that *Salmonella* serogroup C_1 serovars were significantly

less motile (p=0.0027) than serovars of other serogroups. **Conclusion:** We have shown that *Salmonella* serogroup C_1 serovars are phagocytosed by macrophages at higher levels and are less motile than strains of other serogroups.

Control Number:	2021-A-7785-MICROBE
Session Type:	**iPoster**
Session Number:	**FEMSP115**
Session Title:	**FEMS: Infectious Diseases (Bacteria)**
Topic 1:	FEMS - Infectious diseases
Keyword 1:	Shigella
Keyword 2:	Shigellosis
Keyword 3:	Type 3 Secretion System
Abstract Title:	**Evaluation Of The Impact Of *Shigella* Virulence Genes On The Basis Of Clinical Features Observed In Patients With Shigellosis**
Author Block:	**V. Chowdhury**[1], I. J. Azmi[1], M. Haque[1], A. S. G. Faruque[1], K. A. Talukder[2]; [1]icddr,b, Dhaka, Bangladesh, [2]Mawlana Bhashani Sci. and Technology Univ., Dhaka, Bangladesh
Abstract Body:	As *Shigella* is still causing significant morbidity and mortality each year, mostly in developing-country under-five children, we investigated the association between *Shigella* virulence genes and shigellosis. We randomly selected 61 *S. flexneri* strains isolated from Bangladeshi patients between 2009-2013 and evaluated the presence of 140 MDa large-virulence-plasmid (p140), 22 virulence genes, including *ipaH, ial*, toxin, and T3SS-related genes. We found p140 in 79% (n=48) and ipaBCD in 90% (n=55) strains, while seven of these were missing the p140. The prevalence of *ial* was 89%, *ipgC* and *ipgE* was 85%, and for the rest of the genes, it was below 85%. During the multivariate analysis, we found instead of *sen*, the *Shigella* enterotoxin gene - *set* - along with several other virulence genes, i.e., *ipgA, icsB, ipgB1, spa15*, and *mxiC* were found significantly influencing - in different combinations - several clinical features relevant to shigellosis, including bloody stool, mucoid stool, and rectal straining, while we could use such model for rapid diagnosis and quick management of patients, especially when detecting the causative organisms by direct means is difficult.


Serotypes
1b
1c
2a
2b
3a
4
6a
8.2
14.75
26.23
24.59
13.14
4.92
8.2
0
10
20
Percentage (%)
Genes and Plasmid (p140)
p140
ipaH
ial
set
sen
virB
ipaBCD
ipgC
ipgB1
ipgA
icsB
ipgD
ipgE
ipgF
mxiH
mxiI
mxiK
mxiE
mxiC
spa15
spa47
spa32
spa24
78.69
100
88.52
55.74
62.3
81.97
90.16
85.25
72.13
81.97
44.26
78.69
85.25
77.05
80.33
80.33
70.49
65.57
36.07
80.33
80.33
75.41
70.49
0 10 20 30 40 50 60 70 80 90 100
Percentage (%)
Clinical features
Bloody stool
Mucoid stool
Rectal straining
Fever
Cough
80.33
81.97
73.77
36.07
40.98
0 10 20 30 40 50 60 70 80
Percentage (%)
Variable
set
mxiC
ipgB1_spa15
ipgA_icsB
(Intercept)
-6
-3
0
3
6
Coefficient
modelName
Bloody stool
Mucoid stool
Rectal straining

Control Number:	2021-A-7870-MICROBE
Session Type:	**iPoster**
Session Number:	**FEMSP115**
Session Title:	**FEMS: Infectious Diseases (Bacteria)**
Topic 1:	FEMS - Infectious diseases
Keyword 1:	Bovine intramammary infection
Keyword 2:	Intramammary resistome
Keyword 3:	Whole genome sequencing
Abstract Title:	**Analysis Of Bovine Intramammary Resistome And Of The Bacterial Transmission Within Dairy Herds**
Author Block:	**A. Romano'**[1], I. Ivanovic[1], M. Vaccani[2], L. Sesso[2], A. Steiner[2], H. Graber[1]; [1]Agroscope, Bern, Switzerland, [2]Vetsuisse Faculty, Bern, Switzerland
Abstract Body:	**Background:** Mastitis is the most important and costly disease in dairy cows worldwide. Bovine intramammary infection (IMI) caused by pathogenic bacteria is common and well understood, but very little is known about the bacteria and their antimicrobial resistance (AMR) genes present in the mammary gland (= intramammary resistome, IR) of healthy and untreated cows. **Objective**: The goals for the present project are to study: -the bacteriome, all the bacteria that we can isolate from the intrammmary gland -the intramammary resistome of healthy cows meaning the genomic and phenotypic antimicrobial resistance profile **Methods and Results**: Nine dairy herds were chosen and sampled three times during one year; each time ten healthy cows were randomly selected and aseptic milk samples were taken from all quarters. After the bacteriological analysis, the species were identified by MALDI-TOF spectrometry; 1288 isolates were obtained from 90 different bacterial species. In total, 1024 single quarters were analyzed; 824 quarters were colonized with at least one bacterial species representing a quarter prevalence of 80%.The most prevalent species isolated were non-*aureus* staphylococci (NAS), consisting of 14 different species that covered 73.5% of the colonized quarters. To study the presence of AMR genes, the DNA of 475 representative strains (24 different species, 68% Staph *spp.*) were extracted and subjected to whole genome sequencing (Illumina). The obtained reads were assembled against the respective type strain (chromosome). The unassembled reads were then de novo assembled into contigs (mobile elements, ME). The chromosomes and ME were examined for AMR genes using an in-house software with an own database for AMR genes and online softwares (ResFinder, CARD). All the strains sequenced were further analyzed for phenotypical presence of AMR by the Minimum Inhibitor Concentration (MIC) method. *Staphylococcus xylosus* and *Staphylococcus sciuri* were the most abundant bacteria isolated. At the genomic

level, *Staph. xylosus* was mainly characterized by presence of the genes *abc* and *rlmN* variants and *Staph. sciuri* by *mecA1* and *sal*A. Phenotypically, *Staphylococcus* spp. were for the majority resistant to clindamycin (50%) and oxacillin (36%). Further analyses are ongoing to analyze genomic and phenotypic AMR of the other bacteria strains isolated.

Control Number: 2021-A-7888-MICROBE
Session Type: **iPoster**
Session Number: **FEMSP115**
Session Title: **FEMS: Infectious Diseases (Bacteria)**
Topic 1: FEMS - Infectious diseases
Keyword 1: infection
Keyword 2: Alzheimer's disease
Keyword 3: antimicrobial peptide

Abstract Title: **The Role Of Microbial Infection In The Pathogenesis Of Alzheimer's Disease And The Opportunity For Protection By Anti-microbial Peptides**

Author Block: **F. Li**[1], M. Hearn[1], L. Bennett[1], A. Boulier[2]; [1]Monash Univ., Melbourne, Australia, [2]Ingredia company, Arras, France

Abstract Body:

Background: Alzheimer's disease (AD) is a complex neurodegenerative disease. Current rationales to explain the pathogenesis of AD include amyloid cascade, oxidative stress and inflammation, infection defense and anti-microbial protection hypotheses.
Objectives: We aimed to collect and analyze recent research reports of evidence to support the infection hypothesis, in particular those pathogenic microbes that act systematically, via periodontal and gastro-intestinal infection routes.
Methods: Detailed literature searching was conducted using the keywords "Microbes or infection and Alzheimer's disease"; "Amyloid precursor protein and infection" using google scholar. Additional references were found in the cited literature, retrieved documents and review articles.
Results: Collective evidence convincingly supports that pathogenic microbial infection is associated with, and is likely a causative trigger for, AD pathology. Microbes can drive AD pathology by two main pathways: either by directly invading the brain and stimulating amyloid-mediated defence (causative trigger) or indirectly by stimulating the pro-inflammatory effects of infection. In this context, it follows that anti-microbial/anti-infection therapies could be effective for regulating the pathology and symptoms of AD, depending on the stage of disease. As long-term administration of traditional antibiotic therapy is not recommended, alternative antibiotic agents based on bio-mimicry such as anti-microbial peptides could be preferred for intervention and disease management of AD.

Control Number: 2021-A-7710-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP117**

Session Title: **FEMS: Infectious Diseases (Virus)**

Topic 1: FEMS - Infectious diseases

Keyword 1: COVID-19

Keyword 2: genomic epidemiology

Keyword 3: genetic variants

Abstract Title: **The Seqcovid-spain Consortium: Unravelling The Dynamics Of The Covid-19 First Epidemic Wave In Spain**

Author Block: **Á. Chiner-Oms**[1], M. G. López[1], SeqCOVID-Spain consortium, M. Coscolla[2], F. González-Candelas[3], I. Comas[1]; [1]Inst. de Biomedicina de Valencia (IBV-CSIC), Valencia, Spain, [2]Inst. de Biología Integrativa de Sistemas, I2SysBio (CSIC-Univ.t de València), Paterna, Spain, [3]Joint Res. Unit "Infection and Publ. Hlth." FISABIO-Univ. of Valencia I2SysBio, Paterna, Spain

Abstract Body: The COVID-19 pandemic has shaken the world since the beginning of 2020. Spain is among the European countries with the highest incidence of the disease during the first pandemic wave. We established a multidisciplinary consortium to monitor and study the evolution of the epidemic, with the aim of contributing to decision making and stopping the rapid spread throughout the country. We present the results for 2170 sequences from the first wave of the SARS-CoV-2 epidemic in Spain which represents 12% of diagnosed cases until the beginning of lockdown measures (14th March 2020) and almost 1% of the first wave (14th May 2020). This effort allows us to document at least 500 initial introductions, between early February-March 2020 from multiple international sources. Importantly, we document the early rise of two dominant genetic variants in Spain (Spanish Epidemic Clades), named SEC7 and SEC8, likely amplified by superspreading events. In sharp contrast to other non-Asian countries those two variants were closely related to the initial variants of SARS-CoV-2 described in Asia and represented 40% of the genome sequences analyzed. The two dominant SECs were widely spread across the country compared to other genetic variants with SEC8 reaching a 60% prevalence just before the lockdown. Employing Bayesian phylodynamic analysis, we inferred a reduction in the effective reproductive number of these two SECs from around 2.5 to below 0.5 after the implementation of strict public-health interventions in mid March 2020. The effects of lockdown on the genetic variants of the virus are reflected in the general replacement of pre-existing SECs by a new variant at the beginning of the summer season. Our results reveal a significant difference in the genetic makeup of the epidemic in Spain and support the effectiveness of lockdown measures in controlling virus spread even for the most successful genetic variants.

Control Number: 2021-A-8256-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP115**

Session Title: **FEMS: Infectious Diseases (Bacteria)**

Topic 1: FEMS - Infectious diseases

Keyword 1: Gastritis

Keyword 2: Retrosternal- burn

Keyword 3: Helicobacter Pylori

Abstract Title: **Incidence Of Helicobacter Pylori Infection In People With Gastritis Of Rural Area In Nepal**

Author Block: **S. Jaiswal**; Pokhara Univ., Pokhara, Nepal

Abstract Body: *Background: Helicobacter pylori* are a type of microaerophilic gram-negative bacterium in a spiral shape. The purpose of this study was to investigate the Incidence Rate and Risk Factor Assessment of Helicobacter Pylori Infection in People with Gastritis of Rural Area in Nepal. Methods: A community-based crossectional study and characterize H. pylori infection in this population. 800 individuals based on cluster sampling of residential location in Province 2, Nepal. Results: Out of 800 participants 356(44.5%) were from Aurahi and 444(55.5%) were from Bagdampur village which is the rural village of province 2, the Terai region of Nepal. A total of 41(5.1%) age group less than 20, 20(5.6%) were from Aurahi and 21(4.7%) were from Bagdampur, similarly 176(49.4%) and 168(37.8%) were from age group 20 to 40, 110(30.9%) and 168(37.8%) were from age group 41 to 60 and 50(14.0%) and 87(19.6%) were more than 60 age group from Aurahi and Bagdampur respectively. Similarly, 410 (51.2%) were female of which 170(47.8%) and 240(54.1%) were from Aurahi and Bagdampur respectively. Similarly, 390 (48.8%) were male of which 186(52.2%) and 204(45.9%) were from Aurahi and Bagdampur. Antibody was used the screening for H. pylori which showed 427(53.4%) were negative of which 208(58.4%) were from Aurahi and (219(53.4%) were from Bagdampur while 373(46.6%) were positive for *H. pylori* of which 148(41.6%) were from Aurahi and 225(50.7%) were from Bagdampur. Out of 800 participants, 182(48.8%) were female positive for H. pylori antibody test and 191(51.2%) were male positive with 0.194 P-value and 1.202 odds ratio. Similarly, 174(46.6%) was the highest positive in the 21 to 40 age group followed by 134(35.9%) in the 41 to 60 age group increasing age group showed high infection with a P-value of 0.012. Similarly Hindu were positive for 358(96.0%) while Muslim was 15(4.0%) positive with a P-value of 0.021 and odds ratio at 95% CI 2.940(1.129-7.657). Similarly married were positive for 360(96.8%) with P-value 0.000 and 148(39.7%) were positive from Aurahi village and 225(60.3%) were positive from Bagdampur village with a p-value of 0.006 and odds ratio at 95% CI 1.444(1.090-1.913). Conclusion: H. pylorus is one of the most common problems among gastritis (active) individuals in Nepal. Various public awareness and health education programs should be conducted to aware people about gastritis and its consequences like having ulcers.

Control Number:	2021-A-8263-MICROBE
Session Type:	**iPoster**
Session Number:	**FEMSP115**
Session Title:	**FEMS: Infectious Diseases (Bacteria)**
Topic 1:	FEMS - Infectious diseases
Keyword 1:	Staphylococcus aureus
Keyword 2:	Pleural infection
Keyword 3:	Metagenomic sequencing
Abstract Title:	**The Role Of Metagenomics Next-generation Sequencing In Microbiome Etiology Of Pleural Empyema**
Author Block:	**J. Liang**[1], W. Zhang[1], J. Li[1], H. Cheng[2], L. Yang[2], K. Wang[1]; [1]The First Affiliated Hosp. of Guangxi Med. Univ., Nanning, China, [2]Southern Univ. of Sci. and Technology, ShenZhen, China
Abstract Body:	**Background:** Identification of the offending organism and appropriate antimicrobial therapy are crucial for treating empyema. **Objectives:** The purpose of this study was to explore the microbiome etiology of pleural empyema through the Next-generation sequencing technique. **Method:** The metagenomics next-generation sequencing (mNGS) approach was applied to 45 empyema simples. **Result:** Staphylococcus aureus (S.aureus) was found in almost all of the 45 samples and it was the most variable species across samples. The between-sample (beta) diversity (Principle Coordination Analysis, PCoA) showed the samples formed two distinct cluster and we design the two clusters respectively as high and low abundance Staphylococcus aureus(HA-SA, and LA-SA)types, differentiated by the variation in the level of the most abundance species. The alpha diversity of the LA-SA type was much higher than that of the HA-SA type. Heat map of the microbiome species composition visualized the difference microbiome structure between HA-SA and LA-SA.

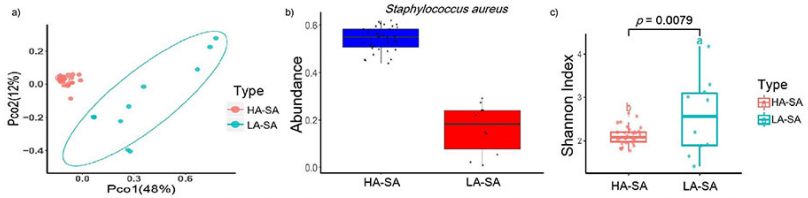


Figure 1. a) Principal Coordinate Analysis. b) The abundance levels of the main contributing species of two microbiome types. c) Alpha diversity estimation.

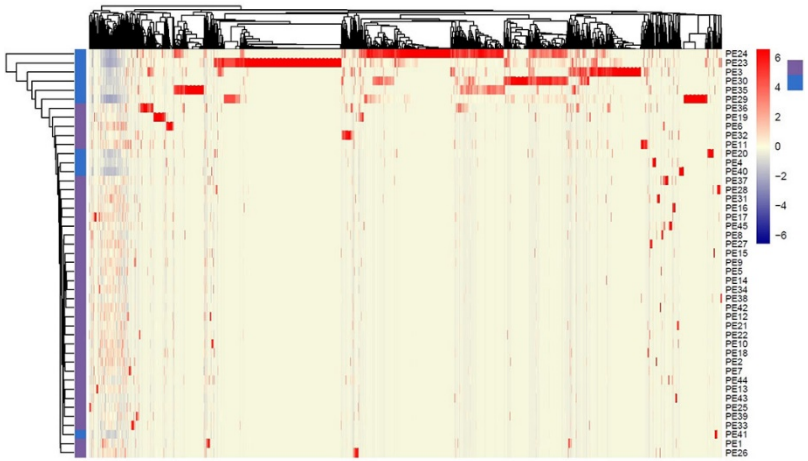


Figure 2. Heat map of the microbiome species composition for all samples.

Control Number: 2021-A-8361-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP115**

Session Title: **FEMS: Infectious Diseases (Bacteria)**

Topic 1: FEMS - Infectious diseases

Keyword 1: magnetic approaches

Keyword 2: bacterial detection

Keyword 3: optical approaches

Abstract Title: **Combining Magnetic And Optical Approaches For The Multiplex Detection Of Bacteria In Blood**

Author Block: **S. Costa**[1], A. Cunha[2], C. Carvalho[3]; [1]Intl. Iberian Nanotechnology Lab.; Ctr. of Biological Engineering, Univ. of Minho;, Braga, Portugal, [2]Intl. Iberian Nanotechnology Lab.; Ctr. of Biological Engineering, Univ. of Minho, Braga, Portugal, [3]Intl. Iberian Nanotechnology Lab., Braga, Portugal

Abstract Body: Background: Bloodstream infections (BSIs) are considered a major cause of death worldwide. *Enterococcus faecalis* and *Escherichia coli* are prevalent pathogens responsible for these infections and have been classified as high priority bacteria due to the increase of multidrug resistance. Thus, specific, sensitive, rapid, and cost- detection of these pathogens is of great importance to prevent BSI from progressing to sepsis. One of the main constraints in the detection of these pathogens is the isolation of the target cells directly from complex biological samples since some components can interfere in the downstream analysis by molecular techniques such as Polymerase Chain Reaction (PCR), flow cytometry, among others.Objective: Rapid and cost-effective detection of bacteria in complex sample matrices such as blood. Methods: In this study, we combined the advantages of bacteriophage receptor binding proteins (RBPs) as specific probes with the ability of magnetic nanoparticles to separate and concentrate the targets from complex matrices. For this, novel identified RBPs were fused with fluorescent genes and used as reporters for the multiplex detection of *Enterococcus faecalis* and *Escherichia coli* through spectrofluorimetry. Results: Overall, the developed assay demonstrated to be a promising approach for separation and detection of sepsis-associated bacteria demonstrating more than 80% of bacterial capture efficiency from spiked blood samples and specific detection in about 2 h. This method presents high versability both in terms of detection of other pathogens and application in different types of clinical samples. Moreover, has the potential to be used in combination with other detection systems like lab-on-chip devices, contributing to the improvement of the overall sensitivity.

Control Number: 2021-A-8506-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP115**

Session Title: **FEMS: Infectious Diseases (Bacteria)**

Topic 1: FEMS - Infectious diseases

Keyword 1: Brucella canis

Keyword 2: Toll-like receptors

Keyword 3:

Abstract Title: **Analysis Of Toll-like Receptors, Genes And Cytokines Expression In *Brucella Canis* Infected Dh82 Cells**

Author Block: **W. Park**, S. Kim, S-W. Choi, S. Kyung, H. Yoo; Coll. of Vet. medicine, Seoul Natl. Univ., Seoul, South Korea, Seoul, Korea, Republic of

Abstract Body: Clinical and zoonotical importance of *Brucella canis* infect in made conducting a variety of researches to understand the immunopathological mechanism of the infection. However, the precious mechanism of the infection is still remained to be resolved. Also, *B. canis* is known to have different immune mechanisms compared to other *brucella* species. In this study, an analysis was conducted on the Toll-like receptors, cytokines and genes expression patterns in the host cell.The time-dependent expression patterns of TLRs were analyzed with immune cells originated canine; DH82: canine cell line. The expression of 10 TLRs and six genes, TNF-α, IL-5, IL-23, CCL4, CD40 and NFkB1 associated with TLRs were analyzed with real-time PCR at 2-hours, 12-hours, and 24-hours after infection of *B. canis* to the cell. The production six cytokines, IFN-γ, IL-1β, IL-4, IL-6, IL-10 and IL-17A, were analyzed with ELSIA at the same time period as TLRs. TLRs 3, 6 and 9 were expressed at 24-hours after stimulation with *B. canis*. In particularly, TLR7 was significantly increased. And the expression of at the all six genes has been increased by the infection. The production of the pro-inflammatory cytokines, IL-1β and IL-6 were confirmed, and production of the Th17-related cytokine, IL-10, was also confirmed. This study found that when an infection of *B. canis* occurred, there was a difference in the appearance of the TLR according to the host. In particular, when infected with the cell line of the canine, the main host, the expression of TLR7 has been confirmed to increase significantly. In addition, the expression of TLR-related genes and cytokines has been identified. These results suggest that unlike other *Brucella* spp., TLR and its associated genes and cytokines are important to the host immune response in the infection of *B. canis*.This work was carried out with the support of CCRC (Project No. PJ013985) RDA, Republicof Korea.

Control Number: 2021-A-8538-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP115**

Session Title: **FEMS: Infectious Diseases (Bacteria)**

Topic 1: FEMS - Infectious diseases

Keyword 1: ACT/MIR/CMH beta-lactamases

Keyword 2: Enterobacter

Keyword 3: phylogeny

Abstract Title: **Reconstruction Of Act/mir/cmh Phylogeny Reveals Species-specific Evolution Within*Enterobacter*Spp.**

Author Block: **A. B. Gonçalves**, T. G. Ribeiro, Â. Novais, L. Peixe; UCIBIO-REQUIMTE, Faculty of Pharmacy Univ. of Porto, Porto, Portugal

Abstract Body: **Background** ACT/MIR/CMH β-lactamases are usually chromosomally located within the *Enterobacter* genus, whereas some variants are increasingly reported in clinical isolates of *Enterobacteriaceae.* However, their origin is poorly understood due to great taxonomic re-structuration's within the genera and lack of reliable and simple species identification tools. We used recent whole-genome based species phylogeny to dissect class C beta-lactamases origin and distribution within *Enterobacter* spp. **Materials/methods** Genes encoding 93 ACT, 22 MIR, and 7 CMH were collected from beta-lactamase database (http://www.bldb.eu/), together with 104 chromosomal AmpC (crAmpC) genes from available *Enterobacter* genomes reliably identified at species level, including type strains. Phylogenetic reconstruction of all genes (and proteins) was performed using neighbor-joining methods, as described. **Results** Phylogenetic tree based on ACT, MIR, CMH, and crAmpC genes showed 21 clusters and 4 branches (sharing ≤96.7% identity, bootstrap ≥81%), corroborated by AmpC-based phylogeny. Eighteen of them comprised a crAmpC from each of the recognized *Enterobacter* species/subspecies (n=19/5), with only one exception (*E. mori* and *E. quasimori*) (Fig. 1Beta-lactamase groups differed in 81% (average), being MIR exclusively associated with *Enterobacter roggenkampii*, CMH with *Enterobacter cloacae*, and groups of highly related ACT with each of the remaining species. Most acquired enzymes (previously described in plasmids or other *Enterobacteriaceae* species) were identified within the *E. asburiae* cluster (n=7/14, 50%) or *E. hormaechei* subsp. *xiangfangensis* cluster (n=4/14, 29%), which are species frequent in human infections. **Conclusions** This study provides a state-of-the-art of ACT/MIR/CMH class C beta-lactamases phylogeny, and alerts for close relatedness between these groups. Besides, it establishes, for the first time, a precise origin for each AmpC cluster within a given *Enterobacter* species, that could be used for reliable molecular-based ID. Finally, our data reveals *E. asburiae* and *E. hormaechei* subsp. *xiangfangensis* are likely sources of most acquired ACT-like β-lactamases.

$$MISSING OR BAD GRAPHIC SPECIFICATION (BC71A84C-A588-4E1F-BB99-472955F8D6C2) $$

Control Number: 2021-A-8673-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP115**

Session Title: **FEMS: Infectious Diseases (Bacteria)**

Topic 1: FEMS - Infectious diseases

Keyword 1: ETEC

Keyword 2: piglets

Keyword 3: bacteriophages

Abstract Title: **Bacteriophages To Tackle Swine Colibacillosis: Assessing The Potential Of This Alternative Therapy**

Author Block: **A. Ferreira**[1], C. Almeida[2], D. Silva[2], I. García-Meniño[3], A. Mora[3], H. Oliveira[1], J. Azeredo[1], A. Oliveira[1]; [1]Ctr. of Biological Engineering, Braga, Portugal, [2]ALS - ControlVet, Viseu, Portugal, [3]Laboratorio de Referencia de Escherichia coli (LREC)/Inst. de Investigación Sanitaria de Santiago de Compostela (IDIS), Santiago de Compostela, Spain

Abstract Body: **Background** Enterotoxigenic and Shiga toxin-producing *Escherichia coli* (ETEC and STEC) are the main agents of swine colibacillosis, causing high morbidity, mortality and economic losses in pig industry. The massive use of antibiotics (AB) in swine greatly contributed to bacterial selective pressure and AB resistances (e.g., to colistin). **Objectives** The prohibition of many active principles in animal production strengths the need of finding alternatives to tackle this pathogen, such as the use of bacterio(phages). **Methods** In this work, 24 *mcr*-positive Portuguese STEC/ETEC strains were characterized for specific O antigen implicated in swine diarrhea, STs, clonotypes, intestinal colonization factors (toxins and fimbriae) and *mcr*-types (1 to 5). Additionally, five phages (vB_EcoM_SP1, vB_EcoS_SP8, vB_EcoM_SP13, vB_EcoM_FJ, vB_EcoM_FN) were isolated from the Portuguese ETEC strains, sequenced (Illumina MiSeq) and characterized. **Results** Predominantly, these strains belonged to serogroups O108 (25 %), O139 (21 %) and the O157 (13 %). The most prevalent fimbriae-encoding gene was F18 (71 %), followed by F4 (17 %). Heat-stable (STa, STb) and heat-labile (LTb) enterotoxin-encoding genes were detected in 58 %, 67 % and 38 % of the strains, respectively. Five strains (21%) carried the shiga toxin gene *stx2e*. CH typing identified 5 clonotypes, being CH11-24 the most prevalent (58 %) and associated with ETEC ST10-CC10, followed by CH2-54 mainly associated with ST1 STEC isolates. The *mcr*-typing identified *mcr-1* in 13 strains, *mcr-2* in 2 strains, and *mcr-4* in 9 strains. All phages have double-stranded DNA genomes encoding no integration genes. They burst, in average, 70 phages per cell and some recognize more than one molecule as a receptor (outer membrane proteins and lipopolysaccharides). All are tailed and mostly belong to the *Myoviridae* family, except for the vB_EcoS_SP8, a *Siphoviridae.* The phage lytic spectrum was carried out against a wider collection of strains, including 63 previously characterized isolates from Spanish pig farms. Overall, ETEC-infecting phages revealed a narrow lytic spectrum, mostly targeting a single isolate. The highest lysis percentage was 3.45 %, obtained for vB_EcoM_FN. **Conclusions** This study suggests the importance of improving knowledge about ETEC-phage interaction and investigating

the mechanisms that are making these strains to wisely escape or decrease sensitivity to phages. This seems to be decisive for designing strategies and predicting the success of phage therapy to fight against ETEC in piglets.

Control Number: 2021-A-8741-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP115**

Session Title: **FEMS: Infectious Diseases (Bacteria)**

Topic 1: FEMS - Infectious diseases

Keyword 1: Bovines

Keyword 2: Paratuberculosis

Keyword 3: PCR

Abstract Title: **Detection Of *Mycobacterium Avium* Subsp. *Paratuberculosis* In Bovines Of District Peshawar, Pakistan**

Author Block: **F. Anwar Khan**; The Univ. of Agriculture, Peshawar, Pakistan, Peshawar, Pakistan

Abstract Body: Paratuberculosis (pTB) also known as Johne's disease (JD), is a chronic infectious disease of animals caused by *Mycobacterium avium* subsp*. paratuberculosis* (MAP). It is also a serious public health concern as MAP is responsible for Crohn's disease in human. This infectious disease is so far unexplored in Khyber Pakhtunkhwa (KPK) Province of Pakistan in bovine. Therefore this study was proposed to investigate the seroprevalence of MAP infection by indirect ELISA (iELISA), associated histopathological lesions, and PCR in cattle and buffaloes at district Peshawar, Pakistan. Serum and fecal samples were collected at random both from commercial farms and abattoirs. Tissue samples (intestine, mesenteric lymph node), were collected at random from cattle and buffaloes at abattoirs of district Peshawar. Analyses of serum samples by iELISA revealed the presence of antibodies against MAP in 9% cattle and 4% buffaloes. Ziehl-Nielsen (ZN) staining exhibited acid-fast bacilli (AFB) in 22% cattle and 10% buffaloes faecal impression smears. In Gross pathology of intestinal samples (thicknes and corrugation) was observed in 35% cattle and 25% buffaloes, while mesenteric lymph node exhibited gross lesions (hemorrhages, edematous swelling) in 32%and 40% in cattle and buffaloes respectively. Histopathological lesions were observed in intestine and MLN from 28%and 22% cattle respectively, whereas 20% and 17% buffaloes showed lesions, respectively, in intestine and MLN. Additionally, PCR revealed the presence of MAP in 4% cattle and 1.33% buffalo's tissue samples, while one cattle sample was confirmed positive for *Mycobacterium bovis*. This study concluded that MAP is present in the large ruminants of district Peshawar. The infection was confirmed by PCR. Importantly iELISA also confirmed the presence of MAP specific antibodies even in treated animals. The presence of MAP could be a serious threat to livestock and public health in the region.

FEMSP117 FEMS: Infectious Diseases (Virus)

Control Number:	2021-A-8229-MICROBE
Session Type:	**iPoster**
Session Number:	**FEMSP117**
Session Title:	**FEMS: Infectious Diseases (Virus)**
Topic 1:	FEMS - Infectious diseases
Keyword 1:	yellow fever virus
Keyword 2:	molecular
Keyword 3:	
Abstract Title:	**Circulation Of Yellow Fever Virus In Benin City Nigeria**
Author Block:	**J. Z. Saidu**[1], N. O. Eghafona[1], M. Y. Tatfeng[2]; [1]Univ. of Benin, Benin City, Nigeria, [2]Niger Delta Univ., Bayelsa, Nigeria
Abstract Body:	Background: Yellow fever (YF) is one of the most acute viral hemorrhagic disease of the 18th and 19th centuries, which continues to cause severe morbidity and mortality in Africa. The etiologic agent is yellow fever virus (YFV). After 21 years of no reported cases of yellow fever in Nigeria, till 2017 where a case was confirmed in Kwara State and in November 2018 WHO was informed of a cluster of suspected yellow fever cases and deaths in Edo state, Nigeria. Objective: Molecular identification of Yellow fever virus among febrile patients in Benin city, Edo state Nigeria. Methods: The study was among all age group attending health centres in Benin city, Edo state. Blood samples were collected from consented febrile patients and were screened for antibodies to Zika virus using rapid diagnostic test (RDT) kits. Blood samples positive to Zika virus (IgM/IgG RDT), were subjected to molecular characterization. Result: Using the *flaviviridae* family primers, 10 samples confirmed positive by reverse transcriptase-polymerase chain reaction (RT-PCR) were sequenced. Nucleotide sequence blast revealed the sequences are similar to Yellow fever virus strains. Phylogenetic analysis revealed that the yellow fever virus sequences are closely related to the African strains. Despite the safe and effective yellow fever vaccine, yellow fever virus is seen to be in circulation, hence the need for continues and mass vaccination.

Control Number: 2021-A-8280-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP117**

Session Title: **FEMS: Infectious Diseases (Virus)**

Topic 1: FEMS - Infectious diseases

Keyword 1: Pleurisy

Keyword 2: Tuberculosis

Keyword 3: Coinfection

Abstract Title: **Tuberculosis Pleurisy And Covid-19 Coinfection**

Author Block: **B. Shurbevska**, B. Ilievska Popovska, M. Damjanovska, A. Aleksoska Gjuzelova, V. Mitreski; Inst. for Lung Diseases and Tuberculosis, Skopje, Macedonia, The Former Yugoslav Republic of

Abstract Body: Background COVID-19 exhibits a diverse range of clinical presentations and our knowledge about this disease is constantly evolving. We report the first case of tuberculosis pleurisy with COVID-19 from Macedonia.Methods A 32 year old male was admitted with dyspnea, temperature, chest pain and a positive Covid-19 antigen test. An X-Ray image shows massive pleural effusion on the left, mediastinum shifted to the right, the parenchyma does not appear to have changes typical for COVID-19 infection. He is pale, obese, tachycardic (100/min) and TA 145/95 mmHg. Aushultatory there is barely audible vesicular breathing with no other abnormal findings. Blood analyzes show elevated sedimentation (29mm/h), CRP 62.27mg/l, glycaemia 8.03mmol/l, D-dymers 3865ng/ml. ECG shows sinus tachycardia 120/min with SVES and RBBB. Chest ultrasound shows large amount of liquid on the left, some taken for analysis through punction. The patient is treated with antibiotics clindamycin, ceftriaxone and ciprofloxacine, dexamethasone, enoxaparin sodium, later also with carvedilol, furosemid and clopidogrel.With this therapy the patient feels better, with improvements in the laboratory findings: sedimentation (8mm/h), CRP 1.2mg/l, glycaemia of 3.88mmol/l, D-dymers 2292ng/ml.The patient was discharged clinically stabile, with improvement in his X-ray and ultrasound findings and without need for supplemental oxygen and further analyzes for the origin of the pleural effusion were recommended.Results Biochemical analysis suggested exudation with inflammatory origin, borderline towards specific (TB) etiology. Microbiology analyses for tuberculosis were performed both on pleural effusion liquid and sputum. Pleural effusion liquid (punctate) was negative for ARB, with growth on Lowenstein-Jensen culture and Middlebrook7H9 to be reported, GeneXpert MTB/Rif Ultra reported trace positive and rifampicine resistance could not be determined leaving room to doubt whether or not patient has active TB. Finally 6 weeks after inoculation a culture positive result with growthon Middlebrook 7H9 medium from the pleural punctate confirmed a TB diagnosis, TBC ID test confirmed that growth is M. tuberculosis complex. The patient has started with 4 tuberculostatics (rifampicine, isoniaside, ethambutol and pyrasinamid), a regimen that is recommended to continue

in the following 6 months.Conclusions When a patient tests positive for SARS-CoV-2 it is a challenge to discover another underlying cause of respiratory symptoms.

Control Number: 2021-A-8499-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP117**

Session Title: **FEMS: Infectious Diseases (Virus)**

Topic 1: FEMS - Infectious diseases

Keyword 1: Clostridioides difficile infection

Keyword 2: Clostridioides difficile

Keyword 3: ribotype

Abstract Title: **Ribotypes Of *Clostridioides Difficile* At The University Hospital During The Covid-19 Pandemic**

Author Block: **K. Curová**[1], A. Toporová[1], L. Ambro[1], M. Novotný[1], M. Krůtová[2], O. Zahornacký[1], L. Siegfried[1]; [1]Med. Faculty Univ. of Pavol Jozef Safarik, Košice, Slovakia, [2]Med. Faculty Charles Univ., Praha 5, Czech Republic

Abstract Body: Background:*Clostridioides difficile* infection (CDI) has become a serious health problem worldwide in recent years. The severity of CDI lies in the ability to spread epidemically in hospitals and in frequent diseases relapses. The novel coronavirus disease (COVID-19), caused by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), has emerged in Wuhan in early December 2019 and it has rapidly spread worldwide causing a major pandemic. The large use of broad-spectrum antibiotics during the actual COVID-19 pandemic raises serious concerns about a consequent possible increase of CDI. *Clostridioides difficile* (CD) is important pathogen in healthcare settings as the leading cause of diarrhea associated with antibiotic treatment and high morbidity and mortality.Objectives: The main objective of this study was to analyse the molecular characteristics of CD isolates from local patients during the COVID-19 pandemic in the period from December 2020 to January 2021.Methods:Stool samples of hospitalized patients were tested for the presence of enzyme glutamate dehydrogenase (GDH) and toxin A/B using the *C.difficile* rapid test. Subsequently, all GDH positive and toxin positive/negative stools were cultured for CD on Brazier´s agar. Identification was confirmed by MALDI TOF mass spectrometry. Genes t*cdA*, *tcdB*, *cdtA* and *cdtB* encoding toxin A, toxin B and binary toxin were investigated by multiplex PCR. CD ribotypes (RTs) were assesed by capillary ribotyping.Results:A total of 314 stool samples were collected. Of these, 35 (33 GDH and toxin positive and 2 GDH positive and toxin negative) non-duplicate stool samples were cultured for CD. Culture was positive in 28 stool samples. A total of 29 CD isolates were identified and analyzed. In one stool sample, two different CD isolates were cultured and molecular analysis confirmed two RTs (RT 176 and RT 001). Molecular typing of CD confirmed toxin A as well as toxin B in 28 isolates (97%) and binary toxin in 15 isolates (52%). RT 176, which is characterized by production of all 3 toxins, was the most prevalent and was detected in 15 isolates (52%). Toxin A and toxin B producing RT 001 was confirmed in 10 isolates (35%), RT 014 in 2 isolates (7%), RT 020 in 1 isolate (3%) and toxin negative RT 010 in 1 isolate (3%). The high incidence of RT 176 in local patients emphasises the need to implement strict epidemic measures and the importance of implementing continuous surveillance programmes for CDI at local and national level.

Control Number: 2021-A-8618-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP117**

Session Title: **FEMS: Infectious Diseases (Virus)**

Topic 1: FEMS - Infectious diseases

Keyword 1: coronavirus

Keyword 2: virology

Keyword 3:

Abstract Title: **Multi-inflammatory Syndrome In Adults With Covid-19: A Report Of Two Cases**

Author Block: **R. Ghafoor**; Aga Khan Univ. Hosp., Karachi, Pakistan

Abstract Body: **Background**SARS CoV-2 acquired by children leads to a Kawasaki like illness that has been titled as multi-inflammatory syndrome in children (MIS-C)A similar state associated with COVID 19 has been described on rare occasion in adults. However there is a a paucity of literature on the topic, and no cases have been described from South Asia.**Methods**We present two cases of MIS-A . Case 1 describes a 32 year old healthy gentleman with no prior chronic illness who came with lethargy and abdominal pain for one week followed by multiple episodes of diarrhea for five days. Patient was received hypotensive in ER. He was not hypoxic and on presentation chest xray did not have any infiltrates. He was fluid resuscutated but remained hypotensive and subsequently started vasopressors due to marked vasoplegia. initial screening SARS CoV-2 PCR was negative. CT chest did not report typical findings of COVID19. He had multiorgan dysfunction with shock, acute kidney injury, hepatitis, pancytopenia and pancreatitis. on Day 7 of admission he was retested for SARS CoV-2 PCR which was positive. He was managed with steroids and improved. Our 2nd case is of a 25 year old gentleman presented with left sided neck pain and swelling for a week followed by high grade fever, abdominal pain and diarrhea. He had unilateral cervical lymphadenopathy. On presentation he was hypotensive and tachypneac but not hypoxic. similar to our first case, he had multiorgan dysfunction with marked leukocytosis, acute kidney injury, myocarditis, hepatitis and pancreatitis. He required vasopressor support. While not hypoxic on arrival he subsequently required Non-Invasive ventilatory support. CT chest showed pulmonary edema. His COVID IgG antibodies were positive. In both cases Viral serology for HIV, EBV, CMV, Hepatitis A, B, C, E, Brucella and Dengue were negative. Malaria was ruled out. Cultures did not yield any growth. autoimmune profiles were negative.
ResultsBoth patients were managed with steroids and made complete recovery**Conclusions**Due to challenges in timely recognizing this unusual presentation of COVID-19, it may be under reported. It is imperative to spread awareness of MIS-A to ascertain its accurate incidence.

FEMSP118 FEMS: Miscellaneous

Control Number:	2021-A-7926-MICROBE
Session Type:	**iPoster**
Session Number:	**FEMSP118**
Session Title:	**FEMS: Miscellaneous**
Topic 1:	FEMS - Eukaryotic microbiology and biotechnology
Keyword 1:	LSDV
Keyword 2:	GTPV
Keyword 3:	Vaccines
Abstract Title:	**Genome Comparison And Structural Proteomics Of LSD Virus And Goat Pox**
Author Block:	**J. Joty**; Univ. of Sci. and Technology Chittagong, Chittagong, Bangladesh
Abstract Body:	Lumpy skin disease virus and Variola caprina (goat pox/sheep pox) viruses, responsible for lumpy skin disease in cattle and pox in goats and sheeps have financial consequences for farming. They are categorized in the genus Capripoxvirus within the Poxviridae family. Due to the genome resemblance, SPPV vaccines have been widely used against LSDV. The present study attempts to validate the use of the SPPV/GTPV vaccines to LSDV by employing computational biology tools and bioinformatics analysis, predicting their genes and protein components same proteins - IL-18 binding protein, G protein coupled receptor, chemokine receptor, epidermal growth factor like proteins responsible for vaccine efficiency. In addition, we also attempted to accomplish gene and protein prediction, localization of targeted proteins, alignment and their 3D structures.

Control Number: 2021-A-8170-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP118**

Session Title: **FEMS: Miscellaneous**

Topic 1: FEMS - Health and food microbiology

Keyword 1: Magnetotactic bacteria

Keyword 2: Hyperthermia

Keyword 3:

Abstract Title: **Magnetotactic Bacteria As Hyperthermia Agents**

Author Block: **L. Gandarias**[1], D. Gandia[2], A. García Prieto[3], A. García Arribas[1], A. Muela[1], M. Fernández Gubieda[1]; [1]Univ. del País Vasco (UPV/EHU), Leioa, Spain, [2]Basque Ctr. for Materials Applications and Nanostructures, Leioa, Spain, [3]Univ. del País Vasco (UPV/EHU), Bilbao, Spain

Abstract Body:
Background: According to the World Health Organization (WHO) cancer is the second leading cause of death worldwide which makes it essential to investigate novel strategies that may complement actual cancer treatments such as surgery, chemo and radiotherapy. There is an outgrowing research on the use of bacteria as nanorobots to treat certain diseases because of their inherent properties such as their ability to self-propel and swim reaching inaccessible regions, their potential to sense and respond to external signals and the possibility to be functionalized with certain drugs or molecules that may enhance their efficacy in certain treatments. Among these nanorobotic microorganisms, magnetotactic bacteria (MTB) show a fundamental advantage, their ability to synthesize magnetic nanoparticles (magnetosomes) that make them not only easy to guide and be detected by using external magnetic fields, but also to be used as hyperthermia agents.
Objectives: In this work we assess the efficiency of magnetotactic bacteria as magnetic hyperthermia agents.
Methods: We studied the performance of *Magnetospirillum gryphiswaldense* MSR-1 in an *in vitro* hyperthermia study with A549 human lung carcinoma cells by applying an alternate magnetic field (150 kHz, 435 Oe, 45 minutes) to cells that had previously internalized MTB, and checking cell viability afterwards. We also studied the endocytic route the cells used for bacterial internalization by means of microscopic techniques and flow cytometry in combination with endocytosis inhibitors, to try and elucidate if it was an active process.
Results: Our results are very promising as they show that human lung carcinoma cells actively internalize magnetotactic bacteria without suffering any decrease in their viability and that the magnetic hyperthermia treatment is effective as it reduces significantly the number of cells present in the culture and their ability to replicate [1].

Control Number: 2021-A-8169-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP118**

Session Title: **FEMS: Miscellaneous**

Topic 1: FEMS - Miscellaneous

Keyword 1: gut microbiota

Keyword 2: reference laboratory

Keyword 3: anaerobiosis

Abstract Title: **Human Microbiota Reference Laboratory: Anaerobiosis Work-station For Optimization Of Microbial Culturing And Its Impact On Nutrition And Health**

Author Block: **K. Cerk**, P. Ortiz, M. Úbeda, A. López-Moreno, A. Torres-Sánchez, A. Ruiz-Moreno, J. Pardo, M. Aguilera; Dept. of Microbiol., Faculty of Pharmacy, Univ. of Granada, Campus of Cartuja, Granada 1, Granada, Spain

Abstract Body: Advances in the current knowledge and research on human microbiota has revealed that our gut microbiota plays a central role in human health and disease development. Dysbiosis, an imbalance in the composition and metabolic capacity of the gut microbiota, is associated with many common diseases such as obesity, inflammatory bowel disease, diabetes and cancer. Therefore, it becomes increasingly important to investigate the parameters and biomarkers of the gut microbiota to contribute and make continuous improvement in aspects of health in order to intervene and palliate dysbiosis. Hence, advances in culturomics that consist on finding multiple culture conditions combined with the rapid identification of bacteria, will extend our understanding of the human microbiota and the potential biotechnological uses linked to their implications for human health. Due to the fact that most of the intestinal bacteria are widely considered to be unculturable, especially strict anaerobes, such bacteria have never been isolated or well characterized in the laboratory yet. Although culture-independent genomic approaches have allowed us to expand our knowledge of the role of the human microbiome in health and disease, culture-dependent approaches are still required to understand their characteristic features and phenotypes so as to identify key microorganisms in the production of bioactive molecules of biotechnological interest. In this sense, it is of major importance to be provided with the appropriate equipment in a reference laboratory that allows the increase of the growth rate of the microbiota considered unculturable, through the combination of anaerobic cultivation techniques in conjunction with the maintenance of the conditions in their natural environments. Basic infrastructure needed can be summarized as follow: anaerobiosis cabinets, CO_2 incubators, positive and negative pressure cabinets, class II biological safety cabinet and autoclave. In addition, this specific infrastructure will allow to work in alignment and according to standardized and explicit Standard Operating Procedures describing microbiota sampling, anaerobiosis culturing, microbiota manipulation and analyses, to obtain harmonized data of higher quality and accuracy in the manipulation of the human microbiota.

Control Number: 2021-A-8185-MICROBE
Session Type: **iPoster**
Session Number: **FEMSP118**
Session Title: **FEMS: Miscellaneous**
Topic 1: FEMS - Miscellaneous
Keyword 1: Gut microbiota
Keyword 2: Metaproteomics
Keyword 3: Host-microbe relationship
Abstract Title: **Metaproteomic Analysis Of Luminal Content Microbiota From Colon Cancer Patients**
Author Block: **A. Tanca**, M. Abbondio, G. Fiorito, G. Pira, R. Sau, M. Muroni, A. Manca, A. Porcu, A. M. Scanu, P. Cossu-Rocca, M. De Miglio, S. Uzzau; Univ. of Sassari, Sassari, Italy
Abstract Body:
Background
Recent studies have provided evidence of interactions among the gut microbiota, local host immune cells and intestinal tissues in colon carcinogenesis. However, little is known regarding possible associations between the functions exerted by the intestinal microbiota and colon cancer, particularly with respect to tumor clinical classification and lymphocyte infiltration. In addition, stool, usually employed as proxy of the gut microbiota, cannot fully represent the original complexity of colon cancer microenvironment.
Objectives
This pilot study was aimed at characterizing the metaproteome of tumor-associated colonic luminal contents and evaluating its potential to identify associations between gut microbial protein functions and colon cancer clinicopathological features, namely tumor stage, tumor grade and Tumor Infiltrating Lymphocytes (TILs).
Methods
Luminal content samples were collected in operatory room before surgery from 24 colon cancer tissue specimens. After protein extraction and digestion, peptide mixtures were analyzed by high-resolution mass spectrometry. Bioinformatic analysis allowed peptide identification, label-free quantification and taxonomic/functional annotation. For each clinical variable, we identified the set of most discriminating peptides through a permutation-based sPLS regression approach. Significantly enriched taxa, functions and metabolic pathways were identified for each set of discriminating peptides, performing a sensitivity analysis considering covariate impact (age, sex and other clinical variables) and adjusting for multiple testing through a premutation-based approach.
Results
We identified 294, 94 and 568 microbial peptides discriminating for tumor stage, grade and TILs, respectively. Proteins produced by *Bifidobacterium* were found significantly enriched in high-stage tumors, whereas those expressed by *Bacteroides* spp. were over-represented in high-grade and TIL-negative tumor samples. Furthermore, microbial enzymes involved in tetrahydrofolate

interconversion, glutamine biosynthesis and galactose catabolism were enriched in the colonic luminal metaproteome of high-stage/grade tumors.

Control Number: 2021-A-8279-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP118**

Session Title: **FEMS: Miscellaneous**

Topic 1: FEMS - Miscellaneous

Keyword 1: Microbial Resources

Keyword 2: Research Infrastructure

Keyword 3: Innovation

Abstract Title: **The European Microbial Resource Research Infrastructure Providing Solutions For A Green, Healthy And Sustainable Future**

Author Block: **R. Aznar**, J. López-Coronado, L. Rodrigo-Torres, P. Ziarsolo, A. Zuzuarregui; CECT-Univ. of Valencia, Paterna, Spain

Abstract Body:

BackgroundThe Microbial Resource Research Infrastructure (MIRRI, www.mirri.org) is the pan-European distributed Research Infrastructure for the preservation, systematic investigation, provision and valorisation of microbial resources and biodiversity. It currently brings together around 50 microbial domain Biological Resource Centres (mBRCs), culture collections and research institutes from ten European countries and one associated country. In the European Strategy Forum on Research Infrastructures (ESFRI) Roadmap since 2010, now on its Health & Food domain, MIRRI is striving to soon establish the European Research Infrastructure Consortium (ERIC).

ObjectivesMIRRI-ERIC aims to improve mBRCs complementarity, reduce redundancy, and increase the capacity of its Partner mBRCs to preserve microbial materials, in a cost-effective and coordinated way, contributing to the reproducibility, integrity and cumulative character of research. The core of the functioning of MIRRI will be the Collaborative Working Environment (CWE), a digital dynamic platform that will bring together MIRRI partners and users of microbial resources and services.

MethodsThe CWE is being built under the Work Package 6 of the ongoing EU project “Implementation and Sustainability of Microbial Resource Research Infrastructure for 21st Century” (IS_MIRRI21, ismirri21.mirri.org), where 14 institutions from 9 European and an associated country are participating in the implementation of the microbial resources database and services, online tools, and procedures that will allow users to communicate and to access information in a time-efficient manner, through 4 Gates:1.- Research Infrastructure Information2.- Microbial Resources, Data and Services3.- Collaboration & Experts4.- Training and Education (T&E)

ResultsThrough the CWE, MIRRI builds a single point of access to a broad range of high-quality microorganisms (including their derivatives and associated data) and services, ranging from general to more application-specific ones (including pipelines, made available as tailor-made, turnkey solutions); expert advice on microbial resources related aspects such as taxonomy, legal use or biosafety aspects, among other; and training programmes on the use and preservation of microorganisms to promote professional

development of mBRC and Culture Collection personnel. Interactions facilitated by the CWE will enable microbe-based innovation supporting the circular bioeconomy for a green, healthy and sustainable future.

Control Number: 2021-A-8340-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP118**

Session Title: **FEMS: Miscellaneous**

Topic 1: FEMS - Miscellaneous

Keyword 1: CRISPRi

Keyword 2: gRNA efficiency prediction

Keyword 3: machine learning

Abstract Title: **Automated Inference Of Crispri Guide Efficiency In Bacteria From Genome-wide Essentiality Screens**

Author Block: **Y. Yu**, S. Gawlitt, C. Beisel, L. Barquist; Helmholtz Inst. for RNA-based Infection Res., Würzburg, Germany

Abstract Body: New CRISPR-based technologies are increasingly being applied to manipulate the genome and transcriptomes of microbes. Despite this, deriving design rules for optimal guide RNAs remains challenging.Genome-wide screens offer one potential source of information, as depletion in a screen reflects a mixture of fitness effects and guide efficiency. By applying automated machine learning to data frompublicly available CRISPRi screens, we show that it is possible to accurately predict the depletion of guides targeting essential genes using a combination of genomic, sequence, thermodynamic, andtranscriptomic features. We then utilized mixed-effects random forest (MERF) model to separate the contribution of features that can be manipulated in guide design from gene-intrinsic features to produce apredictive model for guide efficiency and compared it with median subtracting method. We demonstrate that both MERF and median subtracting models predict guide efficiency in flow-cytometry basedassays and high-throughput screening and outperforms existing tools for guide design intended for genome editing. Our study not only addresses the need for an effective tool for CRISPRi guide design, butalso this robust approach provides a blueprint for the development and interpretation of predictive models for other CRISPR-based technologies.

Control Number: 2021-A-8385-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP118**

Session Title: **FEMS: Miscellaneous**

Topic 1: FEMS - Miscellaneous

Keyword 1: parasites

Keyword 2: climate change

Keyword 3:

Abstract Title: **The Effect Of Climate On Ectoparasite Communities**

Author Block: **K. Davis**; Univ. of Utah, Salt Lake City, UT

Abstract Body: **Background:** Many factors determine where a species lives. One factor currently reshaping species distribution is climate change, which is projected to have a large impact on birds (Foden *et al.* 2019). Climate change may also affect lice, mites, fleas, and flies that parasitize birds (García del Río *et al.* 2020). **Objectives / Methods**: To understand how avian ectoparasite composition might be influenced by environmental change, we compared the ectoparasite communities on songbirds from birds from several arid and humid environments. **Results:** Fleas and flies were only rarely recovered, perhaps because these mobile parasites can leave birds while they are in a mist net. Less mobile parasites like lice and mites were found much more frequently. We found that ectoparasite intensity varied among locations, with ectoparasite prevalence and intensity being lowest at the most arid field site. Our results suggest that ectoparasite diversity will decline in areas where the environment becomes more arid.

Control Number: 2021-A-8603-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP118**

Session Title: **FEMS: Miscellaneous**

Topic 1: FEMS - Miscellaneous

Keyword 1: antimicrobial stewardship

Keyword 2: infection prevention and control

Keyword 3: capacity assessment

Abstract Title: **Antimicrobial Stewardship And Infection Prevention And Control Capacity Assessment Of Three Health Facilities In Ghana.**

Author Block: **O. K. O. Amponsah**, A. Owusu-Ofori, N. Ayisi-Boateng, J. Attakorah, M. N. A. Opare-Addo, K. O. Buabeng; Kwame Nkrumah Univ. of Sci. and Technology., Kumasi, Ghana

Abstract Body:

Background: Tackling antimicrobial resistance (AMR) requires optimal utilization of available resources for sustainable impact. It is therefore important that systems available to support the prevention and control of AMR in health facilities are evaluated and capacity strengthened. This study aimed to assess antimicrobial stewardship capacity and institutional conformance to National and WHO's Infection Prevention and Control (IPC) strategies in three healthcare facilities in the Ashanti region of Ghana coded H1, H2 and H3.

Methods: This was a cross-sectional study conducted by adapting and using the hospital questionnaire in the WHO Methodology for Point Prevalence survey on antibiotic use for antimicrobial stewardship (AMS) capacity assessment. In addition, the Infection Prevention and Control Assessment Framework (IPCAF) at the facility level questionnaire with scoring system to measure the level of IPC implementation according to the eight WHO core components was employed to assess the IPC systems in the facilities. The data obtained was entered into a REDCap® database and exported into Stata™ 14 for analyses.

Results: All the facilities had Drug and Therapeutics (DTC) and IPC Committees with access to microbiology laboratory services. Institutions H3 and H1 did not have a formal AMS or an Organizational structure for AMS. H3 and H1 again did not have a continuously updated antibiotic formulary or guideline, however, the facilities had a formal procedure to review antibiotics on prescriptions for relevance, quality and supply. Institutions H2 and H1 do not participate in any surveillance for data on antibiotic resistance patterns or antibiotic use. In relation to IPCAF, H1 had a basic level system while both H2 and H3 had intermediate level IPC systems scoring 385, 487.5 and 435.8 out of 800 respectively.

Conclusion: All the institutions assessed had gaps identified requiring strengthening to ensure responsible antimicrobial use and efficient IPC strategies implementation. All the institutions need to regularly undertake antimicrobial consumption and microbiological laboratory surveillance on AMR to generate data to support AMS and of IPC guidelines implementation to support global and national efforts on AMR containment.

Control Number: 2021-A-8661-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP118**

Session Title: **FEMS: Miscellaneous**

Topic 1: FEMS - Miscellaneous

Keyword 1: evolution

Keyword 2: lactic acid bacteria

Keyword 3: genomics

Abstract Title: **Evolutionary History And Lifestyle Adaptation Of Lactic Acid Bacteria**

Author Block: **S. Wittouck**[1], E. P. C. Rocha[2], V. van Noort[3], S. Lebeer[1]; [1]Univ. of Antwerp, Antwerpen, Belgium, [2]Pasteur Inst., Paris, France, [3]KULeuven, Leuven, Belgium

Abstract Body:

Background

Lactic Acid Bacteria (LAB) are known for their role in food fermentation and for their association with vertebrate and invertebrate host species. A few different clades are considered to be LAB, but the largest one is the order *Lactobacillales* within the phylum *Firmicutes*. Although one or more genomes from more than 800 species of *Lactobacillales* have been sequenced, the evolutionary history of this order has not yet been extensively characterized and it is yet unclear how they adapted to their wide variety of habitats and lifestyles, ranging from free-living to exclusively adapted to an animal host.

Objectives

Our goal was to identify trends in the evolutionary history of the order *Lactobacillales*, as well as their adaptation to various lifestyles, using large-scale comparative genomics analyses.

Methods

We inferred the pangenome (collection of all gene families) of 4,371 dereplicated genomes of *Lactobacillales* from 838 species and explored it in various ways. First, we categorized all gene families as ancestral (present in the *Lactobacillales* ancestor) or acquired. We explored the loss of ancestral genes over the course of evolutionary history of *Lactobacillales*, as well as the gain of acquired genes. Second, we identified gene families that expanded their copy numbers through multiple gene duplications. And third, we looked for gene families that are associated with a free-living or host-adapted lifestyle.

Results

Compared to the other families of *Lactobacillales*, two families were characterized by an extensive loss of ancestral genes. One of them was the family *Lactobacillaceae*, which is known for its many species with probiotic potential. Further, we identified a number of gene families that expanded heavily over the course of *Lactobacillales* evolution, leading to copy numbers of up to 10-15 in some extant

species. Two of the strongest expanders were both families of amino acid carrier proteins. Finally, we found that the gene content of *Lactobacillaceae* species is associated with their lifestyle. Several gene families were identified as characteristic for a free-living lifestyle, while a smaller number was associated with a host-adapted lifestyle. Most of these lifestyle-associated genes were of unknown function. However, we could identify two of the free-living-specific genes as a methionine synthesis gene and an oxidative stress resistance gene.

Control Number: 2021-A-8793-MICROBE

Session Type: **iPoster**

Session Number: **FEMSP118**

Session Title: **FEMS: Miscellaneous**

Topic 1: FEMS - Miscellaneous

Keyword 1: alcohol dehydrogenase

Keyword 2: oxidative resolution

Keyword 3: microbial biotechnology

Abstract Title: **Revisiting The Secondary Alcohol Dehydrogenases Of Genus Thermoanaerobacter**

Author Block: **E. M. Ingvadottir**, J. Orlygsson, S. M. Scully; Univ. of Akureyri, Akureyri, Iceland

Abstract Body: Genus *Thermoanaerobacter* produces both primary and secondary alcohol dehydrogenases (PADHs and SADHs), with the latter being particularly well described in the literature for *T. pseudoethanolicus* and *T. brockii*. As SADHs allow for the enantioselective oxidation of chiral alcohols, they present a valuable tool for chiral resolution, yielding enantiomerically pure building blocks with uses across industries. Recently, we mapped the substrate specificity of NAD and NADP-linked ADHs within genus *Thermoanaerobacter* and *Caldanaerobacter*. Furthermore, we assessed the impact of culture conditions (including temperature, peptone and/or alcohol supplementation) on both PADH and SADH activities for chosen strains. A number of the strains evaluated exhibited different specificities than those previously reported. Finally, resolution of historically difficult to resolve molecules - such as 1,2-alkanediols - was attempted with partially purified enzymes as well as whole-cell systems.

Control Number:	2021-A-8188-MICROBE
Session Type:	**iPoster**
Session Number:	**FEMSP118**
Session Title:	**FEMS: Miscellaneous**
Topic 1:	FEMS - Molecular microbiology and biochemistry
Keyword 1:	microbiota
Keyword 2:	bioinformatic
Keyword 3:	enzymes
Abstract Title:	**Bioinformatics Analysis On Cultivable Microbiota Species Harbouring Enzymes With Food And Health Biotechnological Potential**
Author Block:	J. Pardo-Cacho, **A. Torres-Sánchez**, A. López-Moreno, K. Cerk, Á. Ruiz-Moreno, M. Aguilera; Univ. of Granada, Granada, Spain
Abstract Body:	Identifying the composition of the human microbiota has always been a challenge due mainly to anaerobiosis. Moreover, the exposure of a wide range of xenobiotics, such as bisphenols (bisphenol A (BPA) and its analogues), has been suggested to affect the microbiome diversity each individual possesses, causing dysbiosis, that has been proved it could be responsible of well-known diseases such as obesity, diabetes and even some hormonal-related cancer. Furthermore, uncover the variety of microorganisms that inhabit the human gut not only could be of great interest for health purposes but also for their enzymatic and biotechnological potential. For instance, α-amylase, carboxymethylcellulase (CMCase), lipases and enzymes involved in the biosynthesis of polyhydroxyalkanoates (PHAs), extracellular polysaccharidic substances (EPSs) and polyketides are notably demanded by the food, health and biotechnological industry. Efforts in simulating these harsh conditions have made possible to isolate and characterise uncultured bacteria which were tolerant to bisphenol A. Additionally, phenotypical assays have also been taken in order to determine specific enzymatic activities from isolated colonies. The phenotypical results proved several bacteria were capable of producing amylase, inulinase, lipase/esterase and DNase. With the aid of phylogenetic studies (16S rRNA gene), interesting bacteria were completely identified. The aim of this study was to verify using bioinformatics tools and genome databanks the enzymatic expected searches and results obtained and to find out their potential to produce valuable enzymes for biotechnology. The bioinformatics analysis from the microbiota isolated specific species showed that some species from *Bacillus, Burkholderia* and *Acinetobacter* genera possess a common enzymatic arsenal that could entirely biodegrade BPA. Additionally, it was also determined that they are potentially capable of synthesising α-amylases, CMCases, and lipases. Biopolymers synthesis was also checked: PHA and EPS biosynthetic gene-encoding enzymes, and finally antimicrobial

potential e.g. polyketides. According to the specific analysis done on the available whole genome sequences of type strains, some species from the *Lactobacillus*, *Microbacterium*, *Kocuria* and *Micrococcus* genus harbour also the genes encoding some enzymes from BPA degrading-pathways, as well as several of the enzymes mentioned that could be useful for food and health biotechnological interests.

FEMS: Late-breaker iPoster Session

Control Number:	2021-A-7803-MICROBE
Session Type:	**iPoster**
Session Number:	
Session Title:	**FEMS: Late-breaker iPoster Session**
Topic 1:	FEMS - Molecular microbiology and biochemistry
Keyword 1:	two-component regulatory systems
Keyword 2:	Pseudomonas putida
Keyword 3:	Acetate metabolism
Abstract Title:	**Physiological Role Of The Mxtr/erdr Two-component System In *Pseudomonas Putida* Kt2440**
Author Block:	**T. Henríquez**, H. Jung; LMU Munich, Planegg-Martinsried, Germany
Abstract Body:	**Background:** Two-component systems are elements that allow bacteria to sense and adapt to changing conditions in the environment. The MxtR/ErdR two-component system, also called CrbS/CrbR, was previously described to regulate the utilization of acetate in *Vibrio cholerae* and selected *Pseudomonas* species. An investigation of MxtR of *Pseudomonas aeruginosa* suggested a more global role of the system including the regulation of virulence factors such as RND efflux pumps and siderophore production. **Aim:** To analyze the physiological significance of MxtR/ErdR in the soil bacterium *Pseudomonas putida* KT2440. **Methods:** *mxtR* and *erdR* were individually deleted, and the impact of the deletions on the utilization of different carbon sources, siderophore production, and the expression of genes related to the utilization of acetate and to RND efflux pumps was analyzed. **Results:** Our results indicated that MxtR/ErdR is active in *P. putida* KT2440 and required for the utilization of acetate. Genes found to be related to the two-component system are *acsA-I* (acetyl CoA synthetase)*, scpC* (putative acyl CoA transferase), and pp_0354 (unknown function). Evidence for an impact of MxtR/ErdR on siderophore production or RND efflux pump related to drug resistance was not found. Taken together, the results indicate a specific role for MxtR/ErdR in the regulation of the utilization of acetate in *P. putida.*

Control Number: 2021-A-7809-MICROBE

Session Type: **iPoster**

Session Number:

Session Title: **FEMS: Late-breaker iPoster Session**

Topic 1: FEMS - Molecular microbiology and biochemistry

Keyword 1: HMOs

Keyword 2: infant microbiota

Keyword 3: glycosidases

Abstract Title: **Consumption Of Human Milk Oligosaccharides By Infant-gut Associated Strains Of Lactic Acid Bacteria**

Author Block: **E. M. Moya-Gonzálvez**[1], A. Rubio-del-Campo[1], J. Rodríguez-Díaz[2], M. J. Yebra[1]; [1]IATA-CSIC, Valencia, Spain, [2]Univ. de Valencia, Valencia, Spain

Abstract Body: Human Milk Oligosaccharides (HMOs) are the third largest solid component in human milk.They are non-digestible carbohydrates that reach the infant large intestine where they aremetabolized by specific bacteria. Consumption of HMOs promotes their growth, shaping anintestinal microbiota with health benefits for the infants. The objective of this work was toevaluate the capability of lactic acid bacteria isolated from breast-fed infant faeces to grow inthe presence of HMOs. In addition, glycosidases involved in HMOs hydrolysis have beencharacterized.Faeces from four infants between one and three months old were plated in different selectivemedia to isolate lactic acid bacteria. One hundred fifty colonies were randomly chosen andsubjected to RAPD-PCR analysis. One representative colony of each band pattern was used forspecies identification by 16S rRNA gene sequencing. We finally selected twelve differentstrains belonging to the genera *Bifidobacterium, Lacticaseibacillus, Lactobacillus,Limosilactobacillus Enterococcus, Staphylococcus a*nd *Streptococcus* and their ability tometabolize HMOs was tested. The strains *Enterococcus faecalis* (Y513, Y533),*Limosilactobacillus fermentum* (Y500), *Lactobacillus gasseri* (Y511), *Lacticaseibacillusparacasei* (Y526), *Limosilactobacillus reuteri* (Y501), *Staphylococcus epidermidis* (Y520),*Staphylococcus hominis* (Y549) and *Streptococcus pasteurianus* (Y529), were not able to growin the presence of the tested HMOs. However, *Bifidobacterium longum* (Y538) was able togrow in all tested HMOs: 2'-fucosyl-lactose (2'-FL), 3-fucosyl-lactose (3-FL), difucosyllactose(DFL), lacto-*N*-tetraose (LNT) and lacto-*N*-neotetraose (LNnT). Both *Bifidobacterium dentium*isolated strains (Y510, Y521) partially consumed LNT and LNnT. Curiously, lacto-*N*-triose(LNTII) was detected in the culture supernatant of both strains when grown in either tetra-saccharide. We have cloned, purified and characterized two β-galactosidases (BDG3 andBDG4) from *B. dentium* strain Y510. Both are exo-β-galactosidases. BDG3 releases galactosefrom LNT with a high specific activity compared to

that observed for the other substrates tested,suggesting a key role in the metabolism of this HMO. BDG4 showed the highest specificactivity for lactose and also efficiently hydrolyses 6’-galactopyranosyl-GlcNAc, LacNAc andLNnT. The results obtained support the hypothesis that HMOs selectively promote the growthof specific bacteria in the gastrointestinal tract of infants.

Control Number: 2021-A-7909-MICROBE

Session Type: **iPoster**

Session Number:

Session Title: **FEMS: Late-breaker iPoster Session**

Topic 1: FEMS - Molecular microbiology and biochemistry

Keyword 1: molecular typing

Keyword 2: spoligotyping

Keyword 3: Mycobacterium bovis

Abstract Title: **Molecular Typing Of Mycobacterium Bovis In Cattle In Bulgaria**

Author Block: **V. Valcheva**[1], T. Savova-Lalkovska[2], A. Dimitrova[2], M. Bonovska[3], H. Najdenski[3]; [1]Inst. of microbiology,Bulgarian Academy of Sci., Sofia, Bulgaria, [2]Natl. Diagnostic and Res. Vet. Med. Inst., Sofia, Bulgaria, [3]Inst. of microbiology, Bulgarian Academy of Sci., Sofia, Bulgaria

Abstract Body: **Background** Bovine tuberculosis (bTB) is an important zoonosis with serious implications for livestock farming and public health. Despite considerable efforts over decades to eradicate bTB by intensive test and slaughter programs, it remains a significant economic burden to the agriculture and *Mycobacterium bovis* infection levels in cattle in Bulgaria continued to rise over recent years.**Objectives** In order to identify the sources of infection as well as the spread of the agents, molecular-epidemiologic tracing by spoligotyping was performed. **Methods** A total of 30 *M*. *bovis* isolates from cattle originating from different regions of Bulgaria were cultured from tuberculous bovine lymph nodes and analyzed by spoligotyping for strain differentiation. The data were compared to the international databases Mbovis.org and SITVIT for shared type and clade assignment. **Results** The isolates were subdivided into 4 spoligotypes: 2 types shared by 20 and 8 isolates and 2 singletons. SITVIT-defined types SIT645 and SIT647 belonged to the common and classical bovine ecotype *M. bovis* (9 isolates) while types SIT120 and SIT339 belonged to the *M. caprae* ecotype (21 isolates). A certain phylogeographic gradient of the spoligotypes and clades at within country level was observed: *M. caprae* was prevalent in the central/southwestern, while classical *M. bovis* in the northeastern Bulgaria. New information was added to the global database in the field of molecular epidemiology of the prevalence of M. bovis strains in the cattle population in Bulgaria. This study provided a first insight into phylogeography of *M. bovis* in Bulgaria and described, for the first time, *M. caprae* isolates identified in this study mostly belong to the Central/Eastern European cluster and is an important infection agent of bTB in Bulgaria.

Control Number: 2021-A-7930-MICROBE

Session Type: **iPoster**

Session Number:

Session Title: **FEMS: Late-breaker iPoster Session**

Topic 1: FEMS - Molecular microbiology and biochemistry

Keyword 1: Mobile genetic element

Keyword 2: antimicrobial resistance

Keyword 3: Fitness

Abstract Title: **Imerm46, A Novel Integrative Mobilizable Element Associated With Antimicrobial Resistance And Pathogenicity In The Zoonotic Pathogen Rhodococcus Equi**

Author Block: **S. Alvarez-Narvaez**, S. Sanchez; Univ. of Georgia, Athens, GA

Abstract Body: Horizontal gene transfer via conjugative plasmids is one of the main mechanisms by which bacteria acquire and exchange antimicrobial resistance genes (ARGs). Recent data suggest that the interactions between plasmids and other mobile genetic elements (MGEs) of the bacterial accessory genome play an important role in aiding the horizontal transfer and expanding the genetic range of ARGs. The recently discovered pRErm46 plasmid from zoonotic pathogen *Rhodococcus equi* is a 90-kb conjugative genetic element that carries ARGs for macrolide, lincosamide, streptogramin B, tetracycline, and sulfamethoxazole. Although pRErm46 is still mainly confined in two clonal populations, pRErm46 can be horizontally transferred to several actinobacteria species, and surprisingly, its presence does not show a fitness cost associated *in vitro*. This study aims to identify and describe critical MGEs in the *R. equi* accessory genome involved in the maintenance and spread of pRErm46. We performed a deep genetic characterization of the accessory genomes of 200 SMRT-sequenced *R. equi* isolates, showing different antimicrobial resistance phenotypes collected between 2010 and 2017 in several US states. Our *in-silico* analysis identified a novel mobile genetic element of 14,293 bp associated with pRErm46. This novel amplicon seems to be an integrative mobilizable element (IME) that we nominated as IMErm46. It carries genes encoding its excision and integration via a serine recombinase but not genes necessary for their conjugative transfer. Additionally, IMErm46 encodes a Toll/interleukin-1 receptor (TIR) protein, a virulence factor that in other bacteria species acts by blocking TLR-mediated NF-kB signaling and thus suppressing innate immunity and increasing virulence; and a toxin-antitoxin (TA) system that in other bacteria be associated with antibiotic resistance/tolerance, virulence and pathogenicity islands, bacterial persistence, and pathogen trafficking.

Control Number: 2021-A-7942-MICROBE

Session Type: **iPoster**

Session Number:

Session Title: **FEMS: Late-breaker iPoster Session**

Topic 1: FEMS - Molecular microbiology and biochemistry

Keyword 1: amyloids

Keyword 2: yeast

Keyword 3: C-DAG

Abstract Title: **Aggregation Of Amyloid Proteins In Different Model Systems**

Author Block: **S. A. Bondarev**[1], A. B. Matiiv[1], X. V. Sukhanova[1], S. E. Moskalenko[2], G. A. Zhouravleva[1]; [1]SPbU, St. Petersburg, Russian Federation, [2]St Petersburg Branch, Vavilov Inst. of Gen. Genetics of the Russian Academy of Sci., St. Petersburg, Russian Federation

Abstract Body: Amyloids are protein fibrils with a cross-β structure. Numerous investigations of amyloids are at the top of interest due to the increasing incidence of amyloid-associated disorders, for instance, Alzheimer's disease, Parkinson's disease, type II diabetes, etc. The microbial cells are often used for the investigation of amyloids. The bacterial C-DAG (Curli Dependent Amyloid Generator) system allows monitoring of the formation of protein aggregates on the cell surface of *Escherichia coli* and analysis of their ability to bind the amyloid specific dye, Congo Red. Yeast *Saccharomyces cerevisiae* cells are one of the most well-known eukaryotic model system, which is also used for screening of amyloids. Aggregation-prone proteins fused with fluorescent proteins lead to the appearance of fluorescent foci in the yeast cells when overproduced. The bacterial and yeast models are well-known. However, the possibility of extrapolation of the results obtained in these systems is debatable. We analyzed several human amyloid proteins in these systems and compare the results. The human HEK293FT and IMR-32 cell lines were used as more complex models. The known amyloids like aSyn, TDP-43, UBQLN1, etc. were included in the test data set. In addition, we analyzed an aggregation of proteins, which are predicted as amyloids (NOS1AP, CHGB, PPP1R10, and UBQLN2). In most cases, the results obtained in microbial systems are consistent with each other, but we found several discrepancies with human model systems. For example, aSyn easily aggregates in yeast and bacterial cells, but not in human cell lines. The same results were previously reported in the literature. CHGB, PPP1R10 form aggregates in bacteria and yeasts, but not in mammalian cells. Only one result inconsistent between microbial systems was found. The UBQLN2 does not aggregate in bacteria. We demonstrated that protein aggregation depends on the model system. Thus, the results obtained in only one system should be considered and interpreted very accurately to escape preliminary conclusions.

Control Number: 2021-A-8081-MICROBE

Session Type: **iPoster**

Session Number:

Session Title: **FEMS: Late-breaker iPoster Session**

Topic 1: FEMS - Molecular microbiology and biochemistry

Keyword 1: Escherichia coli

Keyword 2: Genome-wide association analysis

Keyword 3: Virulence

Abstract Title: **Genetic Determinants Of *E. Coli* Extraintestinal Infection In A Murine Model And In The Clinic Revealed Through A Genome-wide Association Analysis**

Author Block: **M. Galardini**[1], O. Clermont[2], B. Condamine[2], G. Royer[2], M. Esposito-Farese[3], A. Baron[2], B. Busby[4], S. Dion[2], S. Schubert[5], C. Laouénan[2], A. Lefort[2], V. de Lastours[2], P. Beltrao[6], E. Denamur[2]; [1]Twincore, Hannover, Germany, [2]Université de Paris, Paris, France, [3]Assistance Publique – Hôpitaux de Paris, Paris, France, [4]EMBL, Heidelberg, Germany, [5]Ludwig-Maximilians-Univ. München, Munich, Germany, [6]EMBL-EBI, Cambridge, United Kingdom

Abstract Body: *Escherichia coli* is a leading cause of intestinal and extraintestinal infections, including bacteraemia which has a case fatality rate between 10% and 30%. Despite the prevalent role of host factors in determining the outcome of those infections, identifying microbial genetic elements that contribute to it is of interest to 1) better understand the molecular mechanisms of microbial infection, and 2) aid surveillance and screening efforts based on microbial genomics data. We applied a hypothesis-free genome-wide association analysis to two independent datasets spanning a total of 1280 *Escherichia* isolates with matching whole genomes. We first used a murine model of extraintestinal infection and sepsis, which reduces the influence of host factors, to find microbial genetic determinants of infection outcomes. We found that three iron-uptake systems (the High Pathogenicity Island, and the Aerobactin and *sitABCD* operons) present at intermediate frequency in the *Escherichia* pangenome (N=370) were strongly associated with death in mice. We further confirmed the iron-scavenging function of these three gene clusters through a correlation analysis with existing high-throughput growth data on solid agar, also showing that more virulent strains tend to grow better in the presence of sub-inhibitory concentration of various antimicrobials. We then used data from two large clinical observational prospective multicentric studies from the Paris area (Septicoli and Colibafi) aimed at identifying risk factors of mortality due to bacteraemia in a total of 910 adult patients. We used the clinical information from each patient, such as age, comorbidities and treatment, as covariates to reduce the influence of host factors in the association analysis. We find no association between microbial genetic elements and infection outcome, further confirming the overwhelming influence of other clinical factors. We do however find a clear signal between the presence of several genes and SNPs and the urinary and digestive portals of entry. Taken together these two analyses confirm the power of bacterial GWAS and the predominant role of host factors in determining the clinical outcome of *E. coli* bacteraemia.

Control Number: 2021-A-8166-MICROBE

Session Type: **iPoster**

Session Number:

Session Title: **FEMS: Late-breaker iPoster Session**

Topic 1: FEMS - Molecular microbiology and biochemistry

Keyword 1: Salmonella

Keyword 2: Chemical Genomics

Keyword 3:

Abstract Title: **A Deep Chemical Genomics Screen For Salmonella Typhimurium**

Author Block: **M. Galardini**[1], B. Pfalz[2], A. Telzerow[2], M. Zietek[2], G. Kritikos[2], H. Andrews-Polymenis[3], M. McClelland[4], P. Beltrao[5], A. Typas[2]; [1]Twincore, Hannover, Germany, [2]EMBL, Heidelberg, Germany, [3]Texas A&M Univ., Bryan, TX, [4]Univ. of California, Irvine, Irvine, CA, [5]EMBL-EBI, Cambridge, United Kingdom

Abstract Body: Chemical genomics is a valuable high-throughput approach to tackle the lack of functional characterization in microbial genomes. Here we present a deep chemical genomics screen for the important human pathogen *Salmonella typhimurium*. We tested two ordered knockout collections totalling 6'982 mutants belonging to 3'783 individual genes across 544 growth conditions, covering an unprecedentedly large chemical/physical stressors space. We were able to assign at least one gene-condition association for 71.8% of the tested mutants, or roughly half (49%) of the *Salmonella* genome, including members of the SPI pathogenicity islands. By using the correlation of mutants' growth across conditions, we derived putative gene interactions, which we validated with known physical and functional interactions such as protein complexes and metabolic modules. Even though these known interactions can be recovered with a fraction of the growth conditions we actually tested, we showed how interactions between known functional modules can only be recovered when performing a "deeper" screen with a larger number of conditions. Importantly, we found that this observation also applies when looking at the functional interaction between known genes and those of unknown function, for which a "guilt-by-association" annotation approach could be followed. As a comparable chemical genomic screen is available for another species in the order *Enterobacterales* (*Escherichia coli*), we combined the two screens, showing how we could reduce the limitations of each mutant library in recovering functional interactions, and how we could compare them to single out putative gene interactions rewiring between the two species. We anticipate that a similar exercise could be carried out as more similar dataset across species become available. Lastly, we highlight one example in which we followed up a specific growth phenotype and uncovered previously uncharacterized function for the *smvA* gene, which we found to be involved in resistance to the antidiabetic drug metformin.

Control Number:	2021-A-8240-MICROBE
Session Type:	**iPoster**
Session Number:	
Session Title:	**FEMS: Late-breaker iPoster Session**
Topic 1:	FEMS - Molecular microbiology and biochemistry
Keyword 1:	hydrophobin
Keyword 2:	cutinase
Keyword 3:	plastic depolymerization
Abstract Title:	**Assembling A Protein Chimera For Plastic Depolymerization**
Author Block:	**C. Pena Montes**[1], A. Bustos Baena[1], I. Bustos Jaimes[2], R. Oliart Ros[1], V. Urlaher[3]; [1]Technological Natl. of Mexico, Veracruz, Mexico, [2]Natl. Autonomous Univ. of Mexico, Ciudad de Mexico, Mexico, [3]Henrich Heine Univ. of Düsseldorf, Düsseldorf, Germany
Abstract Body:	Hydrophobins (HFB), microbial surfactans, are small hydrophobic proteins with surface activity forming amphiphilic films reducing water surface tension. HFB and cutinases participate in hydrolysis of cutin. Cutinases are hydrolases of esters of carboxylic acid used recently to depolymerize polyesters in plastics. HFB fused to enzymes, increased activity by taking advantage of the assembly mechanism, and exposed the active site. A chimera hydrophobin-cutinase can be helpful in plastic residues depolymerization constituting a current solution to problems of their accumulation. In this work, the objective was the heterologous functional expression of a hydrophobin-cutinase chimera for polyesters depolymerization based on an "*in silico*" structural model and docking. Cutinase gene was amplified by PCR using the pet*mrcut1* plasmid, and hydrophobin gene using pMAT- Hfb1 plasmid, both previously developed. Chimera *mrcut1-hfb1* was generated by Overlap extension PCR and inserted into pET22b plasmid, and *E. coli* BL21 was transformed. Zymograms and western blot corroborated chimera heterologous expression, which was purified by IMAC. Esterase activity on *p*-nitrophenyl butyrate and depolymerization of polycaprolactone (PCL) were evaluated. A structural model was developed in Robetta and I-TASSER. Analysis was carried out in PyMOL. Validation of the predicted model was performed with different servers like COLORADO-3D. Substrate molecule design was conducted in Avogadro-assisted MN-AM server. Chimera docking with designed substrate (PET dimer) was submitted to the DockThor. A recombinant hydrophobin-cutinase chimera with esterase activity was obtained. Its possible use for the depolymerization of synthetic polyesters such as PCL was confirmed. "In silico" modeling of the chimera was achieved with 90% validity (Figure 1). The affinity and possible interaction with the ester bond present in the dimer of the ester bond present in the PET dimer were displayed.

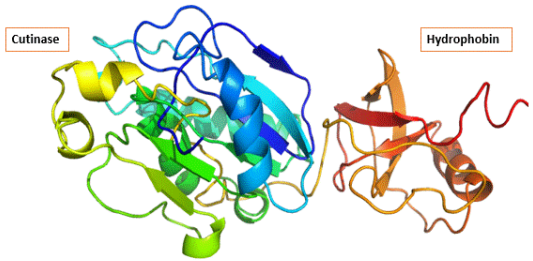


Figure 1. Chimera Proposed structural model with both cutinase and hydrophobin binding domains.

Control Number: 2021-A-8310-MICROBE

Session Type: **iPoster**

Session Number:

Session Title: **FEMS: Late-breaker iPoster Session**

Topic 1: FEMS - Molecular microbiology and biochemistry

Keyword 1: omics

Keyword 2: starters

Keyword 3: resilience

Abstract Title: **FROM STARTER -ASSISTED TO FERMENTOME -DRIVEN: A PARADIGM SHIFT IN SOURDOUGH FERMENTATION**

Author Block: **h. Ameur**; free Univ. of Bolzano, Bolzano (BZ), Italy

Abstract Body:

6th International Conference on Foodomics 2020

From starter-assisted to fermentome-driven: a paradigm shift in sourdough fermentation Hana Ameur[1], Fernandes Lemos Junior Wilson Jose[1], Olga Nikouloudaki[1], Maria De Angelis[2], Raffaella Di Cagno[1] Marco Gobbetti[1] [1]*Free University of Bolzano, Bolzano, Italy*[2]*University of Bari Aldo Moro, Bari, Italy*

The application of omics techniques helps to further unravel sourdough fermentation potential. Meta-genomic, culturomic, metabolomics, and meta-transcriptomics analyses of eight sourdoughs representative of different countries in the world were performed. Cultivable bacteria and yeast species identified by the culture-dependent methods were also identified by the metagenomic approach. Metagenomics analysis described the sourdough metagenome, including dominant bacterial and fungi strains and subdominant populations. The metabolic functions identified by KEGG strongly support the evidence of sourdough fermentation. Multi-copies genes encoding for enzymes involved in key sourdough metabolisms were identified in sourdoughs. Meta-transcriptomic profiles of the different sourdoughs confirmed the expression of core genes encoding for the biosynthesis or catabolism of amino acids by sourdough lactic acid bacteria. From the comparison of all omics data, emerged a clear picture of the potential metabolic background vs. metabolisms expressed under sourdough conditions. The ecological fundaments retrieved will ensure the resilience of sourdough-fermented doughs to various causes of disturbance. The results of this study will allow the industrial development of the most stable and performing mixture of microbes to drive the sourdough fermentation.

Control Number: 2021-A-8343-MICROBE

Session Type: **iPoster**

Session Number:

Session Title: **FEMS: Late-breaker iPoster Session**

Topic 1: FEMS - Molecular microbiology and biochemistry

Keyword 1: racemization

Keyword 2: epimerization

Keyword 3: α-hydroxy acid

Abstract Title: **Uncovering A Superfamily Of Nickel-Dependent α-Hydroxy Acid Racemases And Epimerases**

Author Block: **J. Urdiain-Arraiza**, B. Desguin; Louvain Inst. of Biomolecular Sci. and Technology (LIBST), Louvain-la-Neuve, Belgium

Abstract Body:

α-Hydroxy acids are organic compounds omnipresent in the living world and can be considered as building blocks in organic synthesis. However, very few racemases or epimerases of a-hydroxy acids are known and have been characterized. Among them, lactate racemase (LarA) was shown to require a unique nickel-containing cofactor, the nickel-pincer nucleotide (NPN) [1], in order to catalyze a proton-coupled hydride transfer (Fig. 1).

Interestingly, the genes coding for the NPN-biosynthetic enzymes and LarA homologs (LarAHs) are present in 12 % of bacterial and archaeal genomes and they are even present in some unicellular eukaryotes. An alignment of 2121 LarAHs sequences from 1103 species showed the diversity of sequences in this superfamily (Fig. 2), suggesting that some LarAHs could racemize or epimerize other substrates than lactate.

Our goal is to explore the variety of reactions catalyzed by the LarA superfamily. For this purpose, we heterologously expressed LarAHs of various phylogenetic groups of the LarA superfamily and assayed the activity of these LarAHs in presence of in vitro synthesized NPN and a variety of α-hydroxy acids by enzymatic assay or by capillary electrophoresis.

We discovered that LarA was active on very short chain a-hydroxy acids in addition to lactate. Other phylogenetic groups of LarAH were active on a-hydroxy acids with short side chains, on malate, on hydroxyglutarate, on bulky α-hydroxy acids or on gluconate (Fig. 2) [2]. We also discovered a large phylogenetic group of wide spectrum a-hydroxy acid racemases and epimerases that are active on 10 to 15 different a-hydroxy acids (Fig. 2). Some of these LarAHs could have industrial applications for the synthesis of rare sugars.

In conclusion, we discovered many new NPN-dependent racemases and epimerases of α-hydroxy acid and many are still to be discovered. The LarA superfamily and the LarAHs thus seem to form a superfamily of a-hydroxy acid racemases and epimerases with many promiscuous activities.

Figure 1: NPN cofactor and its catalytic mechanism. The NPN cofactor facilitates a proton-coupled hydride transfer mechanism. During

the reaction, the α chiral carbon of an α-hydroxy acid is transiently oxidized, generating an α-keto acid intermediate. The residues numbers correspond to the residues of *L. plantarum* LarA (PDB code 5HUQ or 6C1W).

Figure 2: Phylogenetic tree of the LarA superfamily. All LarAHs with an identified reaction are indicated as “AH X”. The colors of the phylogenetic groups correspond to the different reactions identified in these groups.

Control Number: 2021-A-8349-MICROBE

Session Type: **iPoster**

Session Number:

Session Title: **FEMS: Late-breaker iPoster Session**

Topic 1: FEMS - Molecular microbiology and biochemistry

Keyword 1: biofim

Keyword 2: rotating magnetic field

Keyword 3: penicilin binding protein

Abstract Title: **Does Structural Differences In Penicillin Binding Proteins Of Staphylococcus Aureus Strains Increase Susceptibility To Beta-lactams In Presence Of Rotating Magnetic Field ?**

Author Block: **R. Drozd**, Magdalena Szymańska, Karol Fijałkowski; West Pomernanian Univ. of Technology in Szczecin, Szczecin, Poland

Abstract Body: Nowadays increasing of pathogenic bacteria antibiotic resistance is one of the biggest public health challenges. The overuse various types of antibiotics over the years has led to among others the emergence of multi-resistant *Staphylococcus* strains (MRSA). In this pathogenic bacteria, one of the reason of beta-lactam antibiotics resistance is expression of a foreign penicillin-binding protein (PBP), PBP2a that has lower affinity to this group of antibiotics. The PBP2a protein is engaged in bacterial cell wall synthesis and tightly cooperate with other PBP4 that was also identified as a next critical factor for *S. aureus* infections. Apart from the traditional therapeutic strategy for treating both planktonic and biofilm infections by more or less specific chemotherapeutic the attention is directed to use electromagnetic field (EMF) as supporting agent. From many types of EMF the rotating magnetic field (RMF) was discovered as force that, can be used for microbial metabolism modification and affecting their ability to colonization of environment. However the molecular mechanism of lower resistance of microbes to antibiotics in presence of RMF is still unclear. The aim of study was to analyse the structural properties the PBP2a and PBP4 proteins of *S. aureus* strains that exhibit different resistance to beta-lactam antibiotics as an effect of exposition on RMF. The PBP2a and PBP4 protein coding genes of analyzed *S. aureus* strains were amplified and obtained cDNA was sequenced. The translated to amino acid sequences cDNA were compared with available at Protein Data Bank resources 3D structures of PBP2a and PBP4 form *S. aureus.* Modified according to discovered amino acid substitution reference *S. aureus* PBP 3D structures, were further analyzed with using DynaMut web-server and scalable molecular dynamics simulation via NAMD software. The obtained results showed that, among the analyzed *S. aureus* strains the main divergence was focused in the structure of regulatory domain of PBP2a protein and any significant differences in structure a PBP4 was not found among compared bacterial strains. The identified mutations sites was mainly located in protein PBP2a structure region that responds for regulation of catalytic functions of its transpeptidase domain. Further *in-silico* analysis revealed that discovered amino acid

substitutions in regulatory region can significantly affect the protein flexibility and influence on PBP2a behavior during the exposition to RMF and finally structure of biofilm matrix of analyzed microbes.

Control Number: 2021-A-8414-MICROBE

Session Type: **iPoster**

Session Number:

Session Title: **FEMS: Late-breaker iPoster Session**

Topic 1: FEMS - Molecular microbiology and biochemistry

Keyword 1: FASII inhibitors

Keyword 2: lipid

Keyword 3: Staphylococcus aureus

Abstract Title: **Alteration Of Lipid Metabolism And Resistance Phenotype To Cell Envelope-antimicrobials In *Staphylococcus Aureus Via* Fasii Inhibitors**

Author Block: **T. Shen**, R. Zhang, Q. Guo, B. J. Werth, L. Xu; Univ. of Washington, Seattle, WA

Abstract Body:

Background: In *Staphylococcus aureus*, cell membrane lipid synthesis is metabolically closely connected to cell wall synthesis, which is a target for vancomycin (VAN). Daptomycin (DAP), on the other hand, targets anionic lipids in the cell membrane, while lipoglycopeptides like dalbavancin (DAL) interact with both. Our lab previously showed that the levels of the membrane lipids, phosphatidylglycerols, correlated with the susceptibility to VAN, DAP, and/or DAL in multiple *S. aureus* strains. **Objectives:** We hypothesized that small molecule lipid synthesis inhibitors can reverse the resistance phenotypes by modulating lipid metabolism. We aimed to test the hypothesis by examining FASII inhibitors because they are antimicrobials currently under clinical development.**Methods:** We grew MRSA strain S7 (VAN MIC:1 µg/mL) and S7-D2 (VAN MIC: 4 µg/mL; DAL MIC: 1 µg/mL; single nucleotide variant in *vraT* after serial passage of S7 in DAL) in the presence and absence of subinhibitory (half-MIC) cerulenin (CER; FabF inhibitor) and AFN-1252 (AFN; FabI inhibitor) and measured the lipidomic changes using mass spectrometry. We also investigated the MIC-lowering effects of subinhibitory concentrations of CER, AFN, and triclosan (TRI; FabI inhibitor) on the MICs of VAN, DAP or DAL. To further interrogate potential synergistic effects that might not be manifested in the combination MIC measurements, we used time-kills to assess for synergy between AFN and VAN against S7 and S7-D2. **Results:** The lipidomics study demonstrated that CER shifted the fatty acid chains of various lipid species to shorter chains, while AFN decreased the lipid synthesis overall, which is expected since CER acts where the fatty acid chains are elongated while AFN acts where the precursors to lipid synthesis are formed. The exception lied in cardiolipins (CLs) in the presence of AFN, where the fatty acid composition shifted to shorter chains in S7, but overall CL levels increased in S7-D2, suggesting that CL synthase was activated with exposure to AFN. Exposure to half-MIC of CER, AFN, and TRI did not reduce the MICs of VAN, DAP, or DAL. In time-kills, S7-D2 survival at 8hr was significantly lower for the AFN+VAN exposed cells than either single agent, but this difference was not apparent at 24 hours due to excessive killing by VAN at 24 hours. In conclusion, CER and AFN altered the

lipid metabolism in *S. aureus* and demonstrated some potential to enhance the killing effects of VAN. Future studies on the synergistic studies between FASII inhibitors and antimicrobials are warranted.

Control Number:	2021-A-8490-MICROBE
Session Type:	**iPoster**
Session Number:	
Session Title:	**FEMS: Late-breaker iPoster Session**
Topic 1:	FEMS - Molecular microbiology and biochemistry
Keyword 1:	Pseudomonas aeruginosa elataseB
Keyword 2:	propeptide
Keyword 3:	
Abstract Title:	**Inhibition Of The *Pseudomonas Aeruginosa* Elastase B By Its Propeptide**
Author Block:	**Y. Shin**; Pusan Natl. Univ., Pusan, Korea, Republic of

Abstract Body:

The extracellular proteases of pathogens are important virulence factors. Among them, three major proteases of *Pseudomonas aeruginosa*, elastase B (LasB) protease IV (PIV), and elastase A (LasA) play a key role in the *P. aeruginosa* infection and pathogenesis. These are activated post-secretionally in a cascade manner in which the initial activation of LasB was controlled by quorum sensing (QS). Once activated, LasB activated PIV, which then sequentially activated LasA. Therefore, when inhibiting these proteases, it is clear that inhibiting LasB is most effective. Since the activities of these three proteases are regulated by their propeptides, in this study, we tried to inhibit LasB using its purified propeptide ($LasB_{pp}$). However, LasB was not inhibited by exogenous addition of $LasB_{pp}$ unlike LasA and PIV that were inhibited by their propeptides. The reason was that once activated, LasB was able to degrade $LasB_{pp}$, so resistance to the inhibition by $LasB_{pp}$. In order to overcome this problem, we tried to find the mutant $LasB_{pp}$ that are resistant to LasB, because the mutant $LasB_{pp}$ that lost the cleavage site of LasB was expected to have resistance to LasB. From the C-terminal deletion series, we obtained a mutant $LasB_{pp}$ that are resistant to LasB ($LasB_{pp}$-R2). By overexpressing $LasB_{pp}$-R2, the LasB activity was inhibited by 30%.

Control Number: 2021-A-8493-MICROBE

Session Type: **iPoster**

Session Number:

Session Title: **FEMS: Late-breaker iPoster Session**

Topic 1: FEMS - Molecular microbiology and biochemistry

Keyword 1: Pseudomonas aeruginosa

Keyword 2: Las B Activity

Keyword 3: Post-secretional activation

Abstract Title: **Lasb Is Not Auto-activated In Pseudomonas Aeruginosa**

Author Block: **C. Lee**, Department of Pharmacy, College of Pharmacy, PusanNational University, Busan, 609-735, South Korea; Pusan Natl. Univiersity, Busan, Korea, Republic of

Abstract Body: **Background:** LasB (elastase B) is the most abundant and quorum sensing (QS)-regulated protease in *Pseudomonas aeruginosa.* LasB is implicated in *P. aeruginosa* infection by causing tissue damage and disrupting host immune response. Previous studies suggested that LasB is auto-activated post-secretionally and successively activates other proteases, protease IV (PIV) and LasA (elastase A) by degrading their propeptides. Meanwhile, we also found that LasB overexpressed in QS mutant has a severe reduction in its activity despite high level expression and secretion. **Objective:** We questioned the suggestion that LasB is auto-activated and intended to reveal the underlying mechanism about the post-secretional activation of LasB. **Methods:** LasB secretion was confirmed by SDS-PAGE in *P. aeruginosa* culture supernatant. Liquid chromatograph was used for protein purification. Elastin-Congo red assay was used to measure the LasB activity. Site-directed mutagenesis was used for genetic analysis. **Results:** Although LasB overexpressed in QS mutant has a reduced activity, the SDS-PAGE analysis showed that LasB in QS mutant was in its mature form, suggesting that the reduction of LasB activity in QS mutant was not caused by the uncomplete processing of LasB. This means that LasB is not auto-activated, but requires some QS-dependent factors for activation. The purified LasA and PIV could not restore the reduced LasB activity in QS mutant, but the addition of whole culture supernatant from a QS^+ *lasB*$^-$ strain could, indicating that there is an unknown QS-dependent factor that activates LasB. Interestingly, the purification of LasB from QS mutant also restored the LasB activity gradually. This means that an unknown inhibitor represses LasB in QS mutant. Taken together, we propose that a QS-independent inhibitor inhibits LasB activity, and another QS-dependent activator eliminates the function of this inhibitor, thereby activating LasB.

Control Number: 2021-A-8496-MICROBE

Session Type: **iPoster**

Session Number:

Session Title: **FEMS: Late-breaker iPoster Session**

Topic 1: FEMS - Molecular microbiology and biochemistry

Keyword 1: ESKAPE

Keyword 2: iron-uptake

Keyword 3: immunotherapy

Abstract Title: **The Use Of Iron Uptake Receptors For The Development Of Broad-range Immunotherapy Against Eskape Pathogens**

Author Block: E. Frutos Grilo, **G. Ortiz**, M. Gaona-Soler, P. Conill, M. Sastre, J. Barbé, S. Campoy; Univ. Autónoma de Barcelona, Cerdanyola del Vallès, Spain

Abstract Body: **Background:** The available therapeutic options against ESKAPE pathogens (*Enterococcus* spp., *Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.) are extremely limited due to their multi-drug resistance phenotype thus alternative treatments against these pathogens are urgently needed. Some of them have been focused on iron, an essential trace element important for bacterial physiology, pathogenicity and metabolism. Due to the limited free-iron availability in the host, pathogenic bacteria must overexpress iron uptake receptors (IUR) to capture host iron and ensure their survival. These proteins are immunogenic and therefore, they are promising targets for immunotherapy approaches. **Objectives:** Further, and as IURs are highly conserved, use them as targets for the development of broad-range therapy strategies has been proposed. **Methods and results:** To validate this possibility, we selected several IUR that were revealed *in silico* to be conserved among phylogenetically related ESKAPE pathogens (*i.e.* present in either the grampositive *Enterococcus* spp. or *S. aureus*; in *A. baumannii* or *P. aeruginosa* or in *Enterobacter* spp. *K. pneumoniae* sequenced isolates) and we have confirmed their prevalence in a collection of clinical isolates from five hospitals. The selected candidates were *in silico* modeled and their outer loops were located and analyzed. Peptide sequences were selected based on their conservation among ESKAPE species, their immunogenicity, and non-toxic and non-allergenic characteristics. The regions with the best scores for each protein were selected and synthetic peptides conjugated to KLH were obtained and used to mice immunization for polyclonal antibody production. Finally, the obtained sera have been tested by western blotting to determine the cross-reactivity against ESKAPE pathogens. The obtained results could lead to the development of broad-range immunotherapy as an alternative to the use of antibiotics to treat and prevent infections caused by these recalcitrant pathogens.

Control Number: 2021-A-8517-MICROBE

Session Type: **iPoster**

Session Number:

Session Title: **FEMS: Late-breaker iPoster Session**

Topic 1: FEMS - Molecular microbiology and biochemistry

Keyword 1: HOCl

Keyword 2: Prions

Keyword 3:

Abstract Title: **Comparison Of HOCl And HOBr For Efficacy Of Inactivation Of Scrapie Prions, Ms2 Bacteriophage And Various Microbes**

Author Block: L. Robins[1], E. Keim[2], A. Hughson[3], K. Isiofia[3], J. Meschke[2], J. Santiago[4], B. Caughey[3], **J. Williams**[4]; [1]Univ. of Washington Bothell, Bothell, WA, [2]Univ. of Washington, Seattle, WA, [3]NIH, Hamilton, MT, [4]Briotech Incoporated, Woodinville, WA

Abstract Body: Expectations of chemical decontamination measures for medical instrumentation and surfaces in hospitals have been eroded by accumulating evidence that certain commonly used formulations are not reliably effective for the full spectrum of contemporary infection control challenges (e.g., HPV, infectious proteins, *Candida auris*). Hypochlorous acid (HOCl) has been shown to rapidly inactivate infectious prions and MS2 bacteriophage. However, hypobromous acid (HOBr) containing mixtures have been reported to be more potent biocides. To directly compare the biological properties of HOCl and HOBr we characterized the hypohalous acids using analytical spectroscopy and spectrophotometry prior to their use. The hypohalous acids were tested against scrapie prions, MS2 bacteriophage, *Escherichia coli, Staphylococcus aureus* and *Aspergillus niger.* HOCl showed no detectable changes in stability after 22 months at 22 °C. At elevated temperatures of 52 °C and 70 °C HOCl concentrations decreased over time, however little change was detected in the pH and ORP of the solution at any temperature. HOBr, in contrast, degraded to form mixtures of aqueous bromine solutions within hours of preparation. HOCl and HOBr inactivated scrapie prions similarly after 5 minutes of incubation. HOBr showed higher efficacy than HOCl at low concentrations against MS2 bacteriophage at both 30s and 60s time points. HOBr was more effective than HOCl against *E. coli* and *S. aureus*. In contrast, *A. niger* was more susceptible to HOCl. The differences in reactivity of these hypohalous acids may be due to the inherent reactivity of the oxidative species. However, it is likely that any modifications (e.g., oxidations) by these hypohalous acids occur at or near locations crucial to the viability of the protein or microbial organism.

Control Number: 2021-A-8527-MICROBE
Session Type: **iPoster**
Session Number:
Session Title: **FEMS: Late-breaker iPoster Session**
Topic 1: FEMS - Molecular microbiology and biochemistry
Keyword 1: Directed evolution
Keyword 2: Antimicrobials
Keyword 3: cyclic peptides
Abstract Title: **Selection Of Bioactive Cyclic Peptides By Bacterial Lysis**
Author Block: **S. Worms**[1], P. Soumillion[2]; [1]UCLouvain, Louvain La Neuve, Belgium, [2]UCLouvain, Louvain-la-Neuve, Belgium
Abstract Body:
Background
In a world where antibiotic resistance is outpacing the discovery of new antibiotics, finding ways to inhibit resistance offers another way to fight antibiotic-resistant bacteria.
Objectives
We aim to use directed evolution to identify new cyclic peptides that potentiate existing antibiotics, either by inhibiting known resistance enzymes such as carbapenemases, or by interfering with the membrane to increase antibiotic permeability, widening the range of molecules such as vancomycin to allow their use against Gram-negative bacteria.
Methods
We use Split-Intein mediated Circular Ligation of Proteins and Peptides (SICLOPP) to assemble large (>10^8) libraries of periplasmic or cytoplasmic cyclic peptides in *Escherichia coli*. To avoid time-consuming screening of library we developed a novel selection method termed Selection by Lysis. Strains expressing the carbapenemases from a genomic loci are transformed with the library. Subsequent addition of antibiotics lyses the cells containing a sensitizing peptide, releasing the encoding plasmid in the culture medium. By isolating and sequencing this free-floating DNA we can select peptides that inflicts a large, negative fitness cost to their hosts.
Results
Initial selections campaigns selected plasmids produced by a freak insertion of parts of the lysis operon of the cryptic prophage DLP12 into our library plasmid, leading to a highly lytic plasmid. This however demonstrates the selecting power of Selection by Lysis. A strain deleted for the DLP12 lysis operon has been engineered and is currently being used for a new selection campaign.

Control Number: 2021-A-8539-MICROBE

Session Type: **iPoster**

Session Number:

Session Title: **FEMS: Late-breaker iPoster Session**

Topic 1: FEMS - Molecular microbiology and biochemistry

Keyword 1: ParB

Keyword 2: segregation

Keyword 3: Streptomyces

Abstract Title: **The Role Of ParB Interactions In The *Streptomyces Coelicolor* Segrosome Assembly**

Author Block: **J. Szymczak**, D. Jakimowicz, M. Szafran; Univ. of Wroclaw, Wroclaw, Poland

Abstract Body:

Streptomyces are Gram-positive, mycelial soil bacteria, that exhibit a complex life cycle, which includes the formation of a multicellular, vegetative mycelium as well as sporogenic hyphae which develop to chains of spores. *Streptomyces* hyphal cells are multigenomic and several copies of the linear chromosomes remain unsegregated during vegetative growth, they undergo segregation in sporogenic hyphae to generate unigenomic spores. The proper distribution of *Streptomyces* linear chromosomes during sporulation is dependent on segregation protein ParB. ParB binds numerous DNA sequences (*parS* sites) and organizes the *oriC* proximal region of chromosome into the large nucleoprotein complex - segrosome. Recent studies, suggest that ParB complex formation starts by specific ParB-*parS* binding. ParB spreads along DNA as well as can bridge distant *parS* sites. Thus, segrosome assembly requires short (ParB-spreading) and long (ParB-bridging) distance interactions between conserved arginine and glycine residues located in the N-terminal domain of ParB. Additionally, it was shown that non-specific ParB spreading along DNA requires cytidine triphosphate (CTP). We hypothesize that mutations in conserved residues may affect ParB-dependent segrosome assembly in *Streptomyces* disturbing chromosome segregation during sporulation. Using *in vitro* analyses (e.g. Electrophoretic mobility shift assay and Bio-layer interferometry) we determined the effect of mutations of conserved arginine/glycine residues in *S. coelicolor* ParB on DNA binding. Interestingly, all tested ParB mutants bound *parS* sites but were not able to spread along DNA. Additionally, our studies showed that the presence of CTP highly stimulates ParB spreading *in vitro*. To test the influence of above mentioned point mutations on ParB function *in vivo*, we constructed a set of *S. coelicolor* strains producing fluorescently labelled ParB-EGFP mutants. Using microscopy analysis of ParB-EGFP complexes in *S. coelicolor* hyphae and analyzing the chromosome segregation of mutants strains we showed that the conserved glycine and arginine ParB residues are necessary for proper *Streptomyces* segrosome assembly ensuring proper and efficient chromosome segregation to spores. Our data showed that conserved motif in the N-terminal domain of ParB is crucial for ParB spreading i*n vitro* and its mutation affects segrosome formation and proper segregation of *S. coelicolor* chromosomes to spores.

Control Number: 2021-A-8540-MICROBE

Session Type: **iPoster**

Session Number:

Session Title: **FEMS: Late-breaker iPoster Session**

Topic 1: FEMS - Molecular microbiology and biochemistry

Keyword 1: chromosome segregation

Keyword 2: ParA segregation protein

Keyword 3: Mycobacterium smegmatis

Abstract Title: **The Novel Component Of *M. Smegmatis*Chromosome Segregation Machinery.**

Author Block: **I. Magierowska**, M. Pióro, D. Jakimowicz; Univ. of Wroclaw, Wroclaw, Poland

Abstract Body:

Chromosome segregation in bacteria is a crucial stage of cell cycle involving a number of proteins.
In most bacteria, chromosome segregation requires activity of ParABS system. This system consists of DNA binding protein ParB, Walker A type ATPase ParA and *parS* sequences. Upon binding *parS* sequences, ParB forms large nucleoprotein complexes which are segregated due to interactions with dynamic ParA protein. Interestingly, in number of model bacterial species (*C. crescentus, C. glutamicum, S. coelicolor*) ParA and ParB were shown to be engaged in genus-specific interactions with proteins involved in other cell cycle processes e.g. cell division or cell extension. In our studies we focus on the chromosome segregation in *Mycobacterium smegmatis*, the model organism for mycobacterial cell cycle studies. The elimination of ParA in *M. smegmatis* causes significant growth inhibition and several defects in chromosome segregation. We have also shown, that in *Mycobacteria*, ParA interacts with polar growth determinant DivIVA. ParA-DivIVA interaction was suggested to coordinate the chromosome segregation with cell elongation. This interaction facilitates segrosome separation, controls positioning of the *oriC* and modulates cell elongation affecting interdivision time. Notably, novel protein, named PapM (ParA partner in *M. smegmatis*) has been identified as the protein interacting with ParA- and also with DivIVA in *M. smegmatis*. Here, we investigate the role of PapM in *Mycobacterium smegmatis*. The function of this protein was studied using a set of *M. smegmatis* mutant strains. We investigated, how deletion and overproduction of *papM* affected cell growth and chromosome segregation. Our results suggested, that deletion of *papM* or its overproduction affected *M. smegmatis* cell length influencing the cell extension rate. Moreover, the overproduction of *papM* partially complemented *parA* deletion, but in wild type background delayed the growth rate and disturbed chromosome segregation. Our data suggest, that *papM* is a novel ParA interaction partner that contributes to chromosome segregation.

Control Number: 2021-A-8545-MICROBE

Session Type: **iPoster**

Session Number:

Session Title: **FEMS: Late-breaker iPoster Session**

Topic 1: FEMS - Molecular microbiology and biochemistry

Keyword 1: Amyloid

Keyword 2: Rhizobium leguminosarum

Keyword 3: host-cell interactions

Abstract Title: ***In Vitro* And *In Vivo* Amyloid Formation By The RopA And RopB Proteins From *Rhizobium Leguminosarum***

Author Block: **A. O. Kosolapova**[1], M. V. Belousov[2], A. I. Sulatskaya[3], M. E. Belousova[2], M. I. Sulatsky[3], K. S. Antonets[2], K. V. Volkov[4], A. N. Lykholay[4], O. Y. Shtark[2], E. N. Vasileva[5], V. A. Zhukov[5], A. N. Ivanova[4], P. A. Zykin[4], I. M. Kuznetsova[3], K. K. Turoverov[3], I. A. Tikhonovich[2], A. A. Nizhnikov[2]; [1]Lab. for Proteomics of Supra-Organismal Systems, All-Russia Res. Inst. for Agricultural Microbiol., St. Petersburg, Russian Federation, [2]All-Russia Res. Inst. for Agricultural Microbiol., St. Petersburg, Pushkin 3, Russian Federation, [3]Inst. of Cytology of the Russian Academy of Sci., St. Petersburg, Russian Federation, [4]St. Petersburg State Univ., St. Petersburg, Russian Federation, [5]All-Russia Res. Inst. for Agricultural Microbiol., St. Petersburg, Russian Federation

Abstract Body: Background: Amyloids represent fibrillar protein aggregates with a “cross-β” spatial structure. Amyloid formation is associated with development of more than 40 incurable diseases in humans and animals. Amyloids can also perform various physiological functions. Most of these functional amyloids have been identified within prokaryotic species where they can act as biofilm matrix structural components and adhesins, regulate activity of toxins, and form extracellular protein layers. While many functional amyloids are used by pathogenic microorganisms in their interaction with multicellular hosts, little is known about the role of amyloids in host-symbiont interactions. Previously, we have identified 54 candidate amyloid-forming proteins in the root nodule bacterium *Rhizobium leguminosarum*.

Objectives: The aim of the work is to analyze candidate proteins amyloid properties *in vitro* and *in vivo.*

Methods: To analyze amyloid properties of proteins we used circular dichroism, transmission electron microscopy, fluorescence measurement upon binding of Thioflavin T dye, polarization microscopy upon binding of Congo red dye, detergent- and proteases resistance analysis. For analysis of localization of RopA and RopB proteins *in vivo* we used immunogold assay. The size and amount of aggregates were measured with the usage of SDD-AGE.

Results: For further analysis we chose two β-barrel proteins - RopA and RopB. We demonstrated that RopA and RopB fibrils obtained *in vitro* possess amyloid properties including high content of β-sheets, Thioflavin T binding, green birefringence upon staining with Congo Red, and resistance to treatment with ionic detergents and proteases. RopA and RopB proteins aggregate in yeast cells and form Congo

Red-binding fibrils while exported to the cell surface of *Escherichia coli*. We demonstrated that the extracellular capsules of the *R. leguminosarum* cells exhibit apple-green birefringence upon Congo Red staining. We also showed that fibrillar matrix on the cell surface of the *R. leguminosarum* free-living culture binds anti-RopA and anti-RopB antibodies. Moreover, size and amount of RopA aggregates increase after addition of flavonoid luteolin. Taking together, we may conclude that RopA and RopB proteins can form amyloid fibrils both *in vitro* and *in vivo*. What is more, RopA is likely to be involved in early stages of symbiotic bacteria-plant interactions as we demonstrated increase in RopA aggregation after the treatment with flavonoids.

Control Number: 2021-A-8558-MICROBE

Session Type: **iPoster**

Session Number:

Session Title: **FEMS: Late-breaker iPoster Session**

Topic 1: FEMS - Molecular microbiology and biochemistry

Keyword 1: disulfide bonds

Keyword 2: Helicobacter hepaticus

Keyword 3: Dsb proteins

Abstract Title: **Understanding Of The *Helicobacter Hepaticus* Dsb System By Biochemical Characterization Of Two Dsb-like Proteins**

Author Block: **P. Roszczenko-Jasinska**[1], M. M. Kosiorek[1], C. Czaplewski[2], A. Gieldon[2], A. Liwo[2], E. K. Jagusztyn-Krynicka[1]; [1]Univ. of Warsaw, Faculty of Biology, Inst. of Microbiol., Warsaw, Poland, [2]Univ. of Gdansk, Faculty of Chemistry, Gdansk, Poland

Abstract Body: **Background:** The virulence of bacterial pathogens often depends on extracytoplasmic proteins, many of which contain two or more cysteine residues and achieve the final structure as a result of disulfide bond formation catalyzed by the Dsb proteins. Recently, *Helicobacter* genus, specified as non-pylori *Helicobacter*, have received a lot of attention. Among them is *Helicobacter hepaticus*, a member of enterohepatic microorganisms. There are evidences which suggest an association of the mice/human infections by this bacterium with the development of hepatobiliary cancers.The *H. hepaticus* Dsb system is potentially novel and different from those operating in *E. coli* and *H. pylori*. **Objective:** The aim of presented work was to characterize biochemically two Dsb-like proteins of *H. hepaticus*. **Methods:** Genes encoding Dsb-like (*hh1141* and *hh1412*) were cloned into pET28a plasmid and overexpressed in *E. coli* Rosetta strain and purified using medium-pressure liquid chromatography system. Purified proteins were used to conduct biochemical assays, including determination of their redox potentials, the insulin reduction assay, and RNase oxidation test. The models of the most probable structures of Hh1141 and Hh1412 proteins were generated with I-TASSER. **Results:** *In silico* analysis indicated that both, Hh1141 and Hh1412, are homologs of the main *H. pylori* oxidoreductase Hp0231. They were classified as members of Dsb family that apart from thioredoxin fold with catalytic domain contain also dimerization domain. Biochemical assays confirmed their oxidoreductase activity and showed that their redox potentials are comparable to that of *E. coli* dimeric DsbC protein. To deepen our knowledge about Hh1141 and Hh1412 functioning, we intend to solve their structures by crystallography.

Control Number: 2021-A-8568-MICROBE

Session Type: **iPoster**

Session Number:

Session Title: **FEMS: Late-breaker iPoster Session**

Topic 1: FEMS - Molecular microbiology and biochemistry

Keyword 1: Panton-Valentine Leukocidin

Keyword 2: toxin-antitoxin system

Keyword 3: Staphylococcus aureus

Abstract Title: **Consequences of the Decoupling between MazEF Toxin-Antitoxin System and SigB Operon in Staphylococcus aureu**s

Author Block: **K. Chlebicka**[1], M. Kosecka-Strojek[2], P. Suder[3], B. Wladyka[1], E. Bonar[1]; [1]Jagiellonian Univ., Krakow, Poland, [2]Jagiellonian Univ., Kraków, Poland, [3]AGH Univ. of Sci. and Technology, Krakow, Poland

Abstract Body:
Staphylococcus aureus is a component of skin and nasal microflora. Simultaneously, it constitutes an opportunistic pathogen responsible for a variety of infections, from mild abscesses to bacteraemia. Its pathogenicity is driven by mechanisms that enable the bacteria to synthesize a wide range of virulence factors, including Panton-Valentine Leukocidin toxin (PVL), which evoke conditions like acute lung and bone marrow inflammation.

The starting point of the research was the detection of two variants of the S. aureus ATCC25923 strain that showed a significant difference in the level of PVL production. Genome sequence analysis of both variants, revealed a transposon insertion in the PVL overproducing variant [1]. The insertion decouples mazEF, encoding a toxin-antitoxin system, and rsbU, which is crucial for the activation of SigB transcription factor, implicated in the control of staphylococcal virulence.

To verify the impact of the insertion on staphylococcal regulatory mechanisms two omic techniques were used. The comparative transcriptomics of mRNA, isolated from logarithmic and stationary bacterial growth phase was performed using RNA-seq. Two-dimensional difference gel electrophoresis (2D DIGE) followed by mass spectrometry was utilized for identification of differentially expressed proteins in intracellular and extracellular proteomes.

In logarithmic phase of growth, S. aureus ATCC25923 strain variants differed only in a few gene transcripts, whereas in the stationary phase the number of differentially expressed genes reached over hundred. Intra- and extracellular proteome profiles differed in 11 and 38 proteins, respectively. Differentiating proteins are mainly implicated in basic metabolism and protein synthesis. Interestingly, in the variant with the transposon insertion, apart PVL, additional virulence factors exhibited increased expression. We conclude that the decoupling of mazEF and rsbU has a broader impact on gene expression and protein profile than initially observed PVL overexpression.The study was supported by the National Science Centre (NCN, Poland), DEC-2018/02/X/NZ2/03551 (to EB).

Control Number: 2021-A-8632-MICROBE

Session Type: **iPoster**

Session Number:

Session Title: **FEMS: Late-breaker iPoster Session**

Topic 1: FEMS - Molecular microbiology and biochemistry

Keyword 1: Nutraceuticals

Keyword 2: Synthetic Biology

Keyword 3: Scaffolding

Abstract Title: **Glucosinolates As Nutraceuticals:optimization Of Bio-based Microbial Production Of Glucobrassicin**

Author Block: **L. Maestroni**, P. Butti, P. Branduardi; Univ. of Milano-Bicocca, Milan, Italy

Abstract Body: Plants can produce a wide range of secondary metabolites, many of which are valuable compounds that when in human organism can interact with the gut microbiota with different effects on our health. We focused the attention on microbial based production of glucosinolates (GLSs), which are naturally produced by members of cruciferous vegetables and possess cancer-preventive properties mainly thanks to their hydrolysis products. Glucobrassicin (GLB), an indolyl-methyl glucosinolate, is the precursor of indole-3-carbinol (I3C), one of the most characterized bioactive compound. The first aim is to construct a *Saccharomyces cerevisiae* strain able to produce GLB. The strategy involves the creation of a collection of more than 50 DNA parts, comprising promoters, terminators and the coding sequences of the enzymes and accessory proteins of the heterologous pathway. These parts will be assembled in devices using the Golden Gate Assembly, so to build up a library of expression cassettes with different promoters, that can be easily integrated in yeast's genome using the CRISPR-Cas9 system. The system will be further improved by targeting the enzymes to a synthetic protein scaffold, to maintain them in proximity and enhance their efficiency. In parallel, we are working on the development of an *in vitro* test on intestinal epithelial cell line Caco-2 to verify if GLSs can exhibit a protective effect on the intestinal barrier. Perspectives of the work will be also presented and discussed.

Control Number: 2021-A-8738-MICROBE

Session Type: **iPoster**

Session Number:

Session Title: **FEMS: Late-breaker iPoster Session**

Topic 1: FEMS - Molecular microbiology and biochemistry

Keyword 1: iCHELLs

Keyword 2: autocatalysis

Keyword 3: polyoxometalates

Abstract Title: **Synthesizing Non Carbon-based Life From Polyoxometalate Materials: Finding The Chemistry That Led To Biology**

Author Block: **E. Bellinger**; Charles Stewart Mott Community Coll., Flint, MI

Abstract Body: Background: A survey of the prospects of synthesizing non carbon-based life with an emphasis on Darwinian evolution and whether it is unique to carbon-based life or can be extended to non carbon-based matter. This report examines the chemical systems capable of supporting non carbon-based life by citing multiple studies that substantiate the concept of inorganic cells displaying biologic properties. This study addresses features specific to the organic world, the candidates for a minimal evolutionary system produced from inorganic materials, and the steps taken to engineer an evolvable system without using organic material. Objectives: The considered research observes inorganic nano-sized molecules based on molybdenum oxide that demonstrate an artificial system of evolution and functional inorganic chemical cells (iCHELLs), which are evidence of simple chemistry becoming self-sustaining and complex. These cells may be the key to non carbon-based life, as they provide the compartment to metabolize reactions and store information. Methods: Templated synthesis of functional molecular nanomaterials is evidence of “lifelike” chemistry and provides the basis for new catalytic organization and self-assembly of such nanomaterials. Molecular-level organization of autocatalysis being displayed in inorganic materials confirms the possibility of spontaneously emerging autocatalytic sets existing from inorganic metal oxide clusters. These autocatalytic sets, which are based on inorganic salts, give rise to spontaneously forming, self-replicating systems; an essential property of life. Results: The formation of such autocatalytic sets that are capable of spontaneous emergence and self-reproduction outside of known biology further demonstrates the possibility of evolvable matter and unveils the inorganic systems capable of being extrapolated to discover other nanomaterials. The considered works ascertain the chemical networks required to construct minimal chemical units capable of autonomous assembly, selection, adaptation, and propagation without DNA, sugars, proteins, etc. and may lead to a general definition of life, it’s origin, discovery and constraints of other life forms.

Control Number: 2021-A-8767-MICROBE

Session Type: **iPoster**

Session Number:

Session Title: **FEMS: Late-breaker iPoster Session**

Topic 1: FEMS - Molecular microbiology and biochemistry

Keyword 1: quorum sensing

Keyword 2: biofilms

Keyword 3: Angelica turcica

Abstract Title: **Determination Of Quorum Sensing Inhibitory Potential Of Angelica Turcica**

Author Block: **K. Erkan Türkmen**[1], D. Erdönmez[2], H. Katırcıoğlu[3], E. Hamzaoğlu[3]; [1]Hacettepe Univ., Ankara, Turkey, [2]Aksaray Univ., Aksaray, Turkey, [3]Gazi Univ., Ankara, Turkey

Abstract Body: **Background:** *Angelica turcica* is an endemic plant for Turkey. Quorum sensing(QS) is a crucial mechanism for microorganisms that play an important role in biofilm formation, violacein production, and swarming motility controlled by the QS mechanism. Biofilm formation on pathogens is one of the virulence factors. Swarming motility is considered one of the bacterial social behavior models. Inhibition of QS has become the center of study for understanding bacterial pathogenesis. **Objectives:** The present study aimed to investigate the anti-quorum sensing activity of methanol extract of *A. turcica.* **Methods:** *A.turcica* obtained from Gazi University Herbarium. Root bark, fruit and root core of *A. turcica* were used for obtaining methanol extract. Methanol extracts were used QS inhibition assessment. For this purpose, *Chromobacterium violaceum*(CV) 026, *Pseudomonas aeruginosa* (PA)01 were used. CV026 and PA01 were cultured in LB broth at 30 and 37 0C for 24h. Biofilm formation was assessed by 24 well microtiter plates for PA01. Biofilm formation was quantified by measuring the absorbance of the Crystal violet solution at 585 nm. Biofilm inhibitory activity (BIA%) was evaluated as a proportion of untreated controls (100%). Inhibition of violacein production was determined by CV026 with disc diffusion method and spectrophotometer quantification. Swarming motility inhibition was investigated by the swarm plate method. All experiments were carried out the sub-mic concentration of methanol extract and repeated three times. **Results:** QS system has been an important role in the regulation of biofilm formation. Our study showed that *A.turcica* methanol extract significantly affects QS mechanism, which regulates biofilm formation, swarming motility, and violacein production. The present study is the first report for *A. turcica* QS inhibitory activity. In the light of our data, *A.turcica* could be considered to natural supporter for fighting phytotherapeutic agents for QS inhibitory activity.

Control Number: 2021-A-8787-MICROBE

Session Type: **iPoster**

Session Number:

Session Title: **FEMS: Late-breaker iPoster Session**

Topic 1: FEMS - Molecular microbiology and biochemistry

Keyword 1: Burkholderia pseudomallei

Keyword 2: colorimetric LAMP

Keyword 3: TTSS1-orf11

Abstract Title: **A *Ttss1-Orf11* Colorimetric Loop-mediated Isothermal Amplification Assay Established For Rapid And Accurate Identification Of *Burkholderia Pseudomallei***

Author Block: **H. T. T. Dinh**, Huong Thi Lan Nguyen, Hung Duy Can, Su Xuan Hoang, Son Anh Ho, Tong Van Hoang; Vietnam Military Med. Univ., Hadong, Hanoi, Viet Nam

Abstract Body: *Burkholderia pseudomallei* is an environment saprophytic Gram-negative bacillus that causes melioidosis in humans and animals with common clinical features of pneumonia and multiple abscesses, and mortality can exceed 40%. Although it was firstly discovered by Alfred Whitmore more than a century ago at Rangoon general hospital, Myanmar, *B. pseudomallei* remains a major challenge to the healthcare system. *B. pseudomallei* is also known as "the great mimicker" that makes confused in clinical diagnosis. The current study focuses on the development of a novel colorimetric loop-mediated isothermal amplification (LAMP) assay targeting *TTSS1-orf11* gene of *B. pseudomallei*. *Materials and Methods:* a *TTSS1-orf11* colorimetric LAMP assay for *B. pseudomallei* detection was established with a LAMP primer set of six primers (BpFIP/BIP, BpB3/F3, BpLF/LB) and evaluated on a bacterial panel consisting of *B. pseudomallei* (40 strains), *P. aeruginosa* (40 strains) and *B. cepacia* complex (19 strains) for specificity, sensitivity and the limit of detection. Positive results of the reactions were determined by the change of color from red to yellow. In addition, *TTSS1-orf11* colorimetric LAMP assay was compared with standard PCR and real-time PCR assays for the detection of *B. pseudomallei* in 200 soil and surface-water samples collected in October 2020, Hue City, in the center of Vietnam. *Results and Conclusion*: The optimized LAMP reaction was established in the volume of 25 µl containing 1X WarmStart Colorimetric LAMP master mix, Bp-FIP/BIP, F3/B3, LB/LF primers with concentrations of 0.8, 0.1, 0.2 µM, respectively, and 3 µl genomic DNA. The LAMP assay was highly specific for *B. pseudomallei* identification, reached the detection limit of 12 copies of *B. pseudomallei* genomic DNA per reaction by incubating at 65 °C for 40 minutes. For *B. pseudomallei* detection in the environmental samples, the result was in accordance with the conventional culture methods as well as real-time PCR assay and better than PCR reaction using BpB3/F3 primer pair. Furthermore, this colorimetric LAMP assay can quickly and accurately detect four *B. pseudomallei* strains from soil samples. In conclusion, the colorimetric LAMP assay has the ability of accurate and rapid identification of *B. pseudomallei* from environmental samples and has great potential for use in the field. [#]These authors contributed equally to this work

Control Number: 2021-A-8801-MICROBE

Session Type: **iPoster**

Session Number:

Session Title: **FEMS: Late-breaker iPoster Session**

Topic 1: FEMS - Molecular microbiology and biochemistry

Keyword 1: Transcriptome

Keyword 2: ncRNA

Keyword 3:

Abstract Title: **The Nuclear Transcriptome In U. Maydis**

Author Block: **J. A. Sanpedro**, E. Anastacio-Marcelino, C. Vazquez, P. Sanchez-Alonso; Meritorius Autonomous Univ. of Puebla, Puebla, Mexico

Abstract Body:

Telomerase activity is essential for the viability of eukaryotic cells. It has a role in genome stability, proliferative potential, and lifespan extension[1, 2]. Telomerase is a multi-subunit complex with two core components, the telomerase reverse transcriptase (TERT) subunit, and the telomerase RNA (TER) subunit[3, 4]. Mutations that affect the genes of the essential core cause progressive telomere shortening, slow growth, trigger senescence and most of the cells die[5]. However, among the dying population, a subpopulation of surviving cells emerges that can maintain the length of telomeres through alternative recombination-based pathways[6, 7]. Still, surviving requires gross changes of global gene expression to cope with this emergence: up-regulation of genes involved in the DNA damage response (DDR) is triggered; changes in transcriptional expression also include genes involved in general response to environmental stress, carbohydrate metabolism, oxidative phosphorylation, regulation of the cell cycle, and apoptosis[8, 9, 10]. Transcriptomes of telomerase-negative organisms of diverse taxa share a group of genes which are crucial for surviving, whereas some other genes are expressed in a species-related way, perhaps reflecting the genome particularities of each phylogenetic group. Recently transcriptome of long noncoding RNAs of *Saccharomyces cerevisiae* lacking telomerase activity was also obtained; the results suggested the possible requirement of lncRNAs in the regulation of genes needed for the adaptive response[11]. Due to that most of the functional lncRNAs are harbored in the nucleus, in this work, the nuclei of *U. maydis* were obtained and nuclear transcriptomes from polyA(+) RNA and total RNA of the fungus were assembled and examined. *De novo* assembly yielded 42,712 reconstructed transcripts, covering more than 90% of the genome. Number of differentially expressed transcripts (with log2FC ≥ 2 and FDR ≤ 0.1) were 292, of which 157 we hypothesized to correspond to ncRNAs. Among them, the transcript named *ter114* which encodes the putative TER of the fungus seem to present different isoforms, due to splicing, and polyadenilation at the 3'OH end, moreover all isoforms were found in different abundances. Currently our aims are focused on the 5' end modifications as m^7G, 3mG or m^6A to obtain a better structural landscape of the Basidiomycetous TER structures and its metabolism.

Control Number:	2021-A-8802-MICROBE
Session Type:	**iPoster**
Session Number:	
Session Title:	**FEMS: Late-breaker iPoster Session**
Topic 1:	FEMS - Molecular microbiology and biochemistry
Keyword 1:	MDR
Keyword 2:	ESBL
Keyword 3:	Escherichia coli
Abstract Title:	**Antibiotic Resistance And Esbl Production In*Escherichia Coli* From Various Sources In Aba Metropolis, Nigeria**
Author Block:	**O. Kome**, O. Agbagwa, M. Ajuag; Univ. of Port Harcourt, Rivers State, Nigeria
Abstract Body:	Introduction: The increase in multidrug resistance among pathogenic bacteria responsible for infectious diseases has led to lack of effectiveness of some antibiotics. The ability of Escherichia coli to harbor resistant genes has made the treatment of infections a major challenge. This study was carried out to assess antibiotic resistance and extended spectrum beta lactamase (ESBL) production of E. coli from various sources in Aba metropolis, Nigeria. Methodology: Suspected E. coli isolates from clinical and non-clinical sources were presumptively identified by standard phenotypic testing and their identities confirmed molecularly by the detection of E. coli specific 16S rRNA gene fragments. Positive isolates were subjected to antimicrobial testing by Kirby Bauer disk diffusion method. ESBL producing abilities were then assessed phenotypically using the double disc synergy test and the presence of three beta lactamase gene genotypes (blaTEM, blaSHV and blaCTX-M) assessed also. Results: From a total of 350 samples collected 137 were presumptively identified as E. coli and 83 confirmed using genotypic methods, the majority (52, 62.7%) of which were from non-clinical sources. The clinical isolates however exhibited a higher level of resistance against 62.5% of tested antibiotics. Both group of isolates exhibited similar levels (58.1% vs 53.9%) of multidrug resistance (MDR) though. A low rate of ESBL production was observed (1.2%) and none of the three predominant ESBL genotypes was identified in this study. Conclusions: This study reports high levels of antibiotic resistance but low levels of ESBL production and the absence of the three main ESBL genotypes in this locality.

FEMS Rapid Fire Abstracts - World Microbe Forum

21 June 2021 5:45AM

FEMS106 - Rapid Fire: Eukaryotic Microbiology and Biotechnology

Session Room: Channel 2

5:45 AM - 5:53 AM

Abstract Title: **Pseudomonas Aeruginosa Autoinducer, N-(3-oxododecanoyl)-l-homoserine Lactone, Induces Differentiation In AML.**

Author Block: S. Ataei-Kachooei1, M. Rahmatzadeh1, F. Yekani1, S. Tehrani Fateh2, F. Shekari3, E. Salehghamari1, A. Salehi-Najafabadi4; 1Kharazmi Univ., Tehran, Iran, Islamic Republic of, 2Shahid Beheshti Univ. of Med. Sci., Tehran, Iran, Islamic Republic of, 3Royan, Tehran, Iran, Islamic Republic of, 4Univ. of Tehran, Tehran, Iran, Islamic Republic of

Abstract Body: **Background**: The human opportunistic pathogen *Pseudomonas aeruginosa (PA)* governs its gene expression partly via Quorum sensing molecules such as *N*-(3-oxododecanoyl)-L-homoserine lactone (3-Oxo-C_{12}-HSL). The interaction of PA and its secreted metabolites such as lactones on physiology of human cells has been studied. Differentiation and apoptosis are among the most attracting physiological processes as therapeutic targets for many cancers including acute myeloid leukaemia (AML). **Objective**: In this study, the influence of 3-Oxo-C_{12}-HSL, on human leukemia cell lines, HL60 (i.e., promyeloid leukemia cell line) and KG1a (i.e., leukemia stem cell line), was investigated in terms of induction of apoptosis and differentiation. **Methods**: HL60 and KG1a cell lines were treated with 3-Oxo-C_{12}-HSL (50, 100, 150, and 200 µM) and incubated for 24 hours. Cell viability was measured with MTS assay. Maturation of the population was evaluated with Wright-Giemsa stain for morphological changes towards differentiation. Cell cycle progression and apoptosis were also assessed with flow cytometry techniques using an IQ product kit. Gene expressions of FLT3, PU.1 and CEBPα were also assessed with Real-Time PCR. P-value ≤ 0.05 is considered to be statistically significant. **Results**: MTS Results on HL60 and KG1a cells indicate that 3-Oxo-C_{12}-HSL has a variable effect at different time points and concentrations, showing no linear correlations. In contrast, PBMCs (Peripheral Blood Mononuclear Cells) displayed linear correlations in 24h having an approximate IC_{50} of 200 µM. Incubating with 50 and 100 µM of 3-Oxo-C_{12}-HSL, morphological changes towards differentiation was seen in HL60 cells, while no changes at any concentration was detected in KG1a cells. At higher concentrations (150µM) more than 50% of the cells were detected as apoptotic bodies in both cell lines. Moreover, no alterations were detected in the cell cycle at any concentrations on the cell lines. The Real-Time PCR results indicate increase in expression of differentiation genes in HL60 cells. It is proposed that 3-Oxo-C_{12}-HSL may be a worthy candidate for AML management due to its apoptosis and differentiation inducing effects seen in in-vitro conditions. However, more investigations are needed to evaluate the effects of it on cell physiological pathways.

5:53 AM - 6:01 AM

Abstract Title: **Non-conventional Yeast Ogataea Polymorpha as Promising Organism for High-temperature Xylose and L-arabinose Alcoholic Fermentation**

Author Block: R. Vasylyshyn1, O. Kurylenko1, D. Kostyantyn1, T. Aksyniia1, R. Justyna2, B. Kruk2, A. Sibirny2; 1Inst. of Cell Biology, NAS of Ukraine, Lviv, Ukraine, 2Univ. of Rzeszow, Rzeszow, Poland

Abstract Body: Non-conventional yeast *Ogataea polymorpha* as promising organism for high-temperature xylose and L-arabinose alcoholic fermentationVasylyshyn R.[1], Kurylenko O.[1], Dmytruk K.[1], Tsaruk A.[1], Ruchala J.[2], Kruk B.[2], Sibirny A.[1,2]*[1]Institute of Cell Biology, NAS of Ukraine, Lviv; [2]University of Rzeszow, Poland*corresponding author, sibirny@yahoo.com Development of the feasible process for biofuel production from lignocellulose is very important task of modern microbiology and biotechnology. Thermotolerant yeast *Ogataea polymorpha* is the promising organism for further development as it robustly grows on glucose and xylose and weakly grows on L-arabinose at 45-50^{o}C which could be applicable for simultaneous saccharification and fermentation. However, it accumulates 200 times less ethanol from xylose (0.4 g/L) relative to that from glucose and does not ferment L-arabinose at all. Using combination of the methods of metabolic engineering (deletion of *CAT8* gene, overexpression of *XYL1, XYL2, XYL3, DAS1* and *TAL2* genes) and classical selection, mutant strains of *O. polymorpha* have been isolated which accumulated near 16 g/L of ethanol form xylose at 45^{o}C (Kurylenko et al., 2014; 2018; Ruchala et al., 2017). To overcome glucose inhibition of xylose utilization, native Hxt1 transporter of *O. polymorpha* has been engineered and overexpressed (Vasylyshyn et al., 2020). To further enhance ethanol production from xylose, the best ethanol producing strains were selected for evolving the large colonies on plates with L-arabinose as the sole carbon and energy source. The resulted strains accumulated more than 20 g of ethanol/L from xylose and also small amounts of ethanol from L-arabinose. It was also shown that ethanol production from xylose could be activated after deletion of genes *HAP4A* and *MIG1* coding for transcription factors, *TKL1* and *TAL1* coding for cytosolic transketolase and transaldolase. The role of peroxisomes and autophagy-related gene *ATG13* in xylose fermentation has been shown. Approaches for further development of *O. polymorpha* as promising ethanol producers from pentose sugars under elevated temperatures are under development and will be discussed.

6:01 AM - 6:09 AM

Abstract Title: **Expression and Assembly of Human TIR Proteins from TLR Signaling Supramolecular Organizing Centers (SMOCs) in Saccharomyces cerevisiae**

Author Block: J. Coronas-Serna, E. del Val Oriza, M. Molina, V. J. Cid; Complutense Univ. of Madrid, Madrid, Spain

Abstract Body: **Background:** Toll-like receptor (TLR) signaling is one of the key mechanisms to detect infection or damage and elicit innate immunity responses. Upon recognition of a ligand, TLRs amplify the signal via the formation of Supramolecular Organizing Centers (SMOCs) [1]. These are complexes of proteins sharing protein-protein interaction motifs, namely the Toll/interleukin-1 receptor (TIR) domain. Two SMOCs spread the signal from hTLR4, called myddosome and triffosome [2], composed of the TIR-containing proteins TIRAP and MyD88 or TRAM and TRIF respectively. The stoichiometry and regulatory mechanisms of these SMOCs are not fully understood, despite being related to autoinflammatory disease or increased susceptibility to otherwise mild pathogens, and thus representing valuable potential pharmacological targets. **Objectives:** To bring new insight to the field, we aim to reconstruct the TLR4-associated SMOCs through their

heterologous expression in *Saccharomyces cerevisiae,* a eukaryotic model lacking TLR signaling. **Methods and Results:** Several TLR4 signaling SMOC components were expressed in yeast as either GST or fluorescent protein fusions. The myddosome elements TIRAP and MyD88 localize in the plasma membrane (PM) and cytosolic spots respectively. When co-expressed, TIRAP relocates MyD88 to PM spots, while the introduction of a membrane-bound TLR4 TIR domain reinforces their location into PM foci. Point mutations in TIR domain essential residues impair the TIRAP-MyD88 mutual recruitment, but their subcellular localization remained unaltered. On the other hand, the triffosome components, TRAM and TRIF, did not co-localize in yeast. Nonetheless, TRAM assembled into long PM-bound filaments that become disrupted by co-expression of the TLR4 TIR domain. Downstream IRAK kinases were also expressed together with TIRAP and MyD88 in *S. cerevisiae*. TIRAP interacts with IRAK1 and 2 in the yeast cell, in a setting where all other SMOC elements are absent. IRAK4 binds to MyD88, and its presence induces the formation of a slow mobility band, suggesting a posttranslational modification. Subsequent phosphoproteomic analysis led to the discovery of multiple phosphorylated sites in the MyD88 TIR domain, thus raising the idea of MyD88 being a substrate for IRAK4.In sum, these results prove that *S. cerevisiae* is a suitable model to analyze *in vivo* the interactions and functions of human SMOC building blocks.

6:09 AM - 6:17 AM

Abstract Title: **Characterization of Klebsiella pneumoniae VgrG4 T6SS Effector in Saccharomyces cerevisiae as an Eukaryotic Cell Model**

Author Block: S. López Montesino1, I. Rodriguez-Escudero1, J. Bengoechea2, M. Molina1, V. J. Cid1; 1Univ. Complutense de Madrid, Madrid, Spain, 2Queen's Univ. Belfast, Belfast, United Kingdom

Abstract Body: **Background:** *Klebsiella pneumoniae* is an opportunistic pathogen that mainly causes respiratory and urinary tract infections. The increasingly frequent appearance of hypervirulent and multiresistant isolates has made it one of the species with the highest priority for research and development of therapeutic alternatives (Araujo Barbosa & Santos Lery., 2019). One of its key virulence factors is the expression of a type VI secretion system (T6SS). Recently, we have been identified a new effector of the T6SS, a valine- glycine repeat protein (VgrG4) (Storey et al, 2020). **Objectives:** Based on computer analyses of the VgrG4 protein, that revealed its different domains, in the present work we study the effects of toxicity *K. pneumoniae* VgrG4 in the eukaryotic cell model *Saccharomyces cerevisiae*, as well as its localization, as well as the contribution of distinct domains to the effects observed. **Material / Methods:** Effects of the expression of VgrG4 on yeast growth were tested by spotting cells expressing each truncated VgrG4 version fused to GST from a yeast expression vector under the control of the inducible promoter *GAL1*. Then, to better understand the mechanisms of VgrG4 toxicity, we chose the toxic versions for localization studies, by fusing them to GFP and co-expressing them with organellar markers fused to red fluorescent proteins. **Results:** Both full length VgrG4 and the truncated version containing the C-terminal DUF2345 domain were inhibitory for yeast cells, whereas the truncated variants that contained either the N-terminal region or a unique C-terminal of this protein that is absent in other VgrG paralogs did not affect growth.Localization studies showed that toxic VgrG4 versions localized to membranes displaying markers for the endoplasmic reticulum (ER), which were different from both the peripheral endoplasmic reticulum or the perinuclear reticulum, and were close to the mitochondria.

6:17 AM - 6:25 AM

Abstract Title: **The Aminoglycoside Neomycin Activates the Cell Wall Integrity Pathway in Saccharomyces cerevisiae**

Author Block: E. Jiménez-Gutiérrez, T. Fernández-Acero, E. Alonso-Rodríguez, M. Molina, H. Martín; Complutense Univ. of Madrid, Madrid, Spain

Abstract Body: **Background:** Signalling pathways mediated by mitogen-activated protein kinases (MAPKs) are essential for cell survival due to their ability to detect changes in the extracellular environment and adaptively respond to them. MAPK pathways are widely distributed among eukaryotic cells and are composed of a three-tiered-kinase module, comprising a MAPKKK, a MAPKK, and a MAPK, which are activated by sequential phosphorylation. In general, the stimulus is detected by sensors on the cell surface. They transduce the signal to downstream components of the route, usually a GTPase and a protein kinase, which in turn activates the MAPK module. Lastly, the active MAPK phosphorylates its effectors, mostly transcription factors, which elicit the adaptive response. The budding yeast *Saccharomyces cerevisiae* presents five MAPK pathways, including the Cell Wall Integrity (CWI) pathway, which is responsible for the maintenance of the integrity of the fungal cell wall, an essential cell structure that constitutes an interesting antifungal target. **Objectives:** The main objective of this work was to characterize the cellular response against neomycin and the role of the CWI pathway and other signalling pathways in it. **Methods:** To study the CWI signalling pathway, our research group developed a synthetic positive feedback circuit named 'Integrity Pathway Activation Circuit' or 'IPAC'. Its activation under stimulating conditions results in growth inhibition due to the hyperactivation of the CWI pathway. **Results:** Using this genetic tool, we found that, in contrast with other protein synthesis inhibitors, the aminoglycoside neomycin activates the IPAC circuit, inhibiting cell growth. We also demonstrated that the CWI pathway is necessary for yeast cell survival in the presence of neomycin. Moreover, Pkh1 protein kinase from the sphingolipid synthesis pathway and the PIP_2 binding protein Slm1 from the TORC2 pathway take part in the neomycin-induced signalling through the CWI pathway. These findings are in accordance with the fact that this compound interacts with phosphatidylinositol-4,5-bisphosphate (PIP_2) at the plasma membrane in mammalian cells and the requirement of the presence of this phosphoinositide for some components of the CWI pathway to function properly. To sum up, the neomycin constitutes a novel CWI pathway stimulus and seems to activate this pathway through the alteration of the cell membrane PIP_2 pools.

FEMS107 - Rapid Fire: Environmental Microbiology and Ecology

Session Room: Channel 5

5:45 AM - 5:53 AM

Abstract Title: **Microbial Lignin Degradation in the Environment**

Author Block: B. Steel1, L. Stancampiano1, C. Magill1, T. Aspray2, J. Todd3, D. Goevert4, J. Pratscher1; 1The Lyell Ctr., Heriot Watt Univ., Edinburgh, United Kingdom, 2Solidsense Ltd, Glasgow, United Kingdom, 3Univ. of East Anglia, Norwich, United Kingdom, 4Recircle Ltd, Edinburgh, United Kingdom

Abstract Body: Lignocellulosic biomass has attracted attention as a renewable industrial feedstock due to its abundance in nature and wide range of potential applications. Current conversion processes, such as in biofuel production, are complicated by the presence of the recalcitrant lignin within lignocellulose. Research has identified several microorganisms capable of degrading lignin, which could potentially be exploited for use in bioprocessing, however, still little is known of how microorganisms, specifically bacteria, contribute towards the degradation of lignin in the environment. Therefore, in this project we investigated microbial lignin degradation in a wide range of oxic and anoxic environments, including soil, herbivore animal faeces, compost and lake sediment. Using a combined approach of biochemical and molecular biological techniques, such as stable isotope probing (SIP) with 13C labelled lignin, SSU rRNA gene amplicon sequencing, and metagenomics, we identified the main microbial players, including key enzymes and biochemical pathways that contribute to the degradation of lignin in these ecosystems. SSU rRNA gene community analysis combined with metagenomics of the environmental samples further highlighted specialised microbial communities with the genetic potential for diverse lignin degradative mechanisms. Lignin-degrading bacterial isolates from these environments and their phylogenetic characterization and ligninolytic enzyme activity analysis, as well as gas chromatography-mass spectrometry (GC-MS) analysis of their lignin-oxidised products revealed specific utilization of oxidative enzymes by different isolates and diverse lignin breakdown. These results provide new insights into microbial degradation of recalcitrant plant material in the environment.

5:53 AM - 6:01 AM

Abstract Title: **The Function and Activity of Microbial Communities in Subarctic Soils**

Author Block: S. Viitamäki, I. Pessi, E. Eronen-Rasimus, J. Hultman; Univ. of Helsinki, Helsinki, Finland

Abstract Body: Soil microbial communities have critical role in biogeochemical processes on Earth, but our understanding of their response to the ongoing climate change is poor. Arctic and subarctic soils harbor approximately 50 % of Earth’s below ground carbon. Warmer climate leads to increased rate of soil organic matter decomposition in polar regions, but the overall impact to carbon and other biogeochemical cycles is

difficult to predict without a deeper understanding of the soil microbial ecology. Our aim is to improve the knowledge of the ecology of microbial communities in subarctic soil in changing climate conditions, couple the data to extensive set of metadata, and to use this data to predict the microbial feedback to environmental change. Metatranscriptomics with double RNA approach was applied to elucidate the active functions and activity of microbial communities in subarctic soils collected from Kilpisjärvi, northern Finland. The sampling site forms a climate gradient with 78 sites, representing the possible scenarios of the impact of climate change to soil microbial community. Various metadata including pH, carbon content, soil organic matter, was collected and analyzed together with the metatranscriptomic data. Our data shows differences in the composition and activity of the microbial communities along the climate gradient. Soil pH, organic matter and moisture were the main drivers of activity in organic layer, whereas pH was in the mineral layer. These results give information on how environmental factors contribute to microbial activity and again its feedback effect to warming climate.

6:01 AM - 6:09 AM

Abstract Title: **Glacier Ice Archives Fifteen-thousand-year-old Viruses**

Author Block: Z. Zhong; The Ohio State Univ., Columbus, OH

Abstract Body: Glacier ice archives information, including microbiology, that helps reveal paleoclimate histories and predict future climate change. Though glacier ice microbes are studied using culture or amplicon approaches, more challenging metagenomic approaches, which provide access to functional, genome-resolved information and viruses, are under-utilized. We expand existing clean sampling procedures using controlled artificial ice core experiments to establish metagenomic approaches to study glacier viruses. Controlled sampling experiments drastically reduced contaminating bacteria, viruses, and free DNA to background levels. Amplicon sequencing from eight depths of two Tibetan Plateau ice cores revealed common glacier ice lineages including *Janthinobacterium*, *Polaromonas, Herminiimonas*, *Flavobacterium*, *Sphingomonas* and *Methylobacterium* as the dominant genera. Separately, ~520- and ~15,000-year-old ice were subject to viral enrichment and low-input quantitative sequencing, yielding genomic sequences for 33 vOTUs, representing 28 novel genera and not a single species shared with 225 environmentally diverse viromes. Further, 42.4% vOTUs were identifiably temperate, significantly higher than that in gut, soil and marine viromes, indicating that temperate phages are possibly favored and selected in glacier environments before being frozen. *In silico* host predictions linked 18 vOTUs to co-occurring ice-abundant bacteria *Methylobacterium*, *Sphingomonas,* and *Janthinobacterium*, indicating the infection to abundant bacterial groups before being archived. Functional genome annotation revealed four virus-encoded auxiliary metabolic genes, particularly two motility genes suggest viruses facilitate nutrient acquisition for their hosts. Finally, we focused on the dominant *Methylobacterium* viruses by contextualizing our ice-observed viruses against 123 viromes and prophages extracted from 131 *Methylobacterium* genomes, revealing that the archived viruses might originate from soil or plants. Together these efforts further microbial and viral sampling procedures for glacier ice, reveal microbiological findings concordant with ice core climate records, and provide a first window into viral communities and functions in ancient glacier environments. Such methods and datasets should enable researchers to contextualize new discoveries and begin to incorporate glacier ice microbes and their viruses relative to past and present climate change in geographically diverse regions globally.

6:09 AM - 6:17 AM

Abstract Title: **Omics Metrics for Ecological Niche Potential and Metabolic Interactions**

Author Block: V. Mataigne1, N. Vannier2, P. Vandenkoornhuyse1, S. Hacquard2; 1UMR 6553 ECOBIO - Université de Rennes 1, Rennes, France, 2Max Planck Inst. for Plant Breeding Res., Köln, Germany

Abstract Body: Metabolic interactions among microbes are frequently investigated to explain microbiome dynamics and structure. We explored bacterial genomes with phylogenetic and metabolic metrics, in an attempt to investigate niche potential and find patterns for putative metabolic dependencies. In this aim, genomes of a culture collection of 193 bacteria of the *Arabidopsis thaliana* root microbiome were used. We found that the absence or presence of chemical reactions within genomes is clustered by phylogeny, highlighting metabolism specialization and differentiation respectively within and between taxonomic groups. We also modeled all strains' production ability of relevant compounds (amino acids, vitamins, phytohormones) in a non-constrained rich medium and a constrained minimum medium mimicking a rhizosphere environment. Many strains could produce most targeted compounds on the non-constrained medium, and lost this ability on the constrained-one medium, suggesting dependencies on the secretome of producer strains (i.e. cross-feeding). Moreover, the differentiation of metabolism according to taxonomy weakens when considering only the capacity of producing the targeted compounds. This suggests the importance of available nutrients in the media for niche -potential, -realization, -differentiation, and -overlap. We can also hypothesize that the chosen targets are too ubiquitous to distinguish the strains, i.e. part of their core metabolism, and that niche differentiation appeared to be linked to secondary metabolism.From the 193 strains collection, several metrics applied on random bacterial communities indicated that the number of different reactions in the genomes notably increases with average phylogenetic distance, with an optimum reach for small-sized communities (community and genomes sizes also have an influence). The shared reactions (core metabolism) decreases along the same metrics, without optimum. The biological meaning of these findings is still unclear, but the observed optimum could be interpreted as a niche overlap optimum, a trade-off between metabolic dependencies and competition.Despite these patterns, the relevance of these metrics obtained by microbial system ecology has to be demonstrated. Experimental validation of some synthetic communities are currently being carried out. In any case, it opens new routes of research to understand co-existence and functional interdependence in the root microbiome.

6:17 AM - 6:25 AM

Abstract Title: **Unravelling Bacterial Plant Colonization at the Genome Scale: Insights Into the Rhizosphere**

Author Block: M. Torres Bejar1, K. Zhalnina1, P. W. Kim2, T. K. Owens1, H. K. Carlson1, T. R. Northen1, A. M. Deutschbauer1; 1Lawrence Berkeley Natl. Lab., Berkeley, CA, 2Sandia Natl. Lab., Livermore, CA

Abstract Body: **Background:** As plants perform photosynthesis, they transfer up to 20-40% of the fixed C to the soil through root exudates. These contain nutrients such as sugars and amino acids that are leaked into the rhizosphere, providing an enticing environment for the microorganisms that reside in soil. Amongst them, diverse soil bacteria can influence plant growth and development. In spite of the availability of data on the functional and phylogenetic diversity of plant-associated bacteria, the molecular mechanisms shaping their successful establishment

and enabling them to effectively colonize plants continue to be poorly understood. **Objectives:** The aim of this research is to identify a comprehensive set of genes that are involved in rhizosphere colonization using a genome-wide transposon mutagenesis approach, and to assess if the identified colonization genes are reproductible amongst different types of plant assays in hydroponics and several types of solid substrates. **Methods:** In the last decade, a number of methodologies for transposon insertion site sequencing (Tn-Seq) have been developed for measuring fitness and assigning phenotypes and functions to bacterial genes. In our study we have used RB-TnSeq (random barcode transposon-site sequencing), that combines the advantages of Tn-Seq and rapid quantification of each transposon mutant using unique barcodes that can be amplified by PCR. We have constructed mutant libraries of different plant-associated bacteria and tested their growth with different sugars and amino acids usually found in root exudates. We have also conducted rhizosphere colonization experiments using the *Brachypodium* model grass and different types of substrates in order to assess data reproducibility amongst experimental setups. **Results:** The RB-TnSeq approach has proven to be a very efficient approach for understanding gene functions involved in rhizosphere colonization, as shown in our preliminary results. The outcome of our study will allow for a more accurate understanding of rhizosphere colonization at the genome scale.

6:25 AM - 6:33 AM

Abstract Title: **Plant miRNAs Interfere with Nitrogen Use of Soil Microorganisms**

Author Block: J. Dozois; INRS, Laval, QC, Canada

Abstract Body: Nitrogen is the most limiting element for plants. In order to acquire nitrogen, plants need to compete with soil microorganisms. Plant micro ribonucleic acids (miRNAs) are a group of molecules that can potentially interfere with the nitrogen use of soil microorganisms, alas they are missing from the plant-microbiome paradigm. We hypothesize that miRNAs are key players in plant-microbe interactions, and that they are especially released by plants into the rhizosphere in poor nitrogen soil conditions. Our experimental designed included 5 blocks where 2 plants (*Arabidopsis* and *Brachypodium*) were grown alongside unplanted controls under three nitrogen regimes (organic, inorganic and no added nitrogen) for 21 days. Nucleic acids were extracted and sequenced for microbiome and small RNA profiling. The bacterial community was strongly influenced by the fertilizer treatments whereas the fungal community was mainly influenced by the type of plant. Biostatistics revealed 53 miRNAs with significant responses to nitrogen fertilization, 10 of which were only found in the rhizosphere or bulk soil of planted pots. Target prediction analyses of these 10 miRNAs showed that 9 of them potentially affected important microbial nitrogen transporters or genes involved in the nitrogen cycle such as nitrite reductases, nitrous oxide reductases, peptidases and proteases. The abundances of the 10 miRNAs were correlated with bacterial abundances to identify possibly responding taxa. The next steps are to quantify the miRNAs and genes linked to the nitrogen cycle via RTqPCR and to confront the rhizosphere bacteria targeted by the 9 miRNA candidates in vitro. In brief, our findings imply that plant miRNAs targeting microbial N-related genes are released under various nitrogen availability conditions and may therefore enable plants to competitively acquire nitrogen in a variety of circumstances. Lastly, the release of miRNAs into the rhizosphere to modulate microbial activities is probably a widespread phenomenon within the plant kingdom.

21 June 2021 5:45AM

FEMS109 - Rapid Fire: Health and Food Microbiology

Session Room: Channel 4

5:45 AM - 5:53 AM

Abstract Title: **Can Bacteriophages Contribute Massively to the Food Safety Future? Bacteriophages as a Biosensor Tool for the Detection of Foodborne Pathogens with Emphasis on Immobilization of Bacteriophage for the Detection of Non O157:H7 Shiga Toxin-producing E. coli**

Author Block: N. Alasiri, M. Griffiths, A. Kropinski, H. Anany; Univ. of Guelph, Guelph, ON, Canada

Abstract Body: **Introduction:** Foodborne pathogens are a major cause of disease and death among the global population. Illnesses related to contaminated food may vary from person to person from temporary to long-term complications. Nevertheless, the rapid rise of multidrug-resistant bacteria worldwide with a declination in antibiotics developments and production make bacteriophages an attractive tool to overcome bacterial resistance. Bacteriophages have become widely recognized for several potential applications in food industry. They represent an ideal tool for a rapid and sufficient diagnostic assays with great potentials in controlling the spread of harmful pathogens. Their abundance in nature of and high specificity against a specific host bacterium allow them to eradicate, prevent foodborne illness and recalls and provide safe food to consumers. **Purpose:** The purpose of this topic is to shed a light on the existing phage based application such as immobilization of phages and using them as a biosensor for foodborne pathogen detection in food. **Methods:** In our research, a phage capture-amplification assay based on the phage immobilization on bioactive paper were used. Experiments started with as isolation of very specific phage against one or more of Non-O157:H7 *E. coli* in food. Isolated phages are screened and made to undergo a variety of phenotypic and genotypic characterization to make sure that they meet the desirable requirement For example host range experiment, efficiency of plating, phage adsorption, growth curve of phage, whole genome sequencing, immobilization phage into colorlok paper (dipstick approach) using electrostatic properties of phages and the surface. The data were collected from three independents trials where the averages and standard deviations were determined. Detection limits were calculated and compared using an Independent-Samples *t*-Test using IBM SPSS. **Results:** The result have statistically significant differences ($P<0.05$) in the detection of *E. coliO45:H2.* The cycle threshold (Ct) values were averaged for each concentration and compared to the average Ct values for the incubated control paper without phage. Using phages as biosensor enabled the detection of as few as 10CFU/mL of the Big Six Shiga toxin-producing *E. coli* strains in both TSB media and ground beef using both a plaque assay and real-time PCR to detect phage progeny..

5:53 AM - 6:01 AM

Abstract Title: **Antimicrobial Resistance Genes Are Common in Human and Environmental Samples from West Africa**

Author Block: K. Haukka1, M. Markkanen1, A. Sarekoski1, J. Muurinen1, K. Sintondji2, Z. Garba3, J. Ouedraogo4, V. Dougnon2, I. Bonkoungou3, B. Kouriba4, L. Timbine4, K. Pärnänen1, A. Karkman1, A. Kantele1, M. Virta1; 1Univ. of Helsinki, Helsinki, Finland, 2Univ. of Abomey-Calavi, Abomey-Calavi, Benin, 3Univ. Joseph Ki-Zerbo, Ouagadougou, Burkina Faso, 4Ctr. d'Infectiologie Charles Mérieux, Bamako, Mali

Abstract Body: **Background:** Treatment failures due to antimicrobial resistance (AMR) are becoming a serious problem in West Africa. However, the data available concerning the type, occurrence and spreading patterns of the AMR genes in the area are limited. In the AMRIWA project we are gathering information on occurrence of AMR genes and multiresistant bacteria in humans, animals and environment in Benin, Burkina Faso and Mali. **Objectives:** Our aim is to better understand and control the emergence and transfer of AMR in West Africa by identifying potential hotspots of gene transfer. **Methods:** We collected approx. 2500 samples representing various sources and sites, estimated to be important for the transfer of resistance genes in the West African context. We collected human and animal faecal samples, human hand swabs, hospital waste waters and samples from environmental sources that people and animals interact with and within. We characterized the resistome in the samples using DNA-based techniques and also isolated strains from chromogenic plates specific for carbapenemase-producing Enterobacteriaceae. **Results:** Metagenomic sequence analysis of waste waters from nine African and four Finnish hospitals showed that AMR genes were more common in the African than in the Finnish hospitals. Similar clinically significant AMR genes were detected in both, but in different proportions. Furthermore, using SmartChip qPCR array for detection of 34 AMR genes, environmental samples both in the cities and in the countryside were found to contain high abundance of AMR genes. These included several carbapenemase genes, especially *bla*NDM, *bla*VIM and *bla*OXA-48, and colistin resistance genes. Isolation of carbapenemase-producing bacteria from the same samples showed opportunistic pathogens such as *Escherichia coli*, *Acinetobacter baumannii* and several *Pseudomas*, *Klebsiella* and *Enterobacter* species to be prevalent. They were isolated also from the faeces and hands of healthy people. **Conclusions:** Based on our results, it appears that AMR is a wide-spread phenomenon in West Africa, aggravated by defective hygienic conditions and poor or non-existing waste water management. Currently it is not known how seriously the carriage of AMR genes by healthy population compromises the treatment choices available in health care. Therefore, there is an urgent need to develop risk assessment methods to understand the risks caused by the wide-spread occurrence of opportunistic pathogens that carry AMR genes.

6:01 AM - 6:09 AM

Abstract Title: **Daily High-protein, Drained Yoghurt Consumption Alters the Fecal Abundance of Selected Functional Microbial Groups**

Author Block: F. Ghiamati Yazdi1, L. B. Dalgaard2, Q. Li1, H. J. Ruscheweyh3, M. Hansen4, C. Schwab1; 1Dept. of Biological and Chemical Engineering, Aarhus Univ., Aarhus, Denmark, 2Dept. of Publ. Hlth., Aarhus Univ., Aarhus, Denmark, 3Swiss Inst. of Bioinformatics and Microbiol., ETH Zürich, Zürich, Switzerland, 4Dept. of Biology, Aarhus Univ., Aarhus, Denmark

Abstract Body: **Background:** Fermented foods consumption has gained much attention due to their ability to deliver live microbes to the gut, which might play a role in health-promotion or disease prevention. The high protein, drained yoghurt Skyr is fermented by *Streptococcus thermophilus* and *Lactobacillus delbrueckii* sp. *bulgaricus.* It was the overall goal of this study to determine whether there is a relationship between regular Skyr consumption, fecal microbial profile and microbial fermentation activity. **Methods:** The study cohort encompassed 38 over-weighted women; 26 participants omitted eating breakfast (non breakfast group) and 12 who consumed 300 gr/day Skyr for 120 consecutive days (Skyr group). Age, body mass index (BMI), and total body fat were determined before the start of the study. Fecal samples were collected on days 0, 45, and 120 for microbiota and metabolite analysis. Quantitative microbiome profiling was performed using 16S

rRNA gene amplicon sequencing and quantitative polymerase chain reaction, while MetaLonDa was used to identify temporarily differentially abundant features. Short chain fatty acids concentration as indicator of microbial fermentation activity was measured by high-pressure liquid chromatography with refractive index detection. **Results:** The age of the cohort ranged from 20-29 years old, the BMI from 26-44 kg/m^2 and total body fat was 33-57%. Overal microbiota composition, based on alpha and beta-diversity, did not change between 0, 45 and 120 days in either group. MetaLonDa identified a significant higher abundance of Amplicon Sequence Variants (ASV) assigned to *Streptococcus*, *Ruminococcus torques*, *Eubacterium hallii,* and *Desulvovibrio,* and *Anaerostipes* in the Skyr group, which was confirmed by quantitative microbiome profiling. There was also a reduction in the absolute abundance of *Faecalibacterium*, *Butyricicoccus* in the Skyr group at day 45 compared to day 0. The concentration of acetate was significantly lower in the Skyr group at day 120, while there was no difference in butyrate or propionate levels between groups. **Conclusion:** This study shows that daily Skyr consumption had immediate impact on the fecal microbiota. Skyr consumption successfully transferred the *Streptococcus* starter culture to the gut and enhanced the abundance of selected lactate utilizers. Together, our results suggest that daily Skyr consumption might impact intestinal microbial cross-feeding activities likely due to the nutritive and microbial components of Skyr.

6:09 AM - 6:17 AM

Abstract Title: **Fermented Tomato Extract: A New Source of Antimicrobial Compounds**

Author Block: A. Ricci, V. Bernini, E. Neviani, C. Lazzi; Univ. of Parma, Parma, Italy

Abstract Body: **Background:** Tomato is a crop cultivated all over the word. The major producer countries in 2019 were China, followed by India, Turkey, USA, Egypt and Italy (FAOSTAT, 2019). More than 80 % of tomatoes are consumed as processed products and 7-7.5 % of the raw material is discarded after processing (Nour et al., 2018) making the management of tomato by-products one of the most important sustainability related issues for agro-industrial companies (García et al., 2009). So, in the context of a sustainable and circular economy, fermentation can be a valid strategy to exploit agro-food by-products as a potential source of low-cost substrate for fermentation resulting in the production of value added products. **Objectives:** Applying this **Background:** we employed tomato by-products for the production of antimicrobial compounds. Methods: Tomato by-products were used as substrate for solid state fermentation using lactic acid bacteria as starter. After fermentation an extract was produced and its antimicrobial activity was tested *in vitro* against the main foodborne/spoilage microorganisms applying agar well diffusion assay and minimum bactericidal concentration. Its activity was also tested in real foods matrices through challenge tests in order to confirm the data observed *in vitro*. Finally to find out the compounds responsible for the antimicrobial activity of fermented extract, the phenolic, amino acid and the organic acid composition as well as the steroidal alkaloids were analysed. **Results:** From the data obtained in this work tomato fermented extracts demonstrate antimicrobial activity and to be a promising way for food preservation, but also a great option to produce high-value added products from tomato by-products recovery. This project is part of the Italian patent n. 102019000006815 (14 May 2019) with international extension PCT/IB2020/054520 (13 May 2020).

6:17 AM - 6:25 AM

Abstract Title: **The Use of Non-conventional Native Yeasts to Improve 'Sideritis' Wine Quality, a Late-ripening Greek Cultivar**

Author Block: G. Banilas1, E. Derveni1, M. Filippousi2, A. Nisiotou2; 1Univ. of West Attica, Athens, Greece, 2ELGO-DEMETER, Athens, Greece

Abstract Body: **Background:** In view of the upcoming climate change and the increasingly antagonistic wine market, the exploitation of native grape and microbial genetic resources is currently revisited as an alternative approach to sustainable wine production. In this context, 'Sideritis' is a rare Greek grape variety native to Achaia region, Peloponnese, which due to very late ripening may successfully counteract the annual temperature raise. It gives white delicate wines with high acidity. Nevertheless, it is seldom used for the production of varietal wine due to the relatively low aroma intensity and complexity. **Objectives:** The long-term goal of this study is to improve the organoleptic profile of Sideritis wine by the selection of superior grapevine clones that will be properly vinified. **Methods and Results:** To this end, ten promising Sideritis genotypes from Achaia region were analyzed by microsatellite markers (SSRs) to detect genetic variation. The results were quite interesting showing up to two different alleles for each SSR locus analyzed. In order to improve vinification, as compared to the traditional practice applied in the region, *Hanseniaspora opuntiae* E3SL1 and *Kazachstania hellenica* D9W17, two native non-*Saccharomyces* (NS) yeasts, were inoculated along with *Saccharomyces cerevisiae* E58NW7 in mixed fermentations. Single-inoculated fermentation with E58NW7 (Sc) was also performed. Sc showed the highest fermentation rate. Strain D9W17 exhibited longer persistence than E3SL1. The chemical profiles were largely affected by the NS yeast species applied. The present results are quite promising to improve 'Sideritis' wine quality, towards a sustainable wine production in Western Greece.

6:25 AM - 6:33 AM

Abstract Title: **Community Composition in Sourdough Fermentation Is Dependent on Species and Flour Composition**

Author Block: A. Wittwer, A. Tee, D. Liu, K. Howell; Univ. of Melbourne, Parkville, Australia

Abstract Body: Community composition in sourdough fermentation is dependent on species and flour composition Anna Wittwer, Annli Tee, Di Liu and Kate Howell Background: Sourdough fermentation is a model to study interkingdom communication and interaction between yeast and bacteria. While the species present have been comprehensively catalogued, the interactions and persistence of yeast and bacteria have not been characterised. Objectives: This study investigated the pairwise interactions of yeast and bacteria and considered the impact of nutrient availability to persistence and activity of fermenting microbes and considered the impact on dough and bread composition. Methods and Results: *Saccharomyces cerevisiae* was either dominant or co-dominant in every reconstructed sourdough fermentation, while *Kazachstania humili*s was only able to persist in a simulated dough fermentation if in combination with *S. cerevisiae*. Inoculating *Fructilactobacillus sanfranciscensis* (previously named *Lactobacillus sanfranciscensis*) in reconstructed sourdoughs was not successful, and this isolate did not persist. There were no antagonistic interactions between yeast and bacteria detected. Construction of

the sourdough microbial communities in wheat flour dough was able to produce attractive loaves of bread, with an enrichment of small molecule volatiles. Inclusion of bran particles increased the small molecules produced. Interactions between sourdough microbes may affect longevity and diversity of the fermenting population, and these results have implications for deliberate construction of sourdough communities for bread production.

21 June 2021 5:45AM

FEMS112 - Rapid Fire: Infection Biology and Pathogens

Session Room: Channel 6

5:45 AM - 5:53 AM

Abstract Title: Characterization of Small Non-coding RNAs Expressed by Burkholderia cenocepacia When Infecting Caenorhabditis elegans

Author Block: T. Pita1, J. R. Feliciano1, S. A. Sousa1, J. Vogel2, J. Leitão3; 1Inst. Superior Técnico, Univ.e de Lisboa, Lisbon, Portugal, 2Inst. for Molecular Infection Biology, Würzburg, Germany, 3Inst. Superior Técnico, Univ.e de Lisboa, Lisboa, Portugal

Abstract Body: **Background:** Small non-coding RNAs (ncRNAs) are key regulators of post-transcriptional gene expression in bacteria. These molecules can interact with mRNAs or proteins, affecting a variety of bacterial functions [1]. Despite the identification of hundreds of bacterial ncRNAs mainly due to high throughput techniques, such as RNA sequencing (RNASeq) [2], their roles on bacteria physiology and virulence remain largely unknown. This is the case of bacteria of the *Burkholderia cepacia* complex (Bcc), a group of opportunistic pathogens capable of causing lethal lung infections among cystic fibrosis (CF) patients. This group of bacteria are among the few prokaryotes that encode two Hfq like RNA chaperones in their large genomes, as well as an homolog of the most recently characterized ProQ. Despite recent developments on the identification of ncRNAs from Bcc bacteria, an approach using a host infection model is still missing [3]. **Objective:** The aim of the present work is to characterize ncRNAs expressed by Bcc bacteria when infecting a host. **Methods:** The nematode *Caenorhabditis elegans* was used as an infection model, being infected with the epidemic CF strain *B. cenocepacia* J2315. The RNA extracted from infecting bacteria was sequenced by RNASeq and bioinformatics analyses were performed to identify putative ncRNAs. The predicted ncRNAs were validated by 3' RACE and 5' RACE, and their dependency of Hfq, Hfq2 and ProQ was assessed. **Results:** From a total of 8696 transcription start sites, 98 ncRNAs with a predicted Rho independent terminator were identified, most of them located on chromosome 1. Some of those ncRNAs were previously identified [3], validating the methodology and the results obtained. Previous data from Hfq, Hfq2 and ProQ CLIP-seq analysis were used to predict possible interactions between these chaperones and the identified ncRNAs. Four ncRNAs were selected for further characterization based on their expression levels, conservation among Bcc bacteria and predicted interaction with the mentioned chaperones. Three of the selected ncRNAs show a reduced level of expression in Hfq deletion mutant, which can be linked to the well-known pleiotropic effects of this chaperone, particularly on virulence.

5:53 AM - 6:01 AM

Abstract Title: **Diverse Pseudomonas aeruginosa Isolates Cause an Antigen-specific T Cell Response in Patients with Cystic Fibrosis**

Author Block: V. Chaves Vargas1, A. Galeev1, A. Habich1, N. Benny2, C. Schwarz3, P. Bacher2, D. Unterweger1; 1Max Planck Inst. for Evolutionary Biology, Ploen, Germany, 2Kiel Univ., Kiel, Germany, 3Klinikum Westbrandenburg, Potsdam, Germany

Abstract Body: **Background:** Chronic lung infections are central to the pathogenesis of cystic fibrosis (CF) patients. Those infections can be caused by several pathogens that trigger an inflammatory immune response in CF patients. *Pseudomonas aeruginosa* is an opportunistic human pathogen that often colonizes the lungs of CF patients by early adulthood. While the intraspecific diversity of *P. aeruginosa* within CF lungs has been described, little is known about the effect of such diversity on the adaptive immune responses in the context of CF. **Objectives:** Here, we focus on a CF patient cohort aiming (i) to characterize the *P. aeruginosa* isolates that colonize individual patients and (ii) to determine the adaptive immune response to *P. aeruginosa* in those patients. **Methods and Results:** *P. aeruginosa* was isolated by plating the patients' sputum samples on selective media. Individual isolates were confirmed by PCR using species-specific primers and subjected to whole genome sequencing. The isolates were phenotypically characterized in terms of their growth, colony morphology, and virulence. Most strikingly, we observed two distinct morphotypes among the clinical *P. aeruginosa* isolates: small colony variants (SCV) and strains of a normal morphology (similar to reference strains). The SCVs, which were prevalent in all patients, grew poorly in liquid media and had the tendency to clump. These findings support previous data on SCVs and highlight the intraspecific diversity of *P. aeruginosa* within individual patients.To characterize the T cell responses to *P. aeruginosa*, we used the highly sensitive technology of antigen-reactive T cell enrichment. In short, after several hours of stimulation with bacterial lysate, antigen-activated CD4+ T cells (CD154+) were magnetically enriched and T cell frequencies were determined using multicolor flow cytometry. We detected no increased CD4+ T cell reactivity against *P. aeruginosa* in CF patients compared to healthy controls, but a tendency towards a higher proportion of memory cells within reactive CD154+ cells was observed. Those findings indicate that CF patients generate a T cell mediated immune response to *P. aeruginosa*. **Conclusions:** Taken together, we characterized diverse *P. aeruginosa* isolates that are subject to an adaptive immune response in CF patients. Such analyses will lead to a better understanding of the interplay between bacteria and the immune system during chronic lung infections.

6:01 AM - 6:09 AM

Abstract Title: Investigation Into the Reversibility of the Novel Toxin-antitoxin System Escherichia coli DarTG to Characterise Its Potential Role in Bacterial Persistence

Author Block: T. J. Szeligowski, Tang Laboratory, Sir William Dunn School of Pathology; Univ. of Oxford, Oxford, United Kingdom

Abstract Body: **Background:** DarTG is a recently characterised toxin-antitoxin system found in enteropathogenic *Escherichia coli* - a key cause of persistent diarrhoea in children. The toxin, DarT, inhibits DNA replication and bacterial growth by ADP-ribosylation of single-stranded DNA, while DarG reverses this effect through two mechanistically distinct domains. **Objectives:** Previous experiments showed that DarT exerts a bacteriostatic effect, suggesting that it could be involved in bacterial persistence through reversible growth inhibition. Here we investigate the reversibility of DarTG to further study this hypothesis, and validate chromosomal fluorescence dilution (ChromFD) as a tool for studying heterogeneity in early bacterial growth. **Methods:** $darT^{G49D}$ and *darG* were cloned under inducible promoters and transformed into *E. coli* BL21(DE3). A mutated but biochemically active and toxic version of DarT was used due to high toxicity of the wild-type toxin in *E. coli* BL21(DE3). $darT^{G49D}$ was induced at T = 0 h, while *darG* at T = 0-4 h, and bacterial viability measured by colony forming unit (CFU) counts for 24h. For ChromFD, constitutive *mCherry* and inducible *GFP* were inserted onto the *E. coli* BL21(DE3) chromosome. *GFP* was induced overnight and then repressed to observe dilution using flow cytometry. **Results:** $darT^{G49D}$ induction led to a drastic decrease in

bacterial viability. Co-induction of *darT*^{G49D} and *darG* maintained bacterial growth, demonstrating that DarG counteracts DarT^{G49D}-mediated toxicity. Delayed *darG* induction as late as 4 h after *darT*^{G49D} led to full recovery of bacterial viability by 24 h, with no significant difference in CFU counts compared to samples in which *darG* was induced at T = 0 h. Both domains of DarG are likely required for reversibility, as shown by no recovery when the two domains were cloned and induced separately. ChromFD was validated as a tool to measure early phases of bacterial growth, with no significant difference observed in the predicted number of generations between chromFD, CFU counts, and optical density for the first 2 h following *GFP* repression. Using chromFD, we observed heterogeneity in the time taken to restart replication among cells expressing *darTG,* with a distinct population restarting growth as late as after 6 h. This study provides early evidence that DarTG could be involved in persistence through reversible inhibition of bacterial growth. It also offers a new method for investigating this phenomenon, which allows detection of subpopulations of cells with altered growth dynamics in early stages of regrowth.

21 June 2021 5:45AM

FEMS118 - Rapid Fire: Infectious Diseases

Session Room: Channel 3

5:45 AM - 5:53 AM

Abstract Title: **Plasma Signatures of Necrotizing Soft Tissue Infections Clinical Endotypes**

Author Block: L. M. Palma Medina1, E. Rath2, S. Jahagirdar3, T. Bruun4, M. Madsen5, K. Strålin6, C. Unge1, M. Hansen5, P. Arnell7, M. Nekludov8, O. Hyldegaard5, M. Lourda1, V. Martins dos Santos3, E. Saccenti3, S. Skrede2, M. Svensson1, A. Norrby-Teglund9; 1Karolinska Inst.t, Huddinge, Sweden, 2Haukeland Univ. Hosp., Bergen, Norway, 3Wageningen Univ. & Res., Wageningen, Netherlands, 4Haukeland Univ. Hosp., Bergen, Norway, 5RigsHosp.et, Copenhagen, Denmark, 6Karolinska Univ. Hosp., Stockholm, Sweden, 7Sahlgrenska Univ. Hosp., Gothenburg, Sweden, 8Karolinska Univ. Hosp., Solna, Sweden, 9Karolinska Inst., Huddinge, Sweden

Abstract Body: **Background:** Necrotizing soft tissue infections (NSTI) are life-threatening infections characterized by tissue destruction, rapid progression, and frequently complicated by septic shock. Early diagnosis is critical but challenging due to vague initial symptoms and lack of specific clinical biomarkers. In a recent multicenter study of NSTI (INFECT-project), distinct clinical endotypes were identified involving different co-morbidities, localization, and microbiological etiology. **Objectives:** We explored the inflammatory response profiles in plasma from NSTI patient and sought to identify candidate biomarkers for identification of clinical endotypes and outcome. **Methods:** Concentrations of selected proteins in plasma samples from 251 NSTI patients with positive microbiological cultures were compared to levels measured in two control groups of patients: 20 non-infected surgical controls and 20 non-NSTI patients, i.e. suspected NSTI but with no necrotic tissue after surgical examination. The plasma samples were analyzed by customized Luminex multiplex assays covering 36 analytes including cytokines, adipokines, and tissue remodeling factors. Statistical analyses comprised univariate Mann-Whitney tests, receiver operating characteristic curves, and random forest models to identify potential biomarkers. **Results:** Most measured protein concentrations were significantly different in NSTI patients in comparison to non-infected surgical controls. However, comparison of NSTI with non-NSTI controls (suspected NSTI with no necrosis detected upon exploratory surgery) revealed that Thrombomodulin was a unique biomarker for NSTI cases (AUC 0.95). A distinct profile discriminating mono- (type II) versus poly-microbial (type I) NSTI types was identified based on differential expression of IL-2, IL-10, IL-22, CXCL10/IP-10, Fas-Ligand and MMP9. Notably, each NSTI type displayed a distinct array of biomarkers predicting septic shock, but both shared differences in G-CSF, S100A8 and IL-6 concentrations. The discriminatory power of all selected biomarkers was corroborated using a validation cohort (n=60). Lastly, differential connectivity analysis revealed distinctive networks associated with specific clinical endotypes, reflecting varying underlying pathogenic mechanisms. Taken together this study identifies predictive biomarkers for NSTI clinical endotypes of potential value for novel diagnostic, prognostic, and therapeutic approaches in NSTI.

5:53 AM – 6:01 AM

Abstract Title: **Developing Mycolactone-based Immunoassays For Buruli Ulcer Diagnosis**

Author Block: L. Warryn, G. Pluschke; Swiss Tropical and Publ. Hlth.Inst., Univ. of Basel, Basel, Switzerland

Abstract Body: **Background:**Buruli ulcer (BU) – or *Mycobacterium ulcerans* disease – is a neglected tropical disease of skin and soft tissue, which results in chronic progressive ulceration that can lead to permanent disabilities. The polyketide toxin, mycolactone (ML), is the key virulence factor of *M. ulcerans* and is responsible for the chronic necrotising pathological features of BU. It is currently unknown how *M. ulcerans* is transmitted, although an environmental source of infection associated with stagnant water bodies is strongly indicated. This knowledge gap and lack of an effective vaccine makes prevention next to impossible. Therefore, current control measures hinge on antibiotic treatment of all cases, which is contingent upon early diagnosis. **Objectives**ML is an ideal marker for specific BU diagnosis, given that the toxin is unique to *M. ulcerans.* Consequently, we targeted ML to develop immunodiagnostics for BU. **Methods:**The hapten-like polyketide nature and cytotoxicity of ML precludes its use as an immunogen to elicit antibody responses. Therefore, following extensive structure-activity relationship studies, non-toxic derivatives of ML were designed. Protein conjugates of these derivatives were prepared and used for mouse immunisations to generate series of mAbs via the B cell hybridoma technology. Generated mAbs were carefully characterised using panels of modified synthetic ML derivatives to elucidate the binding specificities and aid selection of appropriate mAbs to develop competitive and antigen capture ELISAs for ML detection and quantification. **Results:**With these mAbs, we have developed and optimised highly specific competitive and capture ELISAs capable of detecting and quantifying ML in biological samples. Development of the capture ELISA specific for the low-molecular weight (≈750 Da) ML molecule was enabled by careful selection of mAbs with complementary ML binding patterns. Both ELISAs can detect 1 – 2 ng of ML in a variety of biological samples, including *M. ulcerans* culture filtrates, tissue samples from animal infection models, as well as clinical samples from suspected BU patients. The ability of these ELISAs to detect ML directly in crude samples, without extensive organic extraction typically required for lipid antigens, greatly simplifies the overall assay procedure. Thus, after final optimisation, the ELISAs may be suitable as diagnostic tests for hospital settings. Furthermore, conversion of the ELISAs into Rapid Diagnostic Test (RDT) formats for use as point-of-care diagnostic tools has been initiated.

6:01 AM – 6:09 AM

Abstract Title: **Identification of Immunodominant Bartonella bacilliformis Proteins for Serodiagnostics and Vaccine Development**

Author Block: A. Dichter1, T. Schultze1, A. Wenigmann1, W. Ballhorn1, A. Latz2, E. Schlüfter2, P. Ventosilla3, H. Guerra Allison3, C. Ugarte-Gil3, P. Tsukayama3, V. Kempf1; 1Univ. Hosp., Goethe Univ., Frankfurt am Main, Germany, 2NovaTec Immundiagnostica, Dietzenbach, Germany, 3Univ. Peruana Cayetano Heredia, Lima, Peru

Abstract Body: **Background:** *Bartonella bacilliformis* is the causative agent of Carrión's disease, a vector-borne biphasic illness restricted to the South American Andes. In the acute phase, the bacteria infect erythrocytes causing severe hemolytic anaemia and transient immunosuppression with high fatality rates (40-90%). *B. bacilliformis* is transmitted by the bite of sandflies (*Lutzomyia* spp.) and asymptomatic infections are assumed to represent the source of new outbreaks. For disease prevention and surveillance strategies, the identification of those

asymptomatic carriers is of particular significance especially in the light of climate change and potential expansion of the vector. Therefore, a reliable serodiagnostic tool and a vaccine are urgently needed. However, with only limited knowledge of the immune response to *B. bacilliformis* infections, antigen candidates for a vaccine are also widely unknown. **Objectives:** This study aims to identify immunodominant proteins of *B. bacilliformis* for serodiagnostic use and vaccine development. **Methods:** Based on the genomes of *B. bacilliformis* strains KC583 and KC584, a reverse vaccinology approach in combination with heterologous genomic expression libraries was used to identify immunodominant proteins. Antigen candidates were recombinantly expressed and their reactivity was systematically assessed by Western blotting, line blotting and ELISAs with a serum collection of Peruvian patients suffering from *B. bacilliformis* infections. **Results:** In total, 21 potentially immunodominant proteins were identified, recombinantly expressed and analysed by Western blotting using a pool of patient sera. Fourteen antigens were found to be immunoreactive with patient sera and were further analysed by line blotting using sera of 26 Carrión's disease patients and 96 healthy German blood donors. Results indicated the use of three antigens as sero-markers to detect IgG antibodies against *B. bacilliformis*. Based on these findings a diagnostic ELISA with a sensitivity of 81% and a specificity of 95% was developed. The combination of reverse vaccinology and heterologous genomic expression libraries has been proven to be effective for the identification of immunodominant proteins. The herein developed line blot assay and ELISA represent useful serodiagnostic tools for future epidemiological studies in endemic areas and provide a solid basis for future vaccine development to prevent the highly lethal Carrión's disease.

6:09 AM - 6:17 AM

Abstract Title: **Ubiquitous Selection for mecA in Community-associated MRSA Across Diverse Chemical Environments**

Author Block: O. Snitser1, D. Russ1, L. K. Stone2, K. K. Wang3, H. Sharir4, N. Kozer4, G. Cohen4, H. M. Barr4, R. Kishony1; 1Technion - Israel Inst. of Technology, Haifa, Israel, 2DSM Biotechnology Ctr., Delft, Netherlands, 3Univ. of Illinois - Metropolitan Group Hosp., Chicago, IL, 4Weizmann Inst. of Sci., Rehovot, Israel

Abstract Body: **Background:** Community- associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has spread worldwide and is threatening public health. Its hallmark is the *mecA* gene, which confers resistance to nearly all β-lactam antibiotics. However, it is unknown whether *mecA* provides a selective advantage across other chemical environments. **Objectives:** To identify *mecA* selective advantages and disadvantages across a diverse chemical library. **Methods:** We developed a competition-based assay to determine the fitness effect of *mecA*, competing wild-type CA-MRSA and a *mecA* deleted strain in the presence of ~57,000 diverse chemical compounds and cefoxitin, a β-lactam, at sub-inhibitory concentration. We determined whether *mecA* selective advantage is dependent on cefoxitin by competing the strains in the presence of a subset of the compounds, with and without subinhibitory concentration of cefoxitin. Cell-envelope permeability was assessed by measuring the fluorescence of propidium iodide on a cefoxitin gradient of the two strains. **Results:** Surprisingly, and in contrast to other resistant mechanisms, we find that *mecA* provides ubiquitous advantage, potentiated by sub-inhibitory levels of cefoxitin, in diverse chemical environments, including antibiotics, non-antibiotic therapeutic drugs and even natural products and synthetic compounds. This advantage is strongly associated with the compounds' physicochemical properties, suggesting that it may be mediated by differential compound permeability into the cell. Indeed, *mecA* confers reduced cell-envelope permeability in the face of subinhibitory cefoxitin concentrations, which allow for increased resistance to compounds with

specific physicochemical properties. Our findings suggest that *mecA* provides a selective advantage across numerous diverse chemical environments, even unrelated to antibacterial drugs, proposing a wider basis to explain its remarkable success and rapid dissemination worldwide. This work was recently published in *Nature Communications*.

6:17 AM - 6:25 AM

Abstract Title: **High-resolution Cervicovaginal Microbiome Profiling**

Author Block: M. A. Molina Beitia1, K. M. Andralojc1, M. Qiu2, B. Spruijtenburg1, M. Rasing1, B. W. B. Pater2, M. A. Huynen2, B. E. Dutilh3, T. H. A. Ederveen2, D. Elmelik2, A. G. Siebers1, D. Loopik1, R. L. M. Bekkers4, W. P. J. Leenders2, W. J. G. Melchers1; 1Radboud Univ. Med. Ctr., Nijmegen, Netherlands, 2Radboud Inst. for Molecular Life Sci., Nijmegen, Netherlands, 3Utrecht Univ., Utrecht, Netherlands, 4Catharina Hosp., Eindhoven, Netherlands

Abstract Body: **Background:** High-risk human papillomavirus (hrHPV) acquisition and persistence have been associated with alterations of the cervicovaginal microbiome (CVM), with distinctive bacterial species and community state types (CSTs) being involved in shaping microbial dominancy, abundance, and dynamics during disease[1]. Current sequencing methods such as multiplex 16S rRNA gene sequencing (16S rRNA-seq) are not able to fully clarify these variations because they generally cannot achieve high-resolution microbiome profiling. To overcome this limitation, recent studies suggest that combining and testing for multiple 16S rRNA variable regions (VRs) may increase the resolution to the species taxonomic level[2]. **Objective:** We designed a method targeting microbial species at multiple VRs and examined its potential for DNA/RNA microbiome profiling. **Methods:** Circular probes-based RNA sequencing (CiRNAseq) was developed using single-molecule molecular inversion probes (smMIPs)[3], targeting and profiling 321 cervicovaginal microbial species. *In vitro* validations were performed on confirmed bacterial species. A small cohort of cervical smears was used to compare CiRNAseq to 16S rRNA-seq. A large cohort of cervical smears (hrHPV cohort), either hrHPV negative or hrHPV positive with cervical intraepithelial neoplasia (CIN2+), was used to further validate CiRNAseq. **Results:** CiRNAseq is a practical technology that can perform high-resolution microbiome sequencing, DNA/RNA profiling, and microbial quantification. Our technique provides similar results to 16S rRNA-seq and superior sequencing sensitivity. Analyses of the hrHPV cohort indorsed known CSTs changes in the CVM of women with hrHPV-induced lesions[1]. Thus, CiRNAseq is a promising tool for exploring the interplay of the CVM and hrHPV in cervical carcinogenesis.

21 June 2021 5:45AM

FEMS124 - Rapid Fire: Molecular Microbiology and Biochemistry

Session Room: Channel 7

5:45 AM - 5:53 AM

Abstract Title: **Inability of Campylobacter jejuni Cells to Communicate with Quorum Sensing Induces Stress Response and Virulence**

Author Block: D. Ramic1, U. Kunej1, M. Kovac2, N. Toplak2, A. Klancnik1, S. Smole Mozina1; 1Biotechnical Faculty, Univ. of Ljubljana, Ljubljana, Slovenia, 2Omega d.o.o., Ljubljana, Slovenia

Abstract Body: *Campylobacter jejuni* is a fragile bacterium outside the host, but still the leading cause of foodborne bacterial gastroenteritis in humans worldwide. The under-researched and under-determined genetic diversity and metabolic capabilities make the biology of *Campylobacter* a mystery, which encourages us to fill the knowledge gap and link it to its phenotype and further to pathogenicity. Therefore, our aim is to gain new insights into the metabolism modulated by the role of the signalling autoinducer-2 production system (*luxS*) at the molecular level using RNA-sequencing and transcriptome analysis of *C. jejuni* 11168Δ*luxS* compared to wild-type *C. jejuni* NCTC 11168. AI-2 is thought to be a universal bacterial signalling molecule synthesized by the LuxS enzyme, which forms an integral part of the activated methyl cycle. After normalising the *luxS* mutant to *C. jejuni* NCTC 11168 mRNA data (false discovery rate, P ≤0.05), we examined the effects of *luxS* knock-out mutation on gene expression measurements. Interestingly, the results showed up-regulation of metabolic pathways involved in oxidative phosphorylation, carbon metabolism, citrate cycle, biosynthesis of secondary metabolites and biosynthesis of different essential amino acids in *luxS* mutant. Thus, we have shown that intercellular signalling and metabolic pathways are interconnected, and we have demonstrated for the first time induced adaptive tolerance in a *luxS* mutant that lacks the ability to express AI-2-like quorum sensing molecules; as seen in up-regulated genes involved in the stress response (*cstA, katA, tpx, trxA, trxB, ahpC*) and in genes that modulate flagellar synthesis and motility. We can conclude that quorum sensing, which is involved in the regulation of multicellular behaviour through communication, is also involved in the regulation of various metabolic processes and stress response. When *Campylobacter* cells are unable to communicate with quorum sensing molecules, metabolism is focused to induce a stress response and virulence potential, enabling the survival, persistence and pathogenicity of this extraordinary foodborne pathogen.

5:53 AM - 6:01 AM

Abstract Title: **Engineered Outer Membrane Vesicles from E. coli: A Platform for Immunotherapy and Drug Delivery Systems**

Author Block: F. Mensitieri1, G. Donadio2, E. P. Lamparelli1, M. Ruggiero2, E. Del Regno3, G. Della Porta1, F. Dal Piaz1, V. Izzo1; 1Università degli studi di Salerno, Baronissi (SA), Italy, 2Università degli studi di Salerno, Fisciano (SA), Italy, 3Università degli studi di Napoli, Napoli, Italy

Abstract Body: Synthetic nanodevices such as liposomes, polymers and metal-based nanoparticles have long been studied for pharmacological applications such as immunotherapies and drug delivery systems. However, the correct production and loading of these structures might be tricky and inefficient. Bacterial outer membrane vesicles (OMVs) are bacterial non-replicating extracellular small-sized proteo-liposomes (20-250 nm) continuously discharged by several bacteria and hold a great potential to be used as engineerable biological carriers. Noteworthy, extracellular vesicles possess adhesion molecules on their surface that render their binding and internalization into target cells highly specific and appealing to be fine-tuned for the regulation of specific ligand-receptor recognition. In this framework, the use of OMV-based vaccines is an upcoming topic in latest years and different OMVs engineering approaches for vaccine development were described so far. In this work, we describe the isolation of OMVs from different commercially available strains of *E.coli* (BL21(DE3), JM109, DH5α). Currently, no hypervesiculating *E. coli* strain is commercially available; therefore, a comparative analysis of OMVs obtained from these commercial strains is of particular interest. Their size and proteomic profile were characterized, respectively, by DLS (dynamic light scattering) and proteomic mass spectrometry analysis. OMVs were preliminary engineered by overexpressing in the corresponding recombinant *E. coli* strain a mutated form of a bacterial membrane protein, the cytolysin A (clyA/hlyE). ClyA enrichment in OMVs was confirmed by mass spectrometry analysis. Beneath the future perspective of this project there is the subsequent modification of clyA for the generation of a recombinant fusion protein that could expose human tumor-specific antigens on OMVs surface.

6:01 AM - 6:09 AM

Abstract Title: **How Is External Cysteine Utilized by E. coli?**

Author Block: Y. Zhou, J. Imlay; UIUC, Urbana, IL

Abstract Body: Sulfur is an essential element for all forms of life. Many sulfur-containing molecules (cystine, sulfate, taurine, glutathione) have dedicated importers that are induced when bacteria are sulfur limited. In *E. coli* these sulfur compounds are first converted into usable cysteine and then are incorporated into biomolecules; thus, one would expect that cysteine import would be the most economical route for cells to acquire sulfur. However, uniquely among the amino acid transporters, cysteine importers are poorly characterized in the microbial world. We have demonstrated that wild-type *E. coli* cannot assimilate cysteine as sulfur source in the presence of other amino acids. Four transporters have adventitious cysteine import activity, but they do not import cysteine when their natural substrates are around. If these four transporters are deleted, *E. coli* can no longer import cysteine. The surprising lack of an effective route to assimilate sulfur from cysteine may be due to the problems that rapid cysteine import can cause. Does this mean that cysteine is of no use for *E. coli*? The story doesn't end like this. *E. coli* possesses a cysteine importer, called YhaO, that is induced only when external cysteine is abundant. Downstream of *yhaO* sits *yhaM*, encoding a cysteine desulfidase that degrades cysteine to pyruvate, ammonia and hydrogen sulfide. This process is fast enough to allow cells to use cysteine as sole carbon or nitrogen source. YhaM is the primary sulfide producer when cells are exposed to cysteine. Expression data showed that *yhaO* was most strongly induced during growth on poor carbon sources. The strong induction did not occur when Crp was deleted, indicating that Crp may induce YhaOM. Crp null mutants could not grow on cysteine as

sole carbon source. We propose that extracellular cysteine is utilized as a carbon or nitrogen source rather than as a cysteine source for protein biosynthesis.

6:09 AM - 6:17 AM

Abstract Title: **HP1021 Is the First Redox Switch Protein Identified in Helicobacter pylori**

Author Block: M. Noszka1, P. Szczepanowski1, D. Żyła-Uklejewicz1, F. Pikuła1, M. Nowaczyk-Cieszewska1, A. Krężel2, K. Stingl3, A. Pawlik1; 1Hirszfeld Inst. of Immunology and Experimental Therapy, Polish Academy of Sci., Wroclaw, Poland, 2Faculty of Biotechnology, Univ. of Wroclaw, Wroclaw, Poland, 3Natl. Reference Lab. for Campylobacter, German Federal Inst. for Risk Assessment, Berlin, Germany

Abstract Body: **Background:** Helicobacter pylori is a Gram-negative, microaerobic, pathogenic bacterium, intensively investigated since the discovery in 1982. H. pylori living in a severe environment of the human stomach has evolved many mechanisms to survive. The immunology system has also developed many strategies to combat pathogens, including the oxidative burst - the rapid release of the reactive oxygen species (ROS) from different immune cells. Curiously, thus far, no typical regulator dedicated to the oxygen-stress response has been discovered in H. pylori. **Objectives:** The project aimed to investigate the role of the orphan response regulator HP1021 in H. pylori response to oxidative stress. **Methods:** Extensive studies combining in vivo analyses (H. pylori mutagenesis, RT-qPCR, oxidative stress assays, protein labelling) and in vitro methods (EMSA, SPR, metal-binding assays) allowed to propose that HP1021 is the first redox-dependent regulator in H. pylori. **Results:** Our results showed that the cysteine residues of HP1021 are sensitive to oxidation and that HP1021 DNA-binding activity to oriC depends on the redox state of the protein. We demonstrated that HP1021 is directly and indirectly involved in the oxygen-dependent control of several H. pylori genes (e.g. gluP, fadA, fecA). Finally, we indicate that HP1021 responds to reactive oxygen species: O_2^- and H_2O_2.

6:17 AM - 6:25 AM

Abstract Title: **A Bacterial Membraneless Organelle Robustly Modulates the Cell Cycle Across Environmental Conditions**

Author Block: S. Saurabh, L. Shapiro; Stanford Univ., Stanford, CA

Abstract Body: Free-living bacteria must devise robust regulatory mechanisms to control signaling in response to a variety of changing environmental conditions. Reversible concentration of signaling molecules in membraneless organelles via liquid-liquid phase separation provides a ubiquitous mechanism for living cells to regulate developmental programs. Here, I will discuss recent developments in our understanding of the role of membraneless organelles in cell signaling in the oligotrophic bacterium, *Caulobacter crescentus*. *Caulobacter* divides asymmetrically into a sessile stalked cell and a flagellated swarmer cell by spatially controlling the synthesis, localization and degradation of proteins as a function of the cell cycle. Much of this asymmetric division is regulated through complex signaling pathways that occur in the cell poles that span 100 nm. Specifically, during differentiation the scaffold protein PopZ recruits a lysozyme homolog SpmX, which further recruits the kinase DivJ to the pole. Notably, SpmX and PopZ have long, unstructured regions rich in Proline and negatively charged

amino acids also known as intrinsically disordered regions (IDRs). Reconstitution of this signaling complex on lipid bilayers revealed that SpmX and PopZ can form phase-separated droplets that sequester DivJ and control its kinase activity. Further, I have identified the regulatory elements in SpmX that control the material properties of SpmX condensates and DivJ kinase activity *in vivo*. SpmX exhibits a dual regulatory role on DivJ activity through protein phase separation and allosteric control through co-evolving amino acids. I will discuss how these two aspects of DivJ regulation can be tuned independently, thus providing us with insights into kinase regulation under a variety of environmental conditions that the bacterium may face in its wild habitat. These results underscore the role of regulatory disordered domains in modulating the nanoscale environment containing signaling kinases. In addition to well understood "lock and key" protein domains that lie at the core of cell signaling, my findings add a novel regulatory layer in signal transduction.

6:25 AM - 6:33 AM

Abstract Title: **Investigating the Role of Small RNAs in Copper Resistance in Cupriavidus metallidurans CH34**

Author Block: L. Maertens1, J-Y. Matroule2, R. Van Houdt1; 1SCK CEN, Mol, Belgium, 2UNamur, Namur, Belgium

Abstract Body: **Background:** *Cupravidus metallidurans* CH34 is a well-known model organism for the study of bacterial metal resistance. Its pMOL30 megaplasmid encodes three main clusters for Cu detoxification, with less functional homologous systems on the chromosome and chromid. In a previous study, we have detected a small non-coding RNA in one of these clusters, the *silDCBA* operon, which encodes a HME-RND-type Ag^{+}/Cu^{2+} efflux pump. This efflux pump has been shown to enhance Cu resistance in CH34-derived strains lacking pMOL30. The sRNA shows partial antisense overlap with both *silC* and *silB*, and its expression was confirmed by 5'RACE. Furthermore, tagRNA-Seq has shown repression of this sRNA in Cu-stressed cells. **Objectives:** We are currently investigating the putative regulatory role of this antisense RNA, by studying its interaction with the *silDCBA* mRNA and consequent post-transcriptional regulation. Knowledge of post-transcriptional regulation of metal resistance clusters remains very sparse, and this study could provide valuable insights into the regulation of the Ag/Cu efflux *silDCBA* operon. Possible areas of application would be the development of Ag/Cu antimicrobials and the bioremediation of metal-contaminated environments. **Methods:** A multipronged approach is being employed to study the interaction of the sRNA and its target on the *silDCBA* operon. In addition, we are investigating the metal resistance phenotype resulting from this interaction. **Results:** In a heterologous system in *E. coli* TOP10, the sRNA induces a small but persistent repression of SilC translation, and a similar system is being optimized for use in *Cupriavidus*. In addition, we have shown that the overexpression of the sRNA has an observable effect on the Cu resistance phenotype in *Cupriavidus*.

6:33 AM - 6:41 AM

Abstract Title: **Staphylococcus aureus Senses and Adapts to Ambient Temperatures Through RNA Thermoswitches**

Author Block: A. Catalan-Moreno1, C. Marta1, M-G. Pilar1, I. Naiara1, C. Carlos J1, I. Caldelari2, A. Toledo-Arana1; 1CSIC, Pamplona, Spain, 2CNRS, Strasbourg, France

Abstract Body: Adaptation to the ever-changing environmental conditions and survival outside the host is crucial for dissemination and transmission of pathogenic bacteria. *Staphylococcus aureus* may be spread from human carriers to diverse environments and survive outside the host for long periods of time. This capacity has been linked to reinfections in addition to new host colonisations. One of the main variables that bacteria need to face when leaving the host is a shift in temperature. However, the mechanisms governing cold adaptation during this transition remain poorly understood. In this study, we found that two paralogous RNA-thermoswitches controlled the production of cold-shock proteins CspB and CspC in *S. aureus*. We demonstrated that the *cspB* and *cspC* 5'UTRs adopt alternative RNA structures that shift from one another upon temperature changes. These RNA structures resembled the thermo-responsive elements recently described in *E. coli* and *L. monocytogenes.* One of the conformations facilitated mRNA translation at ambient temperatures (22ºC) while the other folded into a double stranded RNA structure at host-related temperatures (37ºC) that blocked the ribosome binding site (RBS). We found that the structural rearrangements depended on a long RNA hairpin that sequestered the anti-RBS sequence depending on the environmental temperature. At the same time, the remaining *S. aureus* CSP, CspA, recognised a UUUGUUU motif located in this long hairpin. Such interaction favoured the release of the anti-RBS sequence and, as a result, repressed the CspB and CspC production at 37ºC. In addition, when both RNA-thermoswitches were simultaneously deleted, *S. aureus* growth was inhibited at ambient temperatures. All in all, our findings highlight the importance of CspB/CspC thermoregulation when *S. aureus* transitions from the host to the environment.

21 June 2021 5:45AM

FEMS146 - Rapid Fire: Miscellaneous

Session Room: Channel 8

5:45 AM - 5:53 AM

Abstract Title: **Contribution of Single-cell Omics to Microbiology**

Author Block: S. B. Mauger1, P. Vandenkoornhuyse1, C. Monard1, C. Thion2; 1Université Rennes 1, Rennes, France, 2Cellenion, Lyon, France

Abstract Body: From animal gut to plant roots and the depths of the ocean, whether forming blooms visible from space or hidden within our bodies, microorganisms play a key role in ecosystem functioning but are the least known living forms on Earth. Being microscopic, highly diverse and prevalent has made microbes both exciting and challenging to study. To investigate their role and interactions in ecosystems, many strategies are employed. Each is providing complementary information on community composition, metabolic features, or genetic dynamics. The study of microbes is highly dependent on molecular tools with specific technologies, scales and interpretation boundaries. So far, tremendous technological improvements have been made to obtain a more accurate and representative view of microbial diversity, functions and interactions. One of the latest developments that is gaining interest from the microbiological field is single cell analysis. This technique allows us to observe the diversity of microbes at the population level, generating accurate knowledge of genome composition and linking each individual to its functions. Despite theoretical and practical limitations when applied to prokaryotes, single cell omics represent an additional possibility to understand the microbial world. Advances allowed by single-cell omics concern many fields of microbiology such as ecology, industries, health and environment. In this presentation, an overview of the future research opportunities offered by single cell analyses and possible breakthroughs in bacterial systems will be discussed in connexion to the field of ecology. The shift in standpoint from observation and interpretation offered by single cell omics will also be illustrated through examples in ecology.

5:53 AM - 6:01 AM

Abstract Title: **Culturomics and Metagenomics Data Comparisons in Microbiota from Obese versus Non-obese Children Exposed to Endocrine Disruptor Chemicals**

Author Block: A. López Moreno, K. Cerk, Á. Ruiz-Moreno, A. Torres-Sánchez, J. Pardo, M. Aguilera; Univ. of Granada, Granada, Spain

Abstract Body: According to the World Health Organization (WHO), childhood obesity is one of the most serious public health problems. The increase in the obesity is in parallel with the presence of endocrine disruptor chemicals in the environment and our diet. These compounds known

as obesogens interfere with our hormonal system, causing alterations at all levels of our metabolism and are present in plastics for food use and foods like bisphenol A (BPA). Advances in genome-sequencing technologies have shown large amount of unassigned and non-cultivable bacteria. Although these technologies have provided several important new insights, it is essential to isolate and culture species from those uncultured taxa. The main objective of this work was to search for microorganisms of the intestinal microbiota degrading obesogens through combining innovative culture techniques and metagenomics, contributing to fill gaps by reducing the unknown taxa. Culturomics was carried out in anaerobiosis work station with novel and specific media such as Gifu Anaerobic Media (GAM), Gut microbiota medium (GMM), Anaerobe basal broth (ABB), Brain hearts infusion (BHI) and modifications (BPA addition). Taxonomic characterization of 16S rRNA was performed together with phylogenetic studies. In parallel, metagenomics was carried out through analysing fecal microbiota and characterization with microbial 16S rRNA (V3-V4 regions - Illumina MiSeq platform). The culture conditions used for the analysis of the 20 samples allowed us to select total of 50 colonies to be further analyzed by 16S rRNA. The main cultivated BPA tolerant species were *Bacillus* sp.*, Lysinibacillus fusiformis, Lactobacillus sakei, Enterococcus faecalis, Streptococcus salivarius, Staphylococcus pasteuri, Micrococcus yunnanensis, Kocuria rhizophila, Burkholderia contaminans, Rothia dentocariosa*, among others. Moreover, the metagenomics analysis showed statistically significant differences between population groups: *Gemella* sp., *Roseburia hominis, Eggerthella lenta, Flavonifractor plautii, Clostridium innocuum, Ruminococcus* sp., *Desulfovibrio piger, Actinomyces urogenitalis, Slackia isoflavoniconvertens, Akkermansia muciniphila, Blautia* sp., *Lactococcus lactis, Anaerostipes caccae, Acidaminococcus intestine*. In case of *Bacillus*, stadistical significance was found at the level of Bacillales taxa. Our research group is focusing on the demonstration of *Bacillus spp.* diversity and variability in obese vs non-obese due to their potential of BPA degradation capacities.

6:01 AM - 6:09 AM

Abstract Title: **Gut Microbiota Mediates the Outcome of Host Physiological Responses to Toxic Substances in Caenorhabditis elegans**

Author Block: J. Mangu1, A. Mandal2, P-E. Olsson1, J. Jass1; 1Örebro Univerisity, Örebro, Sweden, 2Univ. of Skövde, Skövde, Sweden

Abstract Body: **Background:** Gut microbiota has an important role in the host physiology and development through secretion of metabolites, antimicrobial compounds, competing for space and nutrients. The gut, microbiota creates an interface between the host and environmental contaminants. Accumulation of toxic substances such as metals, and persistent organic pollutants, through contaminated food and water, affects both the gut microbiota and the host. Previous reports indicated that chemical toxins affect the host physiological responses, however the influence of gut microbiota on host physiology during toxin exposures is not clear. **Objectives:** The aim was to investigate the influence of simplified gut microbiota models in the bacterivorous nematode *Caenorhabditis elegans* on the host responses to environmental contaminants by assessing the lifespan and physiology. **Results:** Simplified bacteria gut microbiota models consisting of 3 different bacteria were developed in *C. elegans* by feeding the nematodes with bacterial mixtures. The resulting gut microbiota were defined as beneficial or detrimental based on the *C. elegans* relative lifespan when compared to the standard *Escherichia coli* OP50 food. *C. elegans* were fed mixtures of the 3 different bacteria to develop the gut microbiota prior to exposure to perfluorooctane sulfonate (PFOS) or arsenite (As^{III}). The lifespan, gene expression using qPCR and fat analysis were assessed and compared to *E. coli* OP50 fed worms. Beneficial microbiota models increased median survival by five days or did not alter the worm lifespan when compared to *E. coli* fed

worms, whereas the detrimental model decreases worm lifespan by two days. Nematodes fed with beneficial or detrimental microbiota, presented altered expression in genes associated with stress response (*hsp-16*, *mtl-1*, *gst-4*), life span (*daf-16, pmk-1*), development *(sma-3, dbl-1, daf-12*) and innate immunity (*lys-8*, *spp-1*, *clec-45*). In the presence of PFOS (100 µM) and As^{III} (150 µM), the *C. elegans* showed a reduced lifespan with all the microbiota combinations compared to the unexposed worms. However, the lifespan of worms with beneficial microbiota remained longer when compared to the unexposed worms fed with detrimental microbiota.

Other Sessions

24 June 2021, 8:00 – 9:00 AM

FEMS140 - FEMS Awards Ceremony and Poster Prizes

Session Room: Channel 9

8:25-8:35 AM

Abstract Title: **The Evolution of Mass Self-lysis in Bacterial Warfare**

Author Block: E. Granato, K. Foster; Univ. of Oxford, Oxford, United Kingdom

Abstract Body: Background: Toxin-based warfare among bacteria is central in shaping microbial communities. As a result, many bacteria have evolved to detect foreign toxins and respond with their own toxins in a reciprocal attack. One of the most dramatic responses is seen in bacteria that release toxins via self-lysis, killing themselves in the process. While widely reported, however, we do not know how often this extreme form of toxin release is actually used in competitions. The challenge is that, because reciprocal attacks are stress-induced, it is hard to tell if cells simply died from the stress or because they actively engaged in cell-suicidal toxin release. Objectives: Here, we sought to quantify bacterial self-lysis in colicin toxin-producing Escherichia coli. Methods: We combined single-cell time-lapse microscopy, fluorescent gene-expression reporters and DNA dyes to determine the fraction of self-lysing bacteria during toxin-mediated competitions. Results: We were amazed to find conditions where more than 90% of a clonal population will self-lyse in response to a competitor's toxin. How can these extreme phenotypes be favoured by natural selection? Studying a strain that cannot make toxins provides an answer. While incapable of cell-suicidal toxin production, these "unarmed" cells are ultimately killed under the same level of toxin stress imposed by the competitor. Under these conditions, therefore, engaging in cell-suicidal toxin release has little or no cost, as it is associated with sacrificing cells that have little or no reproductive value. Our work shows how bacterial warfare has resulted in the evolution of one of the most extreme forms of toxin release in microbes.