ICY 15 meets 30 ICYGMB

INTERNATIONAL CONGRESS ON YEASTS

INTERNATIONAL CONFERENCE ON YEAST GENETICS AND MOLECULAR BIOLOGY

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PROGRAMME & ABSTRACTS

Vienna, Austria

23-27 August 2021

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Series on Fungal Genetics & Genomics

Launched across
GENETICS and G3: Genes|Genomes|Genetics
in February 2021, this Series aims to report and thereby further stimulate advances in genetics and genomics across a diversity of fungal species.

Authors are invited to submit manuscripts to the series on an ongoing basis.

academic.oup.com/genetics/pages/fungal-genetics-and-genomics

Fungal Genetics Conference

March 5-10, 2022
Asilomar Conference Grounds
https://genetics-gsa.org/fungal/

Yeast Genetics Meeting

July 2022
Location TBD
https://genetics-gsa.org/yeast/

INTRODUCTION

Yeasts and humans share common culture since millenia. Even in the earliest known human settlements signs of yeast activity have been found, as residues of beer, wine, sake and bread illustrate. Ever since, humans employ yeasts to ferment their food and brew beverages. It is not an overstatement that brewing and bakers' yeast production geared the development of modern industrial biotechnology. In the 21st century, synthetic biology is shaping the development of yeast biotechnology towards a sustainable production of food and feed, materials, chemicals and fuels.

Yeasts serve as valuable models for biological research and led to groundbreaking fundamental discoveries, shaping our understanding of human diseases and leading to better treatments. Some yeasts, however, are foe, not friends, as they are pathogenic to humans.

Over the last decades, research progress in these fields has been discussed in the series of the International Congress on Yeasts (ICY) and the International Conference on Yeast Genetics and Molecular Biology (ICYGMB). As the COVID-19 pandemic enforced to postpone ICY15 to 2021, the two conference series joined forces to organize **ICY15 meets ICYGMB30** as a virtual congress in August 2021, organized by the University of Natural Resources and Life Sciences Vienna. From August 23-27, 600 yeast researchers from 47 countries gather online to discuss the latest news in yeast molecular and cell biology, yeast research supporting food security, circular bioeconomy and human health, and the treasures of natural yeast biodiversity.

Yeasts are inspiring our research, and we inspire yeasts to support humankind. Therefore, the motto of **ICY15 meets ICYGMB30** is **The Spirit of Yeast**.

Hiroshi Takagi, Chair of the International Commission on Yeasts
Jens Nielsen, Chair of the ICYGMB Finance and Policy Committee
Diethard Mattanovich, Chair of the Organizing Committee of **ICY15 meets ICYGMB30**

CONFERENCE ORGANIZERS

LOCAL ORGANIZING COMMITTEE

Diethard Mattanovich – *Conference chair*	
Brigitte Gasser – *Scientific secretary*	
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Hans Marx	Vienna, AT
Birgit Marckhgott	
Marija Stipac	

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Alain Sourabié	Procelys by Lesaffre, FR

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Markus Ralser	Francis Crick Institute, London, UK, Charité, Berlin, DE
Andriy Sibirny	National Academy of Sciences of Ukraine, UA
Hiroshi Takagi	Nara Institute of Science and Technology Graduate School of Biological Sciences, JP
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PROGRAMME AT A GLANCE

ICY15 meets ICYGMB30, August 23-27, 2021

detailed programme

Plenary lecture	30 min lecture + 10 min discussion	40 min session
Invited speaker (InvS) presentation	20 min	1h 40min session
Selected speaker presentation	10 min	
Discussion	20 min	

Time CEST	Events
	MONDAY 23.08.2021
1:00 - 1:20 PM	**Opening**
1:20 - 2:00 PM	**Plenary 1 Lecture - Speaker: Jef Boeke, NYU Langone Health (US) - Chair: Diethard Mattanovich, Univ BOKU (A)**
2:00 - 2:10 PM	Break

2:10 - 3:50 PM	**Session 1.1 Yeast metabolism and its control**	**Session 5.1 Phenotypic evolution in yeasts**	**Session 7.1 Pathogenic yeasts**	**Session 2.1 RNA Metabolism, transcription and translation**
	384 Heinemann Matthias (InvS), Univ Groningen (NL)	**357 Gonçalves** Paula (InvS), Univ NOVA Lisboa, (PT)	**378 Nobile** Clarissa (InvS), Univ California, (US)	**376 Koutmou** Kristin (InvS), Univ Michigan, (US)
	217 Coral Medina Angela, INRAE (FR)	**364 Louis** Ed (InvS), Univ Leicester, (UK)	**375 Konopka** James (InvS), Stony Brook Univ, (US)	**381 Moriya** Hisao (InvS), RCIS, Okayama Univ (JP)
	151 Tate Jennifer, Univ Tennessee HSC, (US)	**109 Čadež** Neža, Univ Ljubljana, (SI)	**126 Alves** Rosana, Univ Minho (PT)	**372 Spang** Anne (InvS), Univ Basel, (CH)
	88 Fontanesi Flavia, Univ Miami, (US)	**82 Andreev** Ilya, NIH Bethesda, (US)	**113 Pedro** Nuno, Univ Lisboa, (PT)	**195 Balarezo Cisneros** Laura, Univ Manchester, (UK)
	62 Geijer Cecilia, Chalmers Univ Technology, (SE)	**143 De Valk** Sophie, Delft Univ Technol (NL)	**13 Pfliegler** Walter, Univ Debrecen, (HU)	**235 Ensinck** Imke, Crick Inst, (UK)
	382 Vickers Claudia (InvS), CSIRO, (AU)	**160 Avram Shperling** Adi, Bar Ilan Univ, (IL)	**293 Brito** Patricia, Univ NOVA Lisboa, (PT)	
	417 Oliver, Steve, Univ Cambridge, (UK)			
	Chair: Borodina Irina, Tech Univ Denkmark, (DK)	**Chair: Hittinger Chris, Univ Wisconsin-Madison, (US)**	**Chair: Kuchler Karl**, Univ Vienna, (AT)	**Chair: Kimata Yukio**, Nara Inst, (JP)

3:50 - 4:00 PM	Break
4:00 - 5:00 PM	**Virtual Get Together**
5:30 - 7:00 PM	ICYGMB Steering Committee (only invited participants)

Time CEST	
	TUESDAY 24.08.2021
10:00 - 12:00 AM	**ITN Network Session**
10:00 - 11:00 AM	**Aromagenesis: Chair: Morrissey John**, Univ Coll Cork, (IE) - **146 De la Cerda García-Caro** Roberto, Trinity College Dublin, (IE) - **177 Aguiar-Cervera** Jose Enrique, Singer Inst, (UK) - **44 Tapia** Sebastian, IATA-CSIC, (ES) - **55 Pérez Dolores**, IATA-CSIC, (ES) - **11 Denat** Marie, LAAE, Univ Zaragoza, (ES)
11:00 - 12:00 AM	**YEASTDOC: Chair: Bond Ursula**, Trinity College, (IE) - **161 Alvarez Rafael**, INRAE, (FR) - **120 Becerra-Rodriguez** Carmen, INRAE, (FR) - **189 Mendonça Bahia** Silva Frederico, Univ Milano-Bicocca, (IT) - **152 Barroso** Liliane, Univ Milano Biccoca, (IT) - **276 Jayaprakash** Pooja, Univ College Cork, (IE)
	Break
1:00 - 1:40 PM	**Plenary 2 Lecture - Speaker: Markus Ralser, F. Crick Institute (UK), Charité Berlin (D) - Chair: Brigitte Gasser, Univ BOKU (A)**
1:40 - 1:50 PM	Break

1:50 - 3:30 PM	**Session 1.4 Metabolic engineering**	**Session 5.2 Yeast ecology and environmental omics**	**Session 7.3 Yeasts in aging research**	**Session 2.2 Protein folding and secretion**	**Session 8.2 Organelle dynamics and intracellular trafficking**
	391 Borodina Irina (InvS), Tech Univ Denkmark, (DK)	**363 Jiranek** Vladimir (InvS), Univ Adelaide, (AU)	**348 Sagot** Isabelle (InvS), CNRS, (FR)	**21 Gerst** Jeffrey (InvS), Weizmann Inst, (IL)	**388 Drubin** David (InvS), UC Berkeley, (US)
	12 Hou Jin (InvS), Shandong Univ, (CN)	**135 Sampaio** José Paulo, Univ NOVA Lisboa, (PT)	**367 Fedor** Severin (InvS), Moscow State Univ, (RU)	**278 Kimata** Yukio (InvS), Nara Inst (JP)	**374 Fenech** Emma (InvS), Weizmann Inst, (IL)
	115 Erian Anna, BOKU Vienna, (AT)	**140 Rossouw** Debra, Univ Stellenbosch, (ZA)	**185 Bisschops Mark**, Wageningen Univ, (NL)	**65 Korpys-Woźniak** Paulina, UAM Poznan, (PL)	**368 Barral** Yves (InvS), ETH Zürich, (CH)
	123 Pozdniakova, Univ Minho, (PT)	**74 Gombert** Andreas, Univ Campinas, (BR)	**46 Bar-Yosef** Dana Laor, Tel Aviv Univ, (IL)	**201 Namba** Shotaro, Okayama Univ, (JP)	**350 van der Klei** Ida (InvS), Univ Groningen, (NL)
	24 Beltrán Leonardo, Goethe Univ, (DE)	**118 Meriggi** Niccolò, Univ Florence, (IT)	**150 D'Angiolo** Melania Jennifer, IRCAN, (FR)	**149 Bouyx** Clara, Toulouse Biotech Inst, (FR)	
	5 Blenner Mark, Clemson Univ, (US)	**Delneri** Daniela (InvS), Univ Manchester (UK)	**307 Zahoor** Hira, Univ Thailand, (TH)		
	Chair: Ledesma Amaro Rodrigo, Imp College London, (UK)	**Chair: Setati Evodia,** Univ Stellenbosch, (ZA)	**Chair: de Winde Han**, Univ Leiden, (NL)	**Chair: Gasser Brigitte**, BOKU Vienna, (AT)	**Chair: Malcova Ivana,** Inst Microbiol, Czech Academy of Sciences, (CZ)

3:30 - 3:40 PM	Coffee Break
3:40 - 4:40 PM	**Poster Session 1** - *odd numbers*
4:00 - 5:30 PM	ICY Commissioner Meeting (only invited participants)
5:45 - 7:45 PM	**Fermenting Futures: Exploring Yeast Biotechnology Through an Artistic Lense**
	details see next page

ICY15 meets ICYGMB30, August 23-27, 2021

detailed programme

Time CEST

TUESDAY 24.08.2021

5:45 - 7:45 PM **Fermenting Futures: Exploring Yeast Biotechnology Through an Artistic Lense**

5:45-5:50 PM **Mattanovich Diethard,** Univ BOKU (AT) - Introduction

5:50 - 6:10 PM **Dumitriu** Anna and **May** Alex **(UK) - Artists**, Brighton, UK

6:10-6:25 PM **Schneider** Florian, Dept of Economic and Social History, Uni Vienna, (AT)

6:25-6:35 PM **Ata** Özge , Univ BOKU, (AT)

6:35 - 6:45 PM **Altvater** Martin, Univ BOKU, (AT)

6:45 - 7:00 PM **Reichle** Ingeborg, die Angewandte, (AT)

7:00-7:15 PM **Sütter** Heike, Goethe Univ Frankfurt/Main, (DE)

7:15-7:45 PM **Q & A and Discussion Session Chair: Papadimitriou** Irini (GR)**, with Ata Özge, Dumitriu Anna, Giegler Wolfgang, Mattanovich Diethard, May Alex, Reichle Ingeborg, Schachinger Sonja, Schneider Florian, Sütter Heike**

WEDNESDAY 25.08.2021

11:00 - 12:00 AM **Poster Session 2** - *odd numbers*

12:00-1:00PM Break

1:00 - 2:40 PM

Session 1.3 Yeast systems biology	Session 5.3 Genome plasticity and complex genetic traits	Session 7.2 Yeasts as model for human health research	Session 2.3 Engineering yeasts for superior recombinant protein production	Session 8.1 Lipids and membranes
359 Hittinger Chris (InvS), Univ Wisconsin-Madison, (US)	**389 Liti** Gianni (InvS), IRCAN, (FR)	**370 Lajoie** Patrick (InvS), Univ Western Ontario, (CA)	**314 Mortensen** Uffe (InvS), Tech Univ Denmark (DK)	**346 Carman** George (InvS), Rutgers Univ, (US)
386 Klipp Edda (InvS), Humboldt Univ, (DE)	**385 Verstrepen** Kevin (InvS), VIB-KU Leuven, (BE)	**19 Chen** Ee Sin (InvS), Nat Univ Singapore, (SG)	**35 van Zyl** Willem (InvS), Univ Stellenbosch, (ZA)	**366 Stefan** Christopher (InvS), Univ College London, (UK)
108 Lahtvee Petri-Jaan, Tallin Univ, (EE)	**172 Timouma Soukaina, Univ** Manchester, (UK)	**59 Thevelein** Johan, KU Leuven, (BE)	**98 Frey** Alexander, Aalto Univ, (FL)	**226 Hanaoka** Kazuki, Hiroshima Univ, (JP)
17 Österberg Linnea, Chalmers Univ, (SE)	**36 Gyurchev** Nikola, Univ Leicester, (UK)	**111 Kucharczyk** Roza, Inst Biochem Biophysics Pas, (PL)	**138 Ben Tahar Imen**, Univ Liège, (BE)	**37 Laframboise** Sarah, Univ Ottawa, (CA)
49 van Leeuwen Jolanda, Univ Lausanne, (CH)	**72 Vega Estevez Samuel**, Univ Kent, (UK)	**127 Li** Sheena, Univ Toronto, (CA)	**342 Staudacher** Jennifer, BOKU Vienna, (AT)	**89 Rendulić** Toni, Univ Minho, (PT)
244 Grigaitis Pranas, Univ Amsterdam, (NL)	**76 Dutta** Abhishek, Univ Strasbourg, (FR)	**54 Żoładek** Teresa, Inst Biochem Biophysics, (PL)	**69 Gast** Veronica, Chalmers Univ, (SE)	**155 Wiersma** Sana, Delft Univ, (NL)
Chair: Lahtvee Petri-Jaan, Tallin Univ, (EE)	**Chair: Delneri Daniela, Uni Manchester (UK)**	**Chair: Kucharczyk Roza**, Inst Biochem Biophysics Pas, (PL)	**Chair: Fickers Patrick**, Univ Liège, (BE)	**Chair: Förster Jochen**, Carlsberg A/S, (DK)

2:40 - 2:50 PM Break

2:50 - 3:30 PM **Plenary 3 Lecture - Speaker Joris Winderickx, KU Leuven (B) - Chair: Kathryn Ayscough, Univ Calfiornia, Berkeley (US)**

3:30 - 3:40 PM Break

3:40 - 5:20 PM

Session 1.2 Signalling	Session 6.1 Mitotic cell cycle	Session 4.1 Yeast for food fermentation and processing	Session 3.1 Synthetic genomes and genome editing	Workshop W1: Yeast Bioprocess Scale up & Scale down
373 Paiva Sandra (InvS), Univ Minho (PT)	**321 Skoneczna** Adrianna (InvS), Inst Biochem Biophysics Pas, (PL)	**353 Jespersen** Lene (InvS), Univ Copenhagen, (DK)	**383 Pretorius** Sakkie (InvS), Macquarie Univ, (AU)	**Nienov** Alvin W (InvS), Univ Birmingham, (UK)
349 Posas Francesc (InvS), Univ Pompeu Fabra, (ES)	**354 Machín** Félix (InvS), HUNCS, SC Tenerife,(ES)	**395 Schwan** Rosane (InvS),Univ Federal Lavras, (BR)	**399 Daran-Lapujade** Pascale (InvS), Delft Univ, (NL)	**Reuss** Matthias (InvS), Univ Stuttgart, (DE)
97 Eknikom Supapid, Nara Inst, (JP)	**57 Litsios** Athanasios, Univ Toronto, (CA)	**221 Jeong** Damin, Chung-ang Univ (KR)	**355 Zhao** Yu, NY Langone Health, (US)	**Takors** Ralf (InvS), Univ Stuttgart, (DE)
58 Walden Elizabeth, Ottawa Univ, (CA)	**159 Maekawa** Hiromi, Kyushu Univ, (JP)	**144 Mietton** Lauriane, INRAE Montpellier, (FR)	**352 Bennis** Nicole, TU Delft, (NL)	**Minden** Steven (InvS), Univ Stuttgart, (DE)
106 Persson Viktor, Lund Univ, (SE)	**90 Liepins** Janis, Univ Latvia, (LV)	**94 Jadhav** Reshma, Agharkar Research Inst (IN)	**71 Badura** Jennifer, Geisenheim Univ, (DE)	**Delvigne** Frank (InvS), Univ Liège, (BE)
47 Inoue Koichi, Kyoto Univ, (JP)	**31 Matos** Gabriel, UFRJ-Brazil, (BR)	**53 Girardi Piva** Giovana, INRAE Montpellier, (FR)	**166 Bosch-Guiteras** Núria, Univ Lausanne, (CH)	
Chair:Sychrová Hana,Inst Physiol, Czechacadsci, (CZ)	**Chair: Bisshops Mark,** Wageningen Univ, (NL)	**Chair: Erten Hüseyin**, Cukurova Univ, (TR)	**Chair: Ellis Tom**, Imp College London, (UK)	**Chair: Takors Ralf**, Univ Stuttgart, (DE)

5:20 - 5:30 PM Break

5:30 - 6:30 PM **Poster Session 3** - *even numbers*

ICY15 meets ICYGMB30, August 23-27, 2021

detailed programme

Time CEST				
	THURSDAY, 26.08.2021			
11:00 - 12:00	**Poster Session 4** - *even numbers*			
	Break			
1:00 - 1:40 PM	**Plenary 4 Lecture - Speaker: Hiroshi Takagi, Tokyo Inst Technol (JP) - Chair: Pietro BUZZINI, Univ Perugia (I)**			
1:40 - 1:50 PM	Coffee Break			
1:50 - 3:30 PM	**Session 3.2 Tools, parts and chassis for yeast**	**Session 6.2 Mating, meiosis, sporulation**	**Session 4.2 Yeast for fermented beverages**	**Workshop W2: Plant biomass based substrates**
	380 Ellis Tom (InvS), Imp College London, (UK)	**365 Wolfe** Ken (InvS), Univ College Dublin, (IE)	**136 Bauer** Florian F (InvS), Univ Stellenbosch, (ZA)	**87 Perpelea** Andreea, Jacobs Univ Bremen, (DE)
	387 Morrissey John (InvS), Univ College Cork, (IE)	**379 Nicholas** Alain (InvS), Insitut Curie, (FR)	**394 Daran** Jean-Marc (InvS), Delft Univ, (NL)	**243 Netto** João HM, Chalmers Univ, (SE)
	114 Gligorovski Vojislav, EPFL Lausanne, (CH)	**412 Klein** Franz, Univ Vienna, (AT)	**117 Measday** Vivien, Univ British Columbia, (CA)	**67 Mastella** Luca, Univ Milano-Bicocca, (IT)
	202 Zimmermann Anna, VIB Gent, (BE)	**245 Heistinger** Lina, BOKU Vienna, (AT)	**130 Balsa-Canto** Eva, IIM-CSIC, (ES)	**34 Saju** Varghese, KU Leuven, (BE)
	163 Ledesma Amaro Rodrigo, Imp College London, (UK)	**61 Krogerus** Kristoffer, VTT Research, (SF)	**242 Molinet** Jennifer, Univ de Santiago de Chile, (CL)	**103 Soontorngun** Nitnipa, King Mongkut's Univ, (TH)
	110 Bonturi Nemailla, Tallinn Univ, (EE)		**79 Henriques** David, IIM-CSIC, (ES)	
	Chair: Daran-Lapujade Pascale (InvS), Delft Univ, (NL)	**Chair: Liti, Gianni**, IRCAN, (FR)	**Chair: Measday Vivien**, Univ British Columbia, (CA)	**Chair: Sourabié Alain**, Lesaffre BU Procelys, (FR)
3:30 - 3:40 PM	Break			
3:40 - 4:20 PM	**Plenary 5 Lecture - Speaker: Judith Berman, Twel Aviv Univ (IL) - Chair: Lisbeth Olsson, Chalmers Research (SE)**			
4:20 - 4:30 PM	Break			
4:30 - 5:30 PM	**Poster Session 5** - *all posters, free presentation*			
5:45 - 7:45 PM	**Workshop W3: Wild Brewing: History and Present**			
5:45 - 5:50 PM	**Mattanovich** Diethard, Univ BOKU (AT)			
5:50 - 6:20 PM	**Libkind** Diego, Univ Nacional del Comahue (AR)**, Hittinger** Chris, Univ Wisconsin-Madison, (US)**, Sampaio** José, Univ NOVA Lisboa, (PT)			
6:20 -6:50 PM	**Van Waesberghe** Willem, Heineken (NL)**, Boer** Viktor, Heineken (NL)			
6:50-7:00 PM	**Moritz** Charles, acib, (AT)			
7:00-7:30 PM	**Q&A and open discussion with Chris HITTINGER, José SAMPAIO, Willem VAN WAESBERGHE, Viktor BOER, Özge ATA, Charles MORITZ**			
	FRIDAY, 27.08.2021			
11:00 AM - 12:00	**Poster Session 6** - ***all posters, free presentation***			
12.00 - 1:00 PM	Break			
1:00 - 2:40 PM	**Session 3.3 Synthetic biology applications**	**Session 6.3 Environmental communication and**	**Session 4.3 Aroma/fragrance production**	**Session 8.3 Chromosome Structure and Function**
	356 Kang Hyun Ah (InvS), Chung-Ang Univ (CN)	**369 Olsson** Lisbeth (InvS), Chalmers Univ, (SE)	**377 Benjamin** Kirsten (InvS), Amyris, Inc, (US)	**358 Marston** Adele (InvS), Univ Edinburgh, (UK)
	397 Kondo Akihiko (InvS), Kobe Univ, (JP)	**361 Ashe** Mark (InvS), Univ Manchester, (UK)	**139 Querol** Amparo (InvS), IATA-CSIC, (ES)	**371 Vader** Gerben (InvS), Amsterdam UMC, (NL)
	100 Ata Özge, BOKU Vienna, (AT)	**29 Sychrová** Hana, Inst Physiol, Czechacadsci, (CZ)	**112 Mira** Nuno P, Inst Superior Técnico, (PT)	**241 Litwin** Ireneusz, Univ Wroclaw, (PL)
	91 Huo Guangxin, KU Leuven, (BE)	**116 Nenciarini Stefano**, Univ Florence (IT)	**81 Valera Maria**, Univ d República Montevideo, (UY)	**272 Cohn** Marita, Lund Univ**, (SE)**
	153 Canadell David, IRB Barcelona, (ES)	**240 Zunar Bojan**, CNRS Orleans, (FR)	**105 de Guidi** Irene, INRAE Montpellier, (FR)	**253 Tanaka** Seiji, Kochi Univ, (JP)
	77 Dacquay Louis, Univ Toronto, (CA)	**60 Saeki** Nozomu, Okajama Univ, (JP)	**68 Daute** Martina, Albertay Univ, (UK)	
	Chair: Francois Jean-Marie, INSA Toulouse, (FR)	**Chair: Branduardi Paola**, Univ Milano-Bicocca, (IT)	**Chair: Bond Ursula**, Trinity College Dublin, (IE)	**Chair: Terrance Cooper, Univ Tennessee HSC, (US)**
2:40 - 2:50 PM	Break			
2:50 - 3:30 PM	**Plenary 6 Lecture - Christina Smolke, Stanford Bioengineering (US) - Chair: Michael Sauer, Univ BOKU (AT)**			
3:30 - 4:30 PM	**Awards, Announcements and Closing Ceremony**			
4:30 -6:00 PM	**Farewell get together**			

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ORAL PRESENTATIONS

Plenary 1 Lecture

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Tormenting the yeast genome

Mr. Jef Boeke[1]

[1]*Institute for Systems Genetics, NYU Langone Health*

Rapid advances in DNA synthesis techniques have made it possible to engineer diverse genomic elements, pathways, and whole genomes, providing new insights into design and analysis of systems. The synthetic yeast genome project, Sc2.0 is well on its way with the 16 synthetic *Saccharomyces cerevisiae* chromosomes now completed by a global team. The synthetic genome features several systemic modifications, including TAG/TAA stop-codon swaps, deletion of subtelomeric regions, introns, tRNA genes, transposons and silent mating loci. Strategically placed loxPsym sites enable genome restructuring using an inducible evolution system termed SCRaMbLE which can generate millions of derived variant genomes with predictable structures leading to complex genotypes and phenotypes. The fully synthetic yeast genome provides a new kind of combinatorial genetics based on variations in gene content and copy number. Synthetic chromomose IV is the largest in the genome with over 1.4 Mb synthesized. We have created an “inside-out” version of this chromosome. Remarkably, the 3D structures of synthetic and native chromosomes are very similar despite the substantial number of changes introduced.

Chromosome I, the smallest *S. cerevisiae* chromosome, was fused to other chromosomes, which was surprisingly easy. This led to larger questions whether it would be possible to radically reduce chromosome numbers. We completely re-engineered the yeast karyotype, by systematically fusing telomere pairs and deleting single centromeres, generating an isogenic series of yeast ranging from n=16 to n=2. These strains show reproductive isolation and a massively altered 3D genome structure but are surprisingly “normal” with high fitness. We have also developed a method to move megabase segments to distant locations in the genome in a single step, again, with surprisingly little impact on fitness.

Yet another form of genome tormentation is switching up the protein packaging of DNA. The substitution of human for yeast nucleosomes leads to a number of unexpected transcriptional and other phenotypes.

Session 1.1 Yeast metabolism and its control

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Intrinsic biosynthetic dynamics as the metronome of the cell?

Mr. Matthias Heinemann[1]

[1]*University Of Groningen, Groningen, Netherlands*

Even further increasing the challenge to understand yeast metabolism and its regulation, using microscopic single-cell analyses, we recently found that metabolism is dynamic during the cell cycle. Strikingly, these oscillatory dynamics are autonomous, meaning that they occur also when the cell cycle is halted, while they seem to provide the rhythm for cell cycle progression. This raises the question on what makes metabolism oscillate in the first place, under constant nutrient conditions. Through sophisticated dynamic analyses in single cells, we could exclude current hypotheses as explanations and instead found that a number of biosynthetic processes are segregated in time. By means of a model-based analysis of these data, we found that the temporal segregation in biosynthesis generates the oscillations in primary metabolism, by pulling different precursors and cofactors from primary metabolism at different moments. The next challenge is to identify the mechanism that causes the temporal segregation of the biosynthetic processes, as another control layer of yeast metabolism.

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Use of alternative Transcription Start Sites regulates the production of cytoplasmic or mitochondrial forms of branched-chain aminotransferase in *Kluyveromyces marxianus*

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The final step in the biosynthesis of the branched chain amino acids (BCAA), leucine, isoleucine and valine and the first step in their catabolism is carried out by a branched chain amino acid aminotransferase (BCAT). In *Kluyveromyces marxianus*, two isoforms with opposing functions are encoded by a single gene *BAT1*. One proteoform, localised in the mitochondria, is involved in biosynthesis, whereas the cytoplasmic proteoform is the first enzyme of the Ehrlich catabolic pathway. We explored the mechanism the cell uses to regulate the production of one isoform or the other depending on its metabolic needs. To investigate whether this was a transcriptional effect, we analysed Transcription Start Site Sequencing (TSS-Seq) data for *K. marxianus* and discovered that *BAT1* is able to produce two mRNA isoforms through the use of alternative TSS. The longer mRNA isoform encodes a protein containing a putative N-terminal mitochondrial localisation sequence, while the shorter isoform produces an identical polypeptide that lacks the signal sequence. Through fluorescence microscopy experiments, we confirmed that Bat1 is localised in the mitochondria or cytosol depending on the presence of the signal peptide, and that the native promoter plays a role in determining localisation in a nitrogen-dependent fashion. Expression of either the long or the short isoform was tested by RT-qPCR with the finding that when growing on ammonium, the long form was made and the protein localised to the mitochondrion, thereby promoting BCAA biosynthesis. The opposite was seen when cells were grown using BCAA as the nitrogen source. Finally, by constructing mutants in candidate transcription factors, we found that both Gcn4 and Leu3 play crucial roles in regulating the choice of TSS. In conclusion, we established that transcriptional regulation enables *K. marxianus* precisely control the synthesis of the two BCAT proteoforms in a condition-dependent way.

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Amino acid biosynthesis and nitrogen catabolite repression control in synthetic complete media

Ms. Jennifer Tate, Ms. Jana Marsikova, Ms. Libuse Vachova, Ms. Zdena Palkova, Mr. Terrance G. Cooper

The experimental control for many experiments, including those involving Nitrogen Catabolite Repression (NCR), is growth in Synthetic Complete (SC) medium. Four SC formulations, SCcsh,1990, SCcsh,1994, SCcsh,2005 and SCme, have been used interchangeably as the nitrogen-rich medium of choice (CSH manuals, Methods in Enzymology). It has been tacitly presumed that all of these formulations support equivalent responses. However, Chen et al. (2018) concluded that: (i) TorC1 activity is down regulated by the higher concentration of primarily leucine in SCcsh2005 relative to SCme. (ii) The Whi2-Psr1/2 complex is responsible for this down regulation. TorC1 is a primary nitrogen-responsive regulator in yeast. Among its downstream targets is control of NCR-sensitive transcription activators Gln3 and Gat1. They in turn control production of catabolic transporters and enzymes needed to scavenge poor nitrogen sources (Pro) and activate autophagy (ATG14). One of the reporters used in Chen et al. was an NCR-sensitive DAL80-GFP promoter fusion. This intrigued us because we expected minimal if any DAL80 expression in SC. Therefore, we investigated the proteomes of wild type and whi2Δ cells cultured in SCcsh and SCme. We found a massive reorientation of amino acid biosynthetic proteins in both wild type and whi2Δ cells even though both media contained high overall concentrations of amino acids. NCR-sensitive DAL80 expression was minimally affected and overall NCR-sensitive protein production marginally if at all affected by the *whi2*Δ. The levels of 58 proteins changed (log2 = >-3 to -8 and >+3 to +8-fold) when Whi2 was abolished relative to wild type. Surprisingly, they were associated with only two GO terms, carbohydrate metabolism and oxidative stress following a shift from SCcsh to SCme for 6 hours. What was conspicuously missing were proteins related by TorC1-associated GO terms. NIH-GM-35642-27, Harriet VanVleet Chair, USA-LTAUSA18- LTAUSA18162.

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Modulation of cellular bioenergetics by mitochondrial respiratory supercomplexes

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The mitochondrial respiratory chain (MRC) plays a central role in cellular energy conversion, a process essential for aerobic life. MRC biogenesis and function involve the dynamic organization of the single MRC complex enzymes (CI to CIV) in ordered structures known as supercomplexes (SCs). Respiratory SCs have been identified in numerous organisms and, after years of controversy, it is currently accepted that they represent the MRC universal organizing principle. Moreover, a growing number of observations suggest that SCs represent a key determinant of healthy mitochondrial physiology. However, the field has suffered from the absence of tightly controlled model systems in which SC formation is the only variable. Recently, informed by the high-resolution yeast SC structure, we have generated yeast strains expressing CIII mutations that exclusively disrupt SC formation. Data obtained with this strain support a model in which SCs enhance electron transport by decreasing cytochrome c diffusion distance between CIII and CIV, and therefore increase cellular fitness. Here, we report the bioengineering of unique yeast strains expressing covalently linked CIII and CIV subunits to obtain functional tethered SCs (T-SCs). Notably, mitochondria isolated from T-SCs and wild-type strains have similar basal and coupled respiratory rates measured in the presence of either succinate or alpha-ketoglutarate as substrates. On the contrary, mitochondria expressing the T-SC CIII2CIV2 respire NADH at a rate lower than wild-type. Together, these observations suggest the existence of preferential substrate utilization by the different SC species. Moreover, to further gauge the physiological impact of respiratory SC in living cells, we assessed the adaptability of T-SC expressing strains to different carbon sources. Our data indicate that the expression of T-SCs delays the transition from respiration to fermentation, a behavior that we interpret as a faster turnover of CIII and CIV when they are not associated into SCs.

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CAZyme prediction in ascomycetous yeast genomes reveals novel xylanolytic species

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Yeast biodiversity present a huge, untapped resource for desirable phenotypes and novel Carbohydrate-Active enZYmes (CAZymes) that can be used in industrial applications such as consolidated bioprocesses. The aim of this study was to identify polysaccharide-degrading yeasts by mining CAZymes in 332 yeast genomes from the phylum Ascomycota. For this purpose, we developed a bioinformatic pipeline to rapidly predict CAZymes in a large number of genomes. The CAZyme analysis revealed a large spread in the number of CAZyme-encoding genes in the ascomycetous yeast genomes. We identified a total of 217 predicted CAZyme families, including several CAZymes likely involved in degradation of plant polysaccharides. Growth characterization of 40 CAZyme-rich yeasts revealed no cellulolytic yeasts, but several species from the *Trichomonascaceae* and CUG-Ser1 clades were able to grow on xylan, β-glucan and xyloglucan. *Blastobotrys mokoenaii, Sugiyamaella lignohabitans*, *Spencermartinsiella europaea* and several *Scheffersomyces* species displayed superior growth on xylan and well as high enzymatic activities. Moreover, additional non-sequenced species with xylan-degrading capacity were identified through phylogenetic association. Many of the species identified and characterized show equal or better xylanolytic activities compared to described species in literature such as *Scheffersomyces* and *Sugiyamaella* species, highlighting the potential of the approach. Collectively, the results expand our current knowledge on polysaccharide degrading ascomycetous yeasts and opens up for numerous follow-up studies on CAZyme characterization and yeast cell factory design.

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Synthetic biology tools to understand and control metabolic flux in yeast

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Effective redirection of carbon at specific metabolic nodes requires a suite of tools that can deliver useful outcomes under a wide variety of different conditions. Flux control mechanisms are commonly exerted at pre-translational levels; however, many metabolic conditions demand protein-mediated solutions that more directly and/or more rapidly influence catalytic conditions. Here, we have used isoprenoid (terpene/terpenoid) production as a model system to investigate these challenges. Isoprenoids are an extremely large and diverse group of natural compounds with myriad industrial uses, ranging from specialized applications (e.g., pharmaceuticals, fine chemicals, additives) through to bulk chemicals (e.g., colourants, fragrances, industrial polymers, agricultural chemicals, and fuel replacements). To control carbon flux at specific metabolic nodes for delivery of different classes of isoprenoids we have developed a variety of tools, including: (1) protein degradation tools control carbon competition at specific nodes (2) feedback controls that exploit native homeostasis mechanisms to balance carbon competition (3) co-location of pathway enzymes using scaffolds (including nanocompartments) to alleviate toxicity effects, increase titers, and alter the product profile of promiscuous enzymes (4) protein-based biosensors to understand metabolite accumulation. Using these approaches, we can effectively control and balance metabolism to deliver g/L titers of target isoprenoids.

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Minimisation of yeast metabolic networks defines a new class of functional genes

Mr. Steve Oliver

Construction of minimal metabolic networks would contribute both to our understanding of the origins of metabolism and to the efficiency of biotechnological processes by reducing the opportunities to divert flux away from desired products. We have designed minimised metabolic networks using a novel in silico synthetic biology pipeline that removes genes encoding enzymes and transporters from genome-scale metabolic models. The resulting minimal gene-set still ensures both viability and a growth rate value close to that of wild type.

The composition of these MMNs has defined a new functional class of genes that we term mandatory. These genes, whilst not essential, are very rarely eliminated in the construction of an MMN, suggesting that it is difficult for metabolism to be re-routed to obviate the need for such genes. We have characterised the place of these genes in the genetic interactions network using complex network theory. The average degree distributions of MMNs for aerobic and anaerobic growth are distinct, but are similar for media with different chemical compositions. Moreover, MMNs with a higher number of mandatory genes removed show a significantly reduced efficiency.

Further analysis of the mandatory genes, using both network and bioinformatic approaches has revealed that not only do these genes have more genetic interactions than the bulk of metabolic genes but their protein products also show more protein-protein interactions. In yeast, mandatory genes are predominantly single-copy and are highly conserved across evolutionarily distant organisms. These features may explain why mandatory genes are so difficult to remove from the metabolic network.

Session 2.1. RNA metabolism, transcription and translation

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Identification and consequences of mRNA modifications

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Cells face the daunting challenge of synthesizing the correct number of proteins at the right time with high fidelity. Messenger RNAs (mRNAs) serve as the blueprints for protein synthesis by the ribosome, and the post-transcriptional modification of mRNA presents one avenue for cells to regulate protein production. The recent discovery of mRNA chemical modifications has generated tremendous excitement because these modifications have the potential to regulate mRNA function and control protein expression levels. Pioneering work on mRNA modifications have implemented sequencing-based methods to map the location of individual chemical modifications across all of the RNA molecules in a cell. Despite a rapidly growing recognition of their importance, fundamental questions regarding the identity, prevalence and functional consequences of mRNA modifications remain to be answered. We are working to fill these critical knowledge gaps and establish a quantitative and mechanistic basis for understanding how mRNA chemical modifications impact protein synthesis at the molecular level. Our lab developed and applied mass-spectrometry based approaches to discover and characterize yeast mRNA modifications, and used in vitro biochemistry and structural biology techniques to demonstrate how uridine mRNA modifications impact the speed and fidelity of the ribosome. Together, these investigations present a biochemical foundation for understanding the consequences of modifications in mRNA coding regions.

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Understanding the potential toxicity of proteins by pursuing protein burden limits

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Overexpression of proteins often has negative effects on cell function. One of the relatively obvious negative effects of overexpression is resource overload. Cells use resources such as synthesis (transcription/translation), degradation, transport, and modification to process proteins, and if a particular protein that consumes these resources is overexpressed, that protein will monopolize the resources. The condition in which the synthetic process is overloaded is called “protein burden”. In principle, protein burden can occur only when harmless proteins are expressed in extremely large amounts because toxic proteins inhibit growth at lower expression levels than those that cause protein burden. However, we do not know what such harmless proteins are, and what the physiological state of protein burden is.

We hypothesized that the proteins with the highest expression levels to cause growth inhibition (expression limits) are the ones that are harmless and cause protein burden. As candidates for such proteins, we focused on a group of proteins that are expressed in large amounts, namely glycolytic enzymes, and measured their expression limits. We found that Gpm1 has the highest expression limits, about 15% of all proteins (Eguchi et al., eLife 2018). At the same time, we found that green fluorescent protein (EGFP) has a similar expression limit. On the other hand, when we profiled the morphology of cells overexpressing Gpm1 and EGFP respectively, we found that only EGFP-overexpressing cells showed an abnormal elongation phenotype. We investigated the cause of this phenomenon and found that the cysteine content in the protein molecule has a strong relationship with the cell elongation phenotype. Since the highly expressed proteins tended to have low cysteine content, the "cysteine-containing" property may be a factor that gives the protein toxicity. Other evidence that overexpressed EGFP misfolds and causes a heat shock response by incorporating Hsp70 will be discussed in this talk.

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Function and regulation of processing bodies

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Cells respond to changes in the environment by remodeling their proteome, which in turn depends on the transcriptome available for protein synthesis. Upon stress, a variety of mRNAs is sequestered into processing bodies (P-bodies). P-bodies are the major mRNA decay compartment in *S. cerevisiae*. Nevertheless, some RNAs appear to be stabilized in P-bodies under stress. The regulation of this process is poorly understood. Moreover, how P-body assembly is regulated upon different stress conditions remains largely enigmatic. We found that nuclear localization of a subset of P-body components provide a readily releasable pool to counter acute stress situations. I will discuss our current efforts to understand P body formation and function.

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Functional and transcriptional profiling of non-coding RNAs in yeast reveal context-dependent phenotypes and in trans effects on the protein network

Ms. Laura Natalia Balarezo Cisneros[1], Mr. Steven Parker[1], Mr. Marcin G Fraczek[1], Ms. Soukaina Timouma[1], Mr. Ping Wang[2], Mr. Raymond T O'Keefe[2], Mrs. Catherine B Millar[2], Ms. Daniela Delneri[1]

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Non-coding RNAs (ncRNAs) are increasingly being shown to play pivotal roles in the transcriptional and post-transcriptional regulation of genes in eukaryotes. Stable Unannotated Transcripts (SUTs) and Cryptic Unstable Transcripts (CUTs) have been shown to affect nearby genes by physically interfering with their transcription (cis mode of action), or interact with DNA, proteins or other RNAs to regulate the expression of distant genes (trans mode of action). Here, we carried out a large-scale screening on the ncRNA *Saccharomyces cerevisiae* deletion collection and provide evidence for SUT and CUT function. Phenotypic data on 372 ncRNA deletion strains in 23 different growth conditions were collected, identifying ncRNAs responsible for significant fitness variations. Transcriptome profiles were collected for 18 haploid ncRNA deletion mutants and 2 essential heterozygote ncRNAs showing a high correlation between altered phenotypes and global transcriptional changes, in an environmental dependent manner. By analysing the expression network new functional ncRNAs acting in trans by modulating transcription factors were identified. Furthermore, we described the impact of SUTs and CUTs in modulating coding genes in response of different environmental conditions, acting on important biological process such as ethanol tolerance, mitochondrial function, and respiration (SUT125, SUT126, SUT035, SUT432), plasma-membrane fluidity and sterol biosynthesis. (CUT494, SUT530, SUT468) or rRNA processing (SUT075 and snR30). Overall, this data captures and integrates the regulatory and phenotypic network of ncRNAs and protein coding genes, support the notion of the involvement of ncRNAs in fine tuning cellular expression via regulation of transcription factors, as an advantageous RNA-mediated mechanism that can be fast and cost-effective for the cells

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A budding writer: exploring the m6A deposition machinery in yeast

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The posttranscriptional mRNA modification N6-methyladenosine (m6A) has emerged as an important player in RNA regulation and in several physiological processes, such as differentiation and gametogenesis. In budding yeast, m6A deposition occurs only during entry into sporulation, prior meiotic divisions.
Although significant progress has been made in understanding the m6A modification in mammalian cells, little is known about the function and regulation of m6A in yeast. Furthermore, how m6A is deposited on messenger RNAs remains poorly understood in general. Only a fraction of known consensus sequences is modified, mostly at the 3'end of messenger RNAs.

In yeast, the m6A writer complex consists of Mum2, Slz1 and catalytic subunit Ime4. In our efforts to understand how m6A is deposited in yeast, we identified two additional components of m6A writer complex: The pheromone response transcription factor Kar4 and an uncharacterised protein YGL036W. Both proteins are essential for m6A deposition and progression into meiosis. Sequence analysis revealed that orthologues for each component exist in mammalian cells. Interestingly, although the writer complex is essential for m6A deposition, we have found that a core complex (Ime4, Mum2, Kar4, and YGL036W) is formed that also affects the onset of meiosis in a m6A-independent manner.

Taken together, we propose that the m6A writer complex is conserved between fungi, metazoans, and plants, and plays multiple roles during entry into sporulation in yeast. Our findings highlight the exciting potential of budding yeast as a model for studying of a highly conserved RNA modification.

Session 5.1 Phenotypic evolution in yeast

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Adaptive evolution by radical change in a fructophilic yeast clade

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The genomic era brought with it a myriad of opportunities to study yeast evolution that often led to fascinating new insights.

Our laboratory has been studying the molecular basis and evolutionary origin of fructophily in yeasts for some time. Fructophily is the preference for fructose over other carbon sources and originated in yeasts in a clade formed by the *Wickerhamiella* and *Starmerella* genera (W/S clade). The cornerstone of fructophily turned out to be a very unusual, high-capacity fructose transporter that was horizontally acquired from a fungus related to *Aspergillus* (1). While looking for additional genetic determinants of fructophily, we subsequently uncovered hundreds of other horizontally acquired genes in W/S-clade species, mostly involved in metabolism and originating both from bacteria and from filamentous fungi. Among these were genes involved in the reinstatement of alcoholic fermentation (2), lost in an ancestor of the W/S clade, and entire bacterial operons fully adapted to their new eukaryotic setting and functioning in thiamine biosynthesis (3). Presently, we are focusing on horizontally acquired genes that confer entirely new metabolic capabilities to their yeast hosts.

Taken together, our observations unraveled a very prominent role for HGT events in the evolution of metabolism in the W/S clade and suggest that there was apparently a period in the evolution of this lineage when these events were intriguingly pervasive.

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Exploring (and exploiting) natural genetic variation underlying quantitative traits

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S. cerevisiae, and its close relatives, are found in a wide variety of niches to which individual populations are well adapted. The population structure of mostly clonal growth with some breeding within but limited intermixing between populations leads to complex co-adapted genetic variants. Outbreeding breaks these up, leading to an expansion of phenotypic diversity and the potential to adapt to new niches previously inaccessible. This underpins the ability to domesticate yeast, intentionally or unintentionally, where strains are found to be well adapted to specific fermentation and industrial situations. We can exploit this genetic architecture to evolve new and improved phenotypes.

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Speciation of *Hanseniaspora* species through loss and gain of genes

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The yeasts of the genus *Hanseniaspora* are wide spread and particularly abundant on various fruits, but also on flowers and bark as their primary habitat. Due to their ability to ferment simple sugars, they contribute at starting phases of various spontaneous food fermentation processes and improve sensorial complexity of fermented products. Recently, it was shown that species of the genus *Hanseniaspora* have evolved unusually rapidly, likely due to the loss of many genes associated with metabolism, DNA repair and maintenance. As increased mutation rates can accelerate adaptation to fluctuating and stressful conditions, they can also represent one of the drivers of intrinsic reproductive isolation among diverging populations.

To gain insight into the ecological causes of gene loss during adaptation and speciation of *Hanseniaspora* species to novel ecological niches, we continued our exploration of the yeast communities associated with *Nothofagus* trees and its parasitic fungi in *Andean Patagonia*. We discovered a group of apiculate yeasts that were genetically distinct from their sister species *Hanseniaspora valbyensis* based on rRNA gene sequence and genomic data. The genomic contents of the new species and its closest relatives revealed that, during diversification, this novel species and its closest relatives, lost mitochondrial and other genes involved in the generation of precursors and energy, which was supported by their slower growth and higher ethanol yields under aerobic conditions. Similarly, in comparison to new apiculate yeast its close relative *Hanseniaspora mollemarum* lost the ability to sporulate, along with genes that are involved in meiosis and mating. Through evidence of loss and gain of genes between two species the process of speciation can be deduced even though in outcrossing yeasts this is not the only driver of their evolution.

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The enigmatic DUP240 yeast gene family plays a role in killer toxin defense

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Killer yeast secrete proteinaceous killer toxins which inhibit the growth of susceptible cells nearby. Although the killing mechanisms of many killer toxins have been well studied, little is known about how non-killer yeast defend themselves against these toxins. Using QTL mapping in *Saccharomyces cerevisiae*, we discovered that YAR028W, a previously uncharacterized gene, plays a vital role in resistance to K28 killer toxin. Analysis of YAR028W alleles showed that it is a major determinant of K28 toxin resistance among geographically and ecologically diverse yeast isolates. Furthermore, YAR028W is a member of the enigmatic DUP240 gene family, one of the largest remaining yeast gene families of unknown function. We found that the composition of DUP240 genes varies substantially across the yeast population, both in the identity of these genes and in their copy number. Using chimeras between YAR028W and a non-protective DUP240 gene, we identified the key region of YAR028W for providing K28 protection. We also found evidence of positive selection concentrated in this region, suggesting that DUP240s may be involved in an evolutionary arms race against killer toxins.

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Identification of novel lactic acid transporters in *Saccharomyces cerevisiae* through evolutionary engineering

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Although much is known about transport of sugars like glucose, fructose, maltose and sucrose, transport of monocarboxylic acids remains enigmatic, while these chemically simple molecules play a central role in the metabolism of many organisms. Transport of monocarboxylic acids such as lactic acid and acetic acid is involved in, for instance, food preservation, weak organic acid tolerance in second generation bioethanol production, metabolic engineering strategies for industrial production of carboxylic acids (which serve as precursors for production of bioplastics) and in development of cancer therapies. In this study, we evolved a *Saccharomyces cerevisiae* strain lacking all known lactic acid transporters (Jen1 and Ady2) on medium with lactate as the sole carbon source. Whole genome sequencing of evolved strains led to the discovery of mutations in Ato2 and Ato3, which are homologs of Ady2 that have not previously been linked to monocarboxylic acid transport. We then showed that the mutated variants of Ato2 and Ato3 catalyzed uptake of lactic acid, and their expression in an unevolved strain background allowed for fast growth (up to 0.15 h-1) in medium with lactic acid as sole carbon source, whereas their native non-mutated counterparts did not. A comparison between (evolved) sequences and 3D models of the transport proteins showed that most of the identified mutations resulted in a widening of the narrowest hydrophobic constriction of the anion channel, which may allow lactic acid to pass through. This study shows that laboratory evolution is a powerful tool for the identification of genes involved in substrate transport. Furthermore, it led to the identification of two novel lactic acid transporters and presents data on transporter structure and function that could potentially aid in future rational engineering and annotation of additional proteins involved in monocarboxylic acid transport.

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A-to-I RNA editing: an overlooked source of temporal mutations that could be beneficial in the life cycle of an organism

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Traditionally, DNA mutations are considered to bear the sole responsibility for alterations in genomic information. Creating diversity at the level of the DNA is costly for the organism, as the majority of mutations are not advantageous. Moreover, changes in the DNA are transmitted between generations, and an adaptive response to an environmental change may result in lower fitness once the environment changes again. Here we suggest that RNA editing enzymes, which modify individual nucleobases within RNA molecules are additional powerful mean for inner transcriptome diversity. In A-to-I RNA editing, genomically encoded adenosines are transformed by the Adenosine Deaminases Acting on RNA (ADAR) protein family and recognized as guanosines in the RNA sequence. When editing occurs within mRNAs, it can recode specific codons, leading to changes in protein structure and function. In addition, RNA editing can create temporal diversity, that unlike DNA mutations does not leave mutation burdens in the genome for many generations of cells.

To test our hypothesis, we created a selection-neutral in vivo system to investigate the effects of massive RNA editing on evolution. This was achieved by the exogenous expression of the ADAR proteins in the yeast *Saccharomyces cerevisiae*, an organism whose origins precede the emergence of ADARs, but can expresses ADAR from different organisms. Remarkably, we identify extensive (thousands) A-to-G changes, genome-wide, without leaving any traces on DNA. Exploration of the proteomics profile changes in response to ADAR activity reveled that RNA editing events are manifested at the proteomic levels and are a source of protein heterogeneity. Using the sophisticated experimental tools available in yeast, revealed that creating massive transcriptome diversification only at the RNA level, and only at a certain stage in the life cycle of an organism, could be much more beneficial in specific scenarios.

Session 7.1 Pathogenic yeasts

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Candida biofilms: importance, regulation, and evolution

Mrs. Clarissa Nobile[1]

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An infection is often treated as if it is composed of a single microbial species in isolation, yet in reality, infections are immensely complex ecosystems composed of many interacting microbes. Research in the Nobile lab is directed towards understanding the molecular and mechanistic bases of biofilm microbial communities. We are most interested in investigating how transcriptional networks underlie the regulation of gene expression during the development of biofilms. Much of this work is carried out in the *Candida* clade species, consisting of some of the most prevalent fungal pathogens of humans. Questions we are currently pursuing and will cover in this talk include: How are *Candida* biofilms regulated? How are they built? How are their unique and specialized properties maintained? How have they evolved?

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MCC/eisosome domains of the plasma membrane promote *Candida albicans* stress resistance and virulence

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The pathogenic effects of *Candida albicans* are caused by its ability to resist stressful conditions in the host and to disseminate to internal organs. Central to these processes is the plasma membrane, an essential barrier that mediates an array of functions needed for virulence including secretion of virulence factors, cell wall synthesis, nutrient uptake and sensing the extracellular environment. The plasma membrane is also on the front line of attack by the host and has developed mechanisms needed to resist stress. Our analysis of MCC/eisosome subdomains of the plasma membrane has revealed novel mechanisms for protecting *C. albicans* against stress. Sur7, a key protein in MCC/eisosomes, is critical for protecting against cell wall stress and resisting permeabilization by copper, which is used as part of the innate immune system attack on pathogens. Mutagenesis studies revealed that the cytoplasmic C-terminus of Sur7 is essential for its ability to promote stress resistance. Interestingly, *sur7*Δ cells contain elevated levels of $PI_{4,5}P_2$, which suggest that Sur7 has broad effects on *C. albicans* cells because it controls the levels of this key signaling lipid that regulates cell wall synthesis and morphogenesis. We hypothesize that Sur7 regulates the function of phosphatidylinositol phosphatases to influence $PI_{4,5}P_2$ levels. In support of this, mutational analysis of the three 5' $PI_{4,5}P_2$ phosphatases in C. albicans (INP51, INP52, and INP54) showed that their phenotypes are similar to the sur7Δ mutant, including abnormal morphogenesis and sensitivity to cell wall stress. While all three of these phosphatases regulate $PI_{4,5}P_2$ levels, Inp52 appears to be most important for stress resistance. Altogether, these studies demonstrate the MCC/eisosome subdomains of the plasma membrane play critical roles in regulating the structure of the plasma membrane in ways that enable it to resist stressful conditions in the host.

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Acetate transporters and channels are an integral part of *Candida* persistence within the human host

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Candida species have evolved distinct strategies to survive and proliferate within the human host (1). These strategies include sophisticated mechanisms to rapidly assimilate the available nutrients. During gastrointestinal and vaginal colonization, or inside immune effector cells, nonfermentable carbon sources, including acetate, are particularly abundant and may support the growth and the proliferation of these human pathogens (2). The presence of acetate has been previously demonstrated to influence biofilm formation and antifungal drug resistance, and to modulate immune recognition, suggesting that acetate transporters and channels have an important impact on these processes (3, 4).

Here, we provide a functional characterization and a detailed view on the role of *Candida* ATO transporters and FPS channels during host colonization. We confirmed by heterologous expression that these proteins restore the growth in acetate, in a strain deleted in acetate transporters. We also addressed their physiological role within human monocyte-derived macrophages. Our data support the view that these transporters are required for adaptation to the human host and, in particular, for *Candida* survival and persistence inside phagocytes.

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Study of the interaction between vaginal and intestinal lactobacilli, *Candida albicans* and *Candida glabrata*

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The success of *Candida glabrata* and *C. albicans* as human commensals/pathogens depends on their ability to cope with the bacterial microflora that has been found to restrain their pathogenic potential. In this work the inhibitory role of *Lactobacillus gasseri* and *L. reuteri*, highly abundant species of the vaginal and intestinal microbiomes, over *C. albicans* and *C. glabrata* was investigated coupling physiological studies with genome-wide scale analyses. The results obtained revealed that while in co-culture *L. gasseri* and *L. reuteri* reduced growth rate and cell viability of the two *Candida* species, however, this could not be attributed to a potential toxic effect exerted by the lactic acid produced by the bacteria. The presence of *L. gasseri* and *L. reuteri* also attenuated virulence traits of *Candida* cells against epithelial cells and also against the wax *Galleria mellonella*. The link between these phenotypic traits and the remodelling of the genomic expression of the *Candida* and lactobacilli species, including the identification of the molecular players governing the interaction, is discussed based on RNA-seq results performed in the co-culture settings.

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Domestication history determines the genome evolution of *S. cerevisiae* upon colonizing and infecting the human host

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Saccharomyces cerevisiae plays an important role in the food industry and as a probiotic, and it may be a member of the human microbiome, or even an opportunistic pathogen. To understand how the species colonizes and infects people, we sampled 62 isolates (both pathogenic and commensal) from 58 patients from Hungary. We subjected these, along with 30 European probiotic and baking yeasts to detailed genotyping, karyotyping, phenotyping, and short-read sequencing and comparative genomics.

Using phylogenomic network analysis based on alignment- and assembly-free methods, amended by comparisons to previously sequenced commercial strains and by extensive analysis of literature, we were able to trace back contemporary baking strains to artificial strain crossing and improvement strategies applied in the middle of the last century.

We show that probiotics, wine and baking yeasts colonize and infect humans frequently. Surprisingly, the domestication-driven genomic characteristics of commercial yeasts has resulted in baking-, probiotic- and wine-yeast-derived human isolates being remarkably different. The former preferably colonize the female genital tract and display large-scale chromosome copy number changes and rearrangements in their mosaic, polyploid genomes. Tetrad analysis showed that this plasticity cannot be attributed to sporulation but arises in clonal populations, leading to high geno- and phenotypic clonal heterogeneity. At the same time, wine- and probiotic-derived human isolates lack such an anatomic niche preference and show lower levels of genome structure variation. The genomic adaptations we observe in human isolates derived from commercial strains are in striking contrast with the indigenous yeasts from non-Westernized populations sequenced in previous studies.

Our study shows that the pathogenic and colonization potential of *S. cerevisiae* has been drastically altered by domestication in the recent past, and even if *S. cerevisiae* is a true member of the human microbiome, it is now mostly present in us in the form of non-natural, domesticated clades.

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Evolution of opportunistic pathogenicity in *S. cerevisiae*

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Saccharomyces cerevisiae is considered a GRAS – generally recognized as safe organism. Yet, reports of *S. cerevisiae* infections have been accumulating in the clinic. Previous studies have addressed the genetic basis for the transition to pathogenicity in this organism, but we still lack a clear understanding of the ecological and evolutionary mechanisms that favor this transition. In this study, we perform whole genome analyses of 250 genomes. We analyzed genomic diversity, population ancestry, and phylogenetic relationship of clinical isolates and non-clinical host-associated isolates within the context of the well-characterized natural and domesticated populations of *S. cerevisiae*. Clinical isolates tend to segregate within Wine and Bread clusters or form isolated clusters characterized by mosaicism with genomic contributions from Wine, Philippine and Sake clades. Within the Wine clade we identified a subclade of highly similar genomes compatible with a disease outbreak likely associated with probiotics. These genomes show a marked increase in whole genome diversity associated with a change in ploidy levels. We also searched for signals of selection using principal component analysis by identifying SNP variants whose differentiation along the top PC is significantly greater than the null distribution under drift. A selection of the most significant SNPs corroborated previous identifications of PDR5 gene as being under selection, but it also points to VTH1 a putative membrane glycoprotein with a function in oxidative stress resistance and vacuolar morphology, and CLN2 a G1 cyclin involved in regulation of the cell cycle as also important in the transition to pathogenicity.

ITN Network Session: Aromagenesis

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Accelerated evolution of lager yeast strains for improved flavor profiles

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Beer is one of the most popular alcoholic beverages in the world. Accounting for 84% of global beer consumption, lager-style beers are very popular due to their clean crisp flavours and are the products of fermentations carried out by *Saccharomyces pastorianus*, a hybrid of *S. cerevisiae* and *S. eubayanus*. As today's consumers favour more flavour diversity, brewers are responding by creating new products. To generate lager yeast strains with improved flavour profiles, specifically with increased concentrations of the aromatic compounds 2-phenylethanol and phenylethyl acetate, the products of phenylalanine metabolism. Using an accelerated evolution approach, mutants were generated using inhibitors of the heat shock response and then selected for resistance to amino acid analogues of phenylalanine, which selects for mutants with decreased feed-back inhibition of phenylalanine biosynthesis. The library of mutants was characterised to identify strains producing the desired changes in flavour compounds. The genomes of two mutant strains, one derived from the Group I *S. pastorianus* strain CBS1538 and the second from the Group II strain *Weihenstephan* 34/70 were sequenced and their transcriptomes analysed during lager-like fermentative conditions. The mutants display chromosomal rearrangements, chromosome loss, and differences in their transcriptome profiles. Furthermore, specific mutations in key genes in the Ehrlich pathway were identified that account for the improved flavour profiles observed in the mutants.

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Improving the aroma profile of beer via non-conventional yeast fermentation

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Non-conventional yeasts have been shown to produce enhanced fermentation characteristics when compared with *S. cerevisiae*, such as texture, reduced alcohol content, and a distinctive aroma profile. In this work, twelve conventional and non-conventional yeasts were studied with the aim of generating alcoholic beverages with improved flavour and aroma profiles.

The yeast strains were screened on solidified wort medium to ensure sufficient fitness in beer fermentative conditions. Then their growth and aroma compound production were assessed in liquid wort. *H. vineae* Y-17530 stood out as an excellent candidate for generating beer with enhanced fruity character, since it produced very high levels of esters: production of ethyl acetate was 10-fold higher than in *S. cerevisiase* WLP 001, a widely used brewing strain. Furthermore, ethanol production of *H. vineae* in wort was very low (1.62 g/L), making it the perfect candidate for the production of beer with reduced alcohol content and a pleasant fruity character. Moreover, if presence of ethanol is desired, the strain could be used in conjunction with an ethanol-producing strain, such as *S. cerevisiase* WLP 095, which was shown to produce relatively high levels of citronellol, a floral component of beverage aromas.

Results generated a vast amount of information on the fitness and aroma compound production of the studied strains in brewing conditions. Additionally, the study of strains during co-fermentations with the proposed candidates would yield valuable knowledge about their combined growth kinetics, and its influence on the final aroma profile of the fermentation. This knowledge could be utilised in the exponentially-growing field of craft brewing.

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A novel aminotransferase gene and its regulator acquired in *Saccharomyces* by a horizontal gene transfer event

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Horizontal gene transfer (HGT) is an important evolutionary mechanism of adaptive importance. HGT has been deeply studied in wine *S. cerevisiae* strains, in which the acquisition of xenologous genes, conferred improved traits related to both transport and metabolism of the nutrients present in the grape must. However, little is known about HGT events occurred in wild *Saccharomyces* yeasts and how they determined their phenotypes. By using a comparative genomic approach among *Saccharomyces* species, we detected an 8-kb subtelomeric segment present in the *S. uvarum*, *S. kudriavzevii*, and *S. eubayanus* species, belonging to the first divergent *Saccharomyces* lineages, but absent in the other S*accharomyces* species. Phylogenetic analysis showed that two predicted genes within this region are closely related to two adjacent genes present in *Zygosaccharomyces*, which encode a putative Zinc-finger transcription factor and an aminotransferase-like protein. The latter showed ~52% amino acid identity with *Burkholderia cepacia* dialkylglicine decarboxylase, whose specific substrate is the non-proteinogenic amino acid 2-aminoisobutyric acid (AIB), a rare amino acid present in some antimicrobial peptides of fungal origin. A growth screening showed that some *S. kudriavzevii* and *S. uvarum* strains grew using AIB as the sole nitrogen source. The deletion of the aminotransferase gene, now called DGD1, totally impaired the growth of the strains in the presence of AIB. Interestingly, the deletion of the putative Zinc-finger transcription factor gene, named DGD2, impaired the growth as well. Moreover, *dgd2* mutants were unable to induce the DGD1 expression in the presence of AIB, proving an AIB-dependent expression of DGD1 by the action of DGD2. These results show evidence of an early HGT event conferring new traits to the ancestor of the *Saccharomyces* genus, that latter were lost in one single event in the ancestor of the more recent lineages of this genus, likely due to adaptation to different environments.

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Effect of non-wine *Saccharomyces* yeasts and bottle aging on the release and generation of aromas in semi-synthetic Albariño wines

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Eight *Saccharomyces* yeasts from different origins and species, including *S. cerevisiae*, *S. uvarum* and *S. kudriavzevii*, were fermented at 16 ºC in a semi-synthetic must containing natural polyphenolic and aroma precursor of cv. Albariño and synthetic mercaptans precursors. The resulting wines were subjected to accelerated anoxic aging simulating bottle aging. Aroma profiles of young and aged wines were analysed by using distinct gas chromatography methodologies.

Cryotolerant strains showed better fermentation performances compared to *S. cerevisiae* and significant differences were observed regarding the level of volatile and non-volatile fermentation products. Given that GABA is a potential precursor of γ-butyrolactone and diethyl succinate, we suggested that the highest levels of the latter two compounds observed in *S. uvarum* strains, together with their greatest succinic acid yields, could be related to greater flux through the GABA shunt. These strains were also characterized by higher production of 2-phenylethyl acetate, geraniol and branched-chain esters, providing floral and fruity notes to wines. The latter compounds were then highly increased by aging, while the original acetates and terpenes of the young wines tended to decrease. *S. kudriavzevii* strains showed a great ability to release the polyfunctional mercaptans from the added non-volatile precursors, with SK1 strain yielding up to 47-fold and 8-fold more 4-methyl-4-mercaptopentan-2-one (4MMP) than *S. cerevisiae* and *S. uvarum* strains, respectively. Finally, the wild *S. cerevisiae* isolate SC2 showed a very particular aroma profile due to the highest production of ethyl 4-methylvalerate (lactic and fruity notes), g-octalactone (coconut notes) and furfurylthiol (roasted coffee notes), the latter compound possibly being produced from the pentose phosphate pathway (PPP) derivative ribose-5-phosphate. Since erythritol, another PPP intermediate was also largely produced by this strain, we hypothetically linked the greater furfurylthiol yield of SC2 to a higher flux trough this metabolic pathway.

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Ability of *Saccharomyces cerevisiae* strains to modulate the aroma of young and aged Tempranillo wines

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AIM: This study aims to evaluate the senso-chemical variability introduced by selected *S. cerevisiae* strains in fermentative and varietal aromatic profiles of young and aged Tempranillo wines.

METHODS: Ten *S. cerevisiae* strains fermented model musts containing a polyphenolic and aroma precursors fraction (PAF) extracted from Tempranillo grapes. Wines were then submitted to accelerated anoxic ageing at 50ºC for 5 weeks. Aroma compounds were studied by Gas Chromatography (GC), GC-Olfactometry and GC-Mass Spectrometry, during fermentation by trapping volatilized aroma, immediately after fermentation and after accelerated ageing.

RESULTS: Odorants lost by evaporation are mostly isobutanal, isopropyl and isoamyl acetates, ethyl propanoate, and isoamyl alcohols, and not grape related aroma compounds. Yeasts exerted a great impact on wine aroma composition, mostly evident only after aging since levels of ethyl esters of branched acids, of most grape-related aroma compounds and of many minor yeast derived aroma compounds mostly increase during aging. Strains could be classified into 3 major clusters. Linalool and geraniol were found to have fermentative origin, reaching high levels with one of the strains.

CONCLUSIONS: This study provides evidence that *S. cerevisiae* strains can efficiently modulate varietal aroma, likely through specific enzymatic activities acting on grape phenolic acids and nor-isoprenoids and may be especially used to mitigate some aging-related odorants, such as massoia lactone, guaiacol or TDN.

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ITN Network Session: YEASTDOC

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Screening of aroma metabolites within a set of 90 *Saccharomyces* strains

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Currently, the main demand in the global wine market relies on products with unique flavour profiles, character, and typicity, and the metabolism of yeasts greatly influences the organoleptic properties of wine. Therefore, the natural diversity of *Saccharomyces* strains rises in interest over the last decade, but a large part of this phenotypic diversity remains unexplored. Moreover, the genetic basis underlying the variation in the production of flavour-active metabolites within the *Saccharomyces* genus remains poorly understood. The main purpose of this project is to provide a better understanding of how the synthesis of these flavour-active compounds is modulated at the genetic level, aiming to identify genes with specific functions in the metabolism of yeasts. This information will be obtained through the generation of novel hybrids between different *Saccharomyces* species and the use of quantitative genetics. In this context, the first step was to assess the phenotypic diversity at the scale of *Saccharomyces* genus, regarding traits of industrial interest.

With this aim, 90 yeast strains of all the eight species which compose the *Saccharomyces* clade were screened for their fermentative capacities and the production of aromas and other compounds of interest, such as glycerol or succinate. Fermentations in oenological conditions were carried out at different temperatures, monitoring the kinetic profiles and analysing the production of the main fermentation metabolites as well as the production of more than 40 aroma compounds. The sporulation ability of the strains, necessary for the hybridization, was also assessed.

Important differences were found in the kinetic and volatile profiles of the strains, and the whole dataset provides a comprehensive picture of the phenotypic diversity within the genus *Saccharomyces*. This information confirms the interest in further development of genetic approaches to identify the molecular basis underlying the studied traits and opens the door for their improvement.

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Fungal oligopeptide transporters have different oligopeptide specificities despite their high sequence identity

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Fungal oligopeptide transporters (FOT) are a novel family of oligopeptide transporters that have been found in *Saccharomyces cerevisiae* wine strains while being absent from the reference laboratory strains. Presence of Fot1, Fot2 and Fot3 in *S. cerevisiae* wine strains is due to a horizontal gene transfer from the yeast *Torulaspora microellipsoides*, which counts with Fot2Tm, FotX and FotY. In the *S. cerevisiae* wine strain EC1118, Fot1 and Fot2 are responsible for a broader range of oligopeptide utilization in comparison with strains not containing Fots, leading to better fermentation efficiency and an increased production of desired organoleptic compounds in wine. Using CRISPR/Cas9, we have constructed *S. cerevisiae* wine strains containing the different Fot members as the unique oligopeptide transporter in order to characterize their oligopeptide specificities. The analysis of substrate consumption by phenotype microarrays has shown a diverse di-/tripeptide specificity among Fot, which brings light into the specialization of these transporters despite their high sequence identity. We have used molecular docking simulations between Fots and their respective preferred substrates to identify potential key residues involved in substrate binding and transport. In addition, phylogenetic analysis of FOT family shows the distribution of these transporters among different fungal groups, contributing to better understand the ecology of oligopeptide uptake systems in yeast.

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Progress in engineering *Saccharomyces cerevisiae* for the fermentative production of succinic acid from glycerol

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Succinic acid (SA) is one of the most promising bio-based platform chemicals. Among the metabolic routes for microbial SA production, a reductive, homo-fermentative, and CO_2-fixing pathway is superior since it allows the highest theoretical yield. With regard to carbon sources, glycerol provides the advantage of a higher degree of reduction compared to sugars, therefore allowing a redox-neutral pathway for SA production. Certain bacteria naturally exhibit a respective pathway, but are not tolerant towards low pH. Fungal organisms can be grown at low pH, which is a significant advantage for downstream-processing in microbial organic acid production. In fact, the yeast *Saccharomyces cerevisiae* was engineered for commercial SA production from glucose via the reductive pathway. However, the yeast-based process has been less efficient compared to the bacterial production. Moreover, the use of glycerol as a carbon source was not considered in the past since wild-type strains do not efficiently utilize glycerol. We previously engineered a *S. cerevisiae* CEN.PK strain with the goal to enable efficient glycerol utilization plus provide the electrons from glycerol oxidation in the form of cytosolic NADH. Here, we show the establishment of the reductive SA production route in the cytosol of the respective glycerol-utilizing strain. The highest titer and yield were obtained in shake flask cultivations in synthetic glycerol medium supplemented with $CaCO_3$. A maximum titer of 34.7 g/L SA was achieved with a maximum yield of 0.54 g/g of consumed glycerol. Notably, significant amounts of the pathway intermediate malic acid were also produced. The total yield of dicarboxylic acids was 0.82 g/g of consumed glycerol, corresponding to 61.5% of the maximum theoretical yield. Optimization of the expressed reductive pathway, SA export, as well as establishing controlled conditions in bioreactors might further shift the observed phenotype towards the target product SA.

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Lessons in the use of CRISPR/Cas9 system in the hybrid yeast *Zygosaccharomyces parabailii*

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Zygosaccharomyces parabailii is a natural hybrid yeast between *Z. bailii* and a yet unidentified yeast. *Z. parabailii* has been described as a robust yeast, highly resistant to weak acids and hence one of the yeasts responsible for food spoilage. However, its poor characterization impairs our capacity to prevent its spoilage activity or to exploit this yeast as a cell factory. Correlation between genotype and phenotype could greatly improve our knowledge, but currently this yeast is not amenable to routine genetic manipulation. This is due in part to its hybrid nature, implying the presence of two similar functional copies of each gene in its genome, and the low efficiency of homologous recombination.

To avoid the use of complex and time-consuming protocols for gene disruption, genome editing technologies such as CRISPR might be useful for manipulating *Z. parabailii*. However, this strategy has not yet been described for non-*Saccharomyces* hybrids, and it is not well characterized for the editing of hybrid yeasts, in general. Therefore, the aim of this project is to implement CRISPR/Cas9 in the hybrid yeast *Z. parabailii*.

Here, we present the successful impairment of the two ADE2 homologs by the use of different gRNA expression cassettes. We show how the efficiency of the simultaneous double disruption is very dependent on the specific gRNA used. Moreover, we also assess the efficiency of using a donor DNA for a targeted insertion, given the prevalence of the NHEJ DNA repair that operates in this yeast. Overall, we describe how the CRISPR/Cas9 approach to knock-out genes of this *Z. parabailii* strain can be used successfully, as well as provide possible guidelines for the editing of non-*Saccharomyces* hybrid yeasts more generally.

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Contribution of PDR12 to weak organic acids resistance in the yeast *Zygosaccharomyces parabailii*

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Yeast biodiversity reflects evolutionary processes that selected different species able to flourish in specific habitats and possess peculiar traits that can be beneficial when exploited in yeast-based biorefineries. Traditionally considered as food-spoilage yeast, *Zygosaccharomyces bailii* is also a potential host for industrial production, owing to promising traits such as the ability to withstand low pH, high osmotic pressure, high ethanol concentrations and weak organic acids. The last trait is of special interest, both because of the potential use of this yeast as a cell factory for organic acids production, and for developing preventative measures against *Z. bailii* food spoilage. While some of the molecular determinants responsible for this robustness have been described, other aspects remain to be studied.

Given its role in counteracting weak organic acids stress in other yeasts, most notably in *Saccharomyces cerevisiae*, we were interested in assessing the role of the ATP-binding cassette transporter PDR12 in *Z. bailii*. One interesting feature of *Z. bailii* genetics is that it is a natural hybrid species, with each parent having contributed a separate copy of each gene. We wanted to determine whether PDR12 is important for organic acid tolerance in *Z. bailii* and, if so, whether both copies of PDR12 contribute equally.

We constructed and characterised a set of single and double PDR12 mutants. These analyses determined that PDR12 is involved in mediating tolerance to butyric and acetic acids, though individual mutants displayed phenotypes consistent with differential roles for each parental allele. Here, we will show and comment on how the possible different contribution of the two alleles was explored through mutagenesis, complementation assays, heterologous expression and transcriptional regulation analysis.

Plenary 2 Lecture

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The metabolic and proteomic landscape of genome-scale genetic perturbation

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Life runs on many thousands of different chemical reactions, known collectively as cell metabolism. Metabolic reactions are vital for keeping cells and organisms growing and alive, but they are equally implicated in cellular sensing, signalling, and its response to environmental challenges. Understanding the interactions between metabolites and the transcriptome and proteome of the cell are hence vital for our understanding of biological phenotypes. In this lecture, I'll summarize our efforts in using systematic yeast perturbation experiments to derive metabolic function principles. We have started by measuring an amino acid metabolome for the genome-scale *S. cerevisiae* knock-out collection, and learned that metabolome profiles provide so far hidden functional information. The desire to understand the metabolic fingerprints, led us to explore the proteome, as a functional layer bridging the genome to metabolism. I'll present a new proteomic platform designed to measure thousands of yeast samples at low cost, and its application to generate a proteome for each knock-out strain. Generating such dataset, allowed us to bridge genome, proteome and metabolome on the scale of genome wide genetic perturbation, and I will illustrate on the basis of several examples, on how genome-scale proteomic data opens new avenues for understanding gene function and interactions within biological systems.

Session 1.4 Metabolic engineering

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Metabolic engineering of yeast to produce fungal secondary metabolites

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Fungal secondary metabolites are a diverse class of natural products with antioxidant, anti-bacterial, anti-fungal, anti-tumor, and plant growth-regulating bioactivities. They have vast potential applications as pharmaceuticals, nutraceuticals, and agrochemicals. Secondary metabolites are typically produced only in small amounts by the native hosts. The native hosts may be challenging to cultivate or genetically engineer, and some may produce toxic by-products. Therefore, it is advantageous for industrial production of these metabolites to transfer the biosynthetic pathway into a yeast chassis that is easy to engineer and ferment at scale. I will share our work on engineering of baker's yeast *Saccharomyces cerevisiae* to produce L-(+)-ergothioneine, psilocybin, and gibberellins. Ergothioneine is an antioxidant present in many edible mushrooms. In animal models, it showed promise against neurological diseases, pre-eclampsia, and some cardiometabolic disorders. Psilocybin is a psychoactive alkaloid present in so-called "magic mushrooms," currently investigated to treat depression, anxiety, and clustered headache. Gibberellins are plant growth hormones used for enhancing productivity and quality of vegetables and fruits.

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Exploring the potential of non-homologous end-joining mediated genome engineering for metabolic engineering applications in *Yarrowia lipolytica*

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As an important oleaginous industrial microorganism, *Yarrowia lipolytica* has been used widely to produce lipid and various value-added compounds. Although genomic editing tools have been developed for *Y. lipolytica,* the tools for pathway optimization and library construction are still lacking. In *Y. lipolytica*, the predominant repair pathway of DNA double-strand break (DSBs) is non-homologous end-joining (NHEJ) repair. Here, the characteristics of NHEJ-mediated genomic integration was systematically analyzed by high-throughput sequencing. Based on that, we developed the tools for both random genomic integration and targeted integration. The tools were applied in constructing modular library of biosynthetic pathway and genome-scale mutation library. The production of succinate, β-carotene and α-farnesene were demonstrated.

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Insight into *Yarrowia lipolytica's* glycerol uptake system

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Plasma membranes build the interface between the cell and its environment, separating the cell's metabolism from molecules essential for its survival. To overcome this hurdle, cells developed specialized transport proteins that allow the exchange of molecules across these membranes. One crucial metabolite that needs to cross the membrane of each living organism, is the carbon source. While many organisms prefer glucose as carbon source, the yeast *Yarrowia lipolytica* seems to favour glycerol. The fast growth of *Y. lipolytica* on glycerol and its flexible metabolism, which allows it to adapt to various environmental conditions, makes this yeast a fascinating organism to study the glycerol metabolism. Although many enzymes involved in the glycerol metabolism have already been identified, information on the proteins essential for glycerol uptake is scarce. Therefore, our aim was to identify proteins that are involved in the glycerol uptake of *Y. lipolytica*. In literature, nine proteins of *Y. lipolytica* can be found that are potentially involved in glycerol uptake based on their sequence similarity to known fungal glycerol transporters. To determine which of these proteins transport glycerol into the cell, we performed complementation assays with glycerol-transporter deficient strains of *S. cerevisiae* and *Y. lipolytica*, the latter of which was obtained during this study. In total, five proteins of *Y. lipolytica* were identified that restore growth of the glycerol-transport deficient *S. cerevisiae* strain. Disruption of these five proteins in *Y. lipolytica* abolished growth on low glycerol concentrations, but growth could be restored by the individual expression of each protein. Surprisingly, the transporter-disrupted strain retained its ability to grow on high glycerol concentrations, indicating the existence of further, not yet identified transport proteins.

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Enzymatic activity of type II fatty acid synthase in *Saccharomyces cerevisiae*

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In bakers yeast (*Saccharomyces cerevisiae*), the biosynthesis of fatty acids is controlled by a complex and difficult to engineer multi-domain type I fatty acid synthase (FASI) where all enzymatic steps of fatty acid synthesis are on one or two large polypeptides. By replacing the native FASI from yeast by bacterial type II fatty acid synthase (FASII), that has each enzymatic step on a different polypeptide, a higher diversity of fatty acids could be produced as the FASII could be easier to manipulate. We have expressed a FAS II from *E. coli* in *S. cerevisiae* in order to replace the native FASI using the yeast pathway kit (Pereira et al. 2016), a protocol for in-vivo assembly of metabolic pathways. To evaluate/select the pathway viability, enzymatic assays have been set up to measure the combined activity of the enzymatic steps.
A discussion of the optimization of the FASII pathway expression underpinned by these measurements will be presented.

Pereira, Filipa, Flávio Azevedo, Nadia Skorupa Parachin, Bärbel Hahn-Hägerdal, Marie F. Gorwa-Grauslund, and Björn Johansson. 2016. “Yeast Pathway Kit: A Method for Metabolic Pathway Assembly with Automatically Simulated Executable Documentation.” *ACS Synthetic Biology* 5 (5) (May 20): 386–394.

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Yeast syntrophic co-culture evolution for improved product synthesis (CoALE)

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The use of microorganisms for production of molecules of interest is highly spread across multiple fields, achieving more effective production while lowering costs and environmental impacts. While the application of rational engineering has allowed an increased production of a variety of molecules of industrial interest, the carbon and energy deregulations produced during the pathways tuning processes can potentially compromise the fitness of the cell forcing to even further genetic modifications. Microbial consortia or co-cultures could allow for the use of naturally occurring mutations to bypass this limitation by linking the production of a metabolite of interest to the consortia survival. Aromatic amino acids such as tyrosine and phenylalanine constitute precursors for the biotechnological production of many important products such as vanillin, cis-cis muconic acid, hydroxymandelic acid among others. This project explores the use of syntrophic consortia and adaptive laboratory evolution (ALE) to generate tyrosine-overproducer yeast strains for their future application as chassis organisms in later production processes. To this end, lysine and tyrosine producing phenotypes were constructed into auxotrophic strains for tyrosine and lysine, respectively, producing complementary phenotypes and conditioning growth in SCD-Lys-Tyr media to an effective complementary cooperation.
To further improve cooperation and growth, the consortia were subjected to several passages of ALE. Isolated strains from the evolved consortia presented performance improvements when compared to their originally engineered ancestors, improved dilution resistance, faster consortia development, and improved production of p-hydroxyphenylethanol as a reporter molecule for tyrosine production. Genome sequencing analysis performed on evolved strains elucidated several mutations with a potential role in the cell fitness as well as into the amino acid transport process.
In our presentation we will highlight the potential of this novel approach which we call CoALE to fuel further efforts into the improved production of molecules of interest.

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Metabolic engineering of the yeast *Cutaneotrichosporon oleaginosus* for valorization lignin and lignin-derived aromatics

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Cutaneotrichosporon oleaginosus, previously known as *Cryptococcus curvatus*, is a non-model oleaginous yeast known for its ability to metabolize many alternative substrates, including xylose, and toxic lignocellulosic hydrolysate inhibitors such as 5-hydroxymethylfurfural and furfural. We discovered C*. oleaginosus* also tolerates and metabolizes lignin-derived aromatics, highlighting this organisms' potential to utilize all components of lignocellulosic biomass. *C. oleaginosus* is able to fully metabolize phenol, 4-hydroxybenzoic acid (pHBA), and resorcinol as sole carbon sources, as well as co-utilization with glucose and xylose. When buffered, cells also metabolize p-coumarate robustly and to a lesser degree ferulate and syringate as sole carbon sources. In a simple batch flask culture, *C. oleaginosus* is able to accumulate nearly 50% of its biomass as lipids while utilizing pHBA, p-coumarate, and resorcinol. Optimizing fed-batch feeding strategies increased lipid accumulation to over 69%, the second highest value in literature for this organism to date. Transcriptomic analyses were used to elucidate aromatic metabolic pathways in *C. oleaginosus*, uncover pathway regulation related to aromatic metabolism and improve the existing genome annotation significantly. Biochemical analysis suggests ortho ring cleavage is used throughout the aromatic metabolic pathways. We also recently demonstrated *C. oleaginosus* metabolizes monomers released from alkaline pretreated cornstover lignin, and the presence of lignin is not inhibitory to growth. With such an exceptionally desirable natural phenotype, this yeast could become a preferred host for oleochemical production if novel synthetic biology tools are developed. We identified both strong constitutive and phenolic-regulated promoters to drive expression of heterologous or native genes. In parallel, we are developing higher efficiency transformation methods for increasing the pace of engineering efforts. Finally, we have engineered production of ricinoleic acid production from lignin-derived aromatics. Overall, our work establishes *C. oleaginosus* as a promising platform to robustly convert all components of lignocellulosic biomass into novel high-value oleochemicals.

Session 2.2 Protein folding and secretion

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RNA targeting and protein secretion

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Studies over the two decades have illustrated that mRNA targeting plays a critical role in protein localization upon translation. This is particularly true for the localization of mitochondrial- and endoplasmic reticulum (ER)-targeted proteins in yeast, which utilize RNA motifs for co-translational mRNA localization to mitochondria and for translation-independent targeting to the ER, respectively. In the latter case, mRNAs encoding soluble secreted and membrane proteins are targeted to the ER via RNA motifs recognized by ER-associated RNA-binding proteins that anchor the RNA and promote translation on ER-bound ribosomes. Importantly, we now show that RNAs encoding secreted proteins can be selectively co-packaged within the nucleus into multiplexes that ensure multicomponent co-translation and secretion. These multiplexes appear to confer an operon-like control gene expression to a number of pathways in yeast, including secreted components of the mating pathway in haploid cells. We will discuss the role of chromatin interactions and RNA motifs in the secretion of proteins from yeast.

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Enhancement of the ER size and function though modification of transcription control systems

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The endoplasmic reticulum (ER) is a membranous organelle in which secretory and transmembrane proteins are folded. Moreover, membrane lipids are mostly biosynthesized on the ER. We thus anticipate that it is possible to generate yeast cells with enhanced production of secretory proteins or lipidic molecules, which can be industrially beneficial, through artificial enlargement of the ER. Impairment and shortage of ER functions are collectively called ER stress and provoke the unfolded protein response (UPR), through which the size and function of the ER is upregulated. In budding yeast *Saccharomyces cerevisiae* cells, the ER-located transmembrane endoribonuclease Ire1 promotes splicing of the HAC1 mRNA when they are ER-stressed. The spliced form of the HAC1 mRNA (HAC1i) is then translated into a transcription factor protein that is responsible for the UPR. In the present study, we employed yeast cells named as HAC1i cells which did not carry the IRE1 gene but constitutively expressed the HAC1i mRNA. As expected, the UPR target genes such as those encoding ER-located molecular chaperones and lipid-biosynthesis enzymes were highly expressed in HAC1i cells, leading to enlarged and stacked ER morphology. Since the growth of HAC1i cells was very slow, which was a critical disadvantage for their industrial usage, we screened for mutations that caused fast growing of HAC1i cells. We found that dysfunction of the HDA complex, which is known to serve as a histone deacetylase, rescued the slow-growing phenotype of HAC1i cells without largely affecting their gene-expression profile. Our study thus presents an intriguing case in which molecular breeding of yeast cells can be accomplished through mutations of two different cellular systems both of which are involved in gene-expression control.

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Insight into *Yarrowia lipolytica's* molecular response to overproduction of heterologous proteins with different biochemical characteristics

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Protein maturation and delivery to various compartments are executed by a secretory pathway, spanning many compartments and involving numerous molecular identities. Multiple processes must be coordinated, e.g., chaperoning capacity, trafficking patterns, availability of resources, like amino acids, redox equivalents, ATP, membranes and glycans, to meet requirements of this metabolically demanding process. Furthermore, based on biochemical characteristics of the proteins that are synthesized at a given time, including those heterologously overproduced, the cell must fine-tune the molecular events. In the present study, we investigated cellular response of *Yarrowia lipolytica* cells challenged with high-level expression of genes encoding reporter proteins having substantially different biochemical traits: i) a small intracellular protein, ii) a small secretory protein with negligible posttranslational modifications, iii) a medium size secretory protein with a high number of disulfide bonds, iv) a medium size secretory protein with a high number of glycosylation sites. The engineered *Y. lipolytica* strains were cultivated in continuous bioreactor cultures to reach steady-state, followed by global transcriptome profiling via RNAseq. The transcriptomics analysis led to some anticipated observations on oxidative stress and unfolded protein response, glycosylation-involved genes, polypeptide folding and translocation, but also yielded many new, previously unreported for *Y. lipolytica*, observations. The conducted research greatly improved our understanding of phenomena taking place inside *Y. lipolytica* cells upon recombinant proteins overproduction. The knowledge gained from this research can be used as a guidance for *Y. lipolytica* strains improvement for more effective production of heterologous secretory proteins.

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Analysis of 3xGFP aggregation and its toxicity, and its utilization for knockdown experiments

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Cellular functions are accomplished through the coordinated action of thousands of proteins. Many proteins are folded while being synthesized as polypeptide chains by the ribosome to specific conformations and perform their functions. If a protein is not folded properly, it may interact nonspecifically with itself or other proteins and form protein aggregates. The aggregates can be toxic, causing cell dysfunction and inducing cell death. In our laboratory, we have found that 3xGFP, which consists of three enhanced green fluorescent proteins (EGFP), forms aggregates in budding yeast cells (Kintaka et al., eLife 2020). In this study, we analyzed the mechanisms of aggregation of 3xGFP and the proteins included in the aggregates.

At first, we hypothesized that 3xGFP aggregates are caused by protein misfolding and disulfide-bonds. To confirm this hypothesis, we created 3xMOX-GFP, which is linked to MOX-GFP with improved folding efficiency and examined its aggregation properties. As a result, no aggregates were formed in the cells. Also, based on the formation of 3xGFP bright spots in oxidative environments and the dissociation of aggregates in reducing environments, we concluded that the cause of aggregate formation was due to misfolding and disulfide-bonds.

Next, we investigated the mechanism of toxicity caused by aggregates by comparing 3xGFP and 3xMOX-GFP. As a result, we found that 1) the aggravated growth of proteasome mutant strain observed with the expression of aggregating 3xGFP did not occur with 3xMOX-GFP, and 2) the insoluble fraction of 3xGFP-expressing cells contained a large amount of the molecular chaperone Hsp70, but this was not found in the moxGFP-expressing cells. These results suggest that 3xGFP aggregates exert their toxic effects by taking in Hsp70, and inhibiting the function of the proteasome, which is essential for cell proliferation.

Finally, we will propose a new gene knockdown system using the aggregative property of 3xGFP.

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Physiological function of Flo11p domains and the particular role of amyloid core sequences of this adhesin in *Saccharomyces cerevisiae*

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Flocculins are a family of glycosylated proteins that provide yeast cells with several properties such as biofilm formation, flocculation, invasive growth or formation of velum. These proteins are similarly organised with a N-terminal (adhesion) domain, a stalk-like central B-domain with several repeats and a C-terminal sequence carrying a cell wall anchor site. They also contain amyloid β-aggregation-prone sequences whose functional role is still unclear.

Previous work of our team unraveled the formation of adhesive patchy structures named 'nanodomains' at the surface of a selected *Saccharomyces cerevisiae* yeast strain [1]. The presence of these nanodomains in this wine yeast grew at industrial scale was associated with an up-regulation by 11-fold of FLO11 and with higher mannan levels in its cell wall.

Therefore, this work aimed at determining the origin and the nature of the nanoscale patches formed on the cell surface of this industrial wine yeast strain, since such abundant nanostructures have never been physically observed before in Saccharomyces species. Using a genome editing approach, we further took the opportunity of this original Flo11p to investigate the contribution from each domain of Flo11p to the cell surface properties of this protein.

We show that Flo11p differs from other flocculins by the presence of unique amyloid-forming sequence, whose the number is critical in the formation of adhesion nanodomains under a physical shear force. Moreover, we elucidate that some phenotypes involve specific domains of Flo11p, whereas others depend on the entire protein and/or its abundance on the cell surface.

Session 5.2 Yeast ecology and environmental omics

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Kazachstania spp: exotic yeasts with winemaking potential?

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Two species of yeast, *Kazachstania* aerobia and *K. servazzii*, first reported in corn silage in Japan and soil in Finland, respectively, were isolated from grapes and uninoculated fermentations in Australia. These isolates were characterised for a range of attributes and stood out from other non-*Saccharomyces* identified in the study. With regard to their fermentative properties, the monocultures achieved significant attenuation of a grape juice containing 250 g/L sugar, with ethanol yields near 70 g/L. To ensure completion of such fermentations a sequential inoculation with *Saccharomyces cerevisiae*, as is commonly practiced in winemaking for other non-*Saccharomyces* strains and starter cultures, was subsequently evaluated. The successfully completed fermentations produced wines that were sensorily distinct from the *S. cerevisiae* controls. Chemical analysis attributed this to altered amounts of major fermentation metabolites and higher contents of desirable volatile aroma compounds. Such isolates therefore have the potential to become the first selected *Kazachstania* strains for application in fermented beverage production, and a response to strong market demands for products with novel sensory attributes.

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An early stage of domestication detected in the variable genome of *Torulaspora delbrueckii*

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Margarida Silva, Ana Pontes, Pedro Soares, Ricardo Franco-Duarte, Maria João Sousa, Patrícia H. Brito, José Paulo Sampaio

The yeast *Torulaspora delbrueckii* is gradually gaining recognition as an industrially important microbe. This species has been associated with winemaking for long and is frequently found in spontaneous wine fermentations. *T. delbrueckii* has several characteristics that are relevant for winemaking, especially if used in combination with *S. cerevisiae*. The most relevant oenological properties of *T. delbrueckii* are the ability to increase the sensorial complexity of wine while simultaneously contributing to ethanol production, but not to the accumulation of undesirable compounds. Here we analyzed the genomes of approximately 60 strains collected worldwide and used also other species of the genus to provide a revised delimitation of *T. delbrueckii*. Phylogenomic analyses suggested a possible separation between wild strains and strains associated with anthropic environments. The latter were consistently associated with the European region. To explore this topic further we assessed the pangenome of *T. delbrueckii*. We identified a core genome of 4151 genes and a variable genome of 1478 genes. Most of the variable genome could be traced to strains found in anthropic environments. Further evidence of genomic changes associated with domestication could be found in GAL genes, and in strains associated with dairy products, and in MAL genes in the case of bread-associated strains. A comparison of domestication in *T. delbrueckii* and in *S. cerevisiae* provided striking differences. Whereas a clear demarcation between wild and domesticated populations can be seen in *S. cerevisiae*, a much more blurred picture is emerging in *T. delbrueckii*, which we view as a case of incipient domestication.

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Directed evolution, synthetic ecology and dual compartment bioreactors: new frontiers and opportunities in multispecies interaction research

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The most ancient, and arguably well characterized example of anthropogenic domestication is provided by the wine ecosystem. It presents a relatively well characterized system with an established evolutionary history. Furthermore, *S. cerevisae* is the perfect model eukaryote for research, not only for traditional, single culture experiments, but increasingly within the context of multispecies, consortia or ecosystem -based research Understanding this ecosystem more fully is important on several fronts: The interspecies interactions therein, whether stimulatory, inhibitory, predatory or neutral, impact microbial population dynamics and perceived quality of the final product.

Here we discuss a novel approach to study cross kingdom interactions between *S. cerevisiae* and *L. plantarum* by designing an artificial, compressed evolutionary timeline with a direct genomic window to observe the evolution of cooperativity on a molecular level. Two parallel approaches are followed, both utilizing principles of directed evolution. In the first strategy, malolactic bacteria are co-evolved by constant reinoculation of a parental, driver yeast strain. Yeast evolution is thus fixed in this scenario, while bacteria are allowed to constantly adapt over successive generations.

In the second, parallel strategy, an additional constraint is provided by the incorporation of synthetic ecology principles in the co-evolution process: In this case, both yeast and bacteria are allowed to co-evolve over numerous successive generations, forced into cooperative growth by obligatory reciprocal nutrient exchange to complement the auxotrophies of each species.

We report the relevant outcomes and their fundamental implications for evolution in complex ecosystems, as well as the potential for intelligent design or regulation of multispecies consortia for use in industrial fermentation processes. Interestingly, the findings of target genes overrepresented in the SNP analyses of both data sets indicate an enrichment for key cell wall -related genes, suggesting that physical contact may play a strong role in interaction mechanisms and fermentation outcomes.

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Tracking yeast population dynamics during industrial bioethanol production using metagenomics and whole genome sequencing

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The production of bioethanol from sugarcane has been an important industrial process in Brazil since the 1970s. In the 70s and 80s numerous process improvements were made by learning-by-doing, leading to substantial increases in production capacity. However, since the 1990s several process indicators, including the ethanol yield on sugar, have remained stable. In order to improve the process further, technological advances are required. One important factor is a better understanding of the yeast strains that conduct the fermentation and their population dynamics in this non-aseptic process, which is carried out with cell recycling during a continuous period of 8 months every year. Ideally, we would understand why some strains are able to persist and eventually dominate in particular biorefineries, so that appropriate starter strains can be employed by each producer, guaranteeing high ethanol yields throughout the industrial period. In the past, chromosomal karyotyping and PCR-based methods have been used for the molecular identification of different *Saccharomyces cerevisiae* strains in industry, but these methods have important limitations. Recent advances in microbial genomics and bioinformatics now allow for investigation into the population dynamics of the strains in such systems that were not possible before. Here we present a first high-resolution description of the yeast population dynamics in two different sugarcane biorefineries across two entire industrial periods (2018 and 2019), based on metagenomic sequencing of samples taken directly from industrial fermentors, without any intermediary cultivation, PCR-amplification, or gel-dependent steps. We also isolated yeast clones from these industrial samples and sequenced their genomes to complement our analysis. Similar to a recent report on the brewery environment (Large et al, bioRxiv 2020.06.26.166157; doi: https://doi.org/10.1101/2020.06.26.166157), our study represents a rare investigation of a natural evolution experiment and provides insight into the population dynamics of different yeast strains.

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Wasps as a model to study yeast evolution

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The study of host-microbe interaction in social wasps has had strong implications in understanding the wasp's behavioral dynamics and yeast ecology and evolution. In the last years the ecological relevance of social wasps in the survival, reproduction and dispersion of *Saccharomycetes*, has been explored. The intestinal tract of social wasps represents a natural reservoir where yeasts belonging to the genus *Saccharomyces* can survive and mate with a greater fitness for interspecific hybrids, thus providing an environment that enhance the fungal biodiversity. These ecological aspects make the social wasps, such as the hornets, an ideal vector able of inoculating yeasts in the ripe grape berries, by biting them to obtain sugars, consequently inducing the fermentation of the grape must. Despite the knowledge acquired so far, it is not actually clear how the environment influences the hornet's microbial communities, an important question remaining is to deeply explore the communication between the environment and these social wasps. We described the effects of different environmental stimuli, inside the vineyard framework, on the intestinal bacterial and fungal communities of the European hornet *Vespa crabro* and social bee *Apis mellifera*, by using targeted-metagenomics approach. The environmental stimuli produced a discrete clustering of the amplicon sequence variants in both insect species datasets according to the environmental pattern considered, with a greater effect on the fungal taxonomic units than the bacterial ones. Interestingly, the hornets sampled at the end of August, after having ripe grapes available for their foraging activities, were significantly enriched in *Saccharomycetes*, compared to the hornets sampled in the same area around 50 days later. This work corroborates the central role of social wasps in the ecology of yeasts, in particular the *Saccharomycetes* class, highlighting their role as ecosystem services in natural environments and their use as a study model in the yeast-host interaction context.

Session 7.3 Yeasts in aging research

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The cell biology of quiescent yeast

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Unlike cell proliferation, cellular quiescence is often ill defined, considered as an inactive default state, and its biological relevance poorly acknowledged. However, an increasing amount of literature reveals that quiescence is central in many biological processes including major human diseases. In fact, quiescence is captivating, as it is diverse, multifaceted, and far from being a “sleeping” cellular state.
In the lab, we are studying quiescence at the individual cell level using *Saccharomyces cerevisiae* for 15 years. We have found that not only many cellular machineries, such as the actin cytoskeleton, microtubules and the proteasome, but also organelles, such as the nucleus and the mitochondria, are drastically remodeled upon quiescence establishment. I will present an overview of these reorganizations and discuss their conservation among species.

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Yeast-based system for the rapid screening of non-toxic substances causing a mild de-energization of the cells

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Chemicals inducing a mild decrease in the cellular ATP/ADP ratio are considered as caloric restriction mimetics as well as treatments against obesity. To our knowledge, all of the well-studied substances of such kind display negative side-effects. Screening for such non-toxic de-energizers in animal model systems requires a lot of time and labor. We have developed yeast-based system for the rapid screening of non-toxic substances decreasing intracellular ATP level. We reasoned that chemicals allowing the growth of yeast lacking trehalose phosphate synthase, tps1-delta mutant strain, on a non-fermentable carbon source in the presence of glucose could be the non-toxic de-energizers. Indeed, tps1-delta cells stop growing upon glucose addition because the cellular phosphate gets re-directed to phosphorylate the sugars in upper glycolysis, while the biosynthesis of bisphosphoglycerate becomes blocked. We reasoned that chemicals decreasing the ATP/ADP ratio could prevent the phosphorylation of the sugars and also boost bisphosphoglycerate synthesis by providing the substrate, inorganic phosphate. We confirmed that a complete inhibition of oxidative phosphorylation alleviates the block of glycolysis. As our system includes a non-fermentable carbon source, only the chemicals that did not cause a complete inhibition of mitochondrial ATP synthesis allowed the initial depletion of glucose followed by respiratory growth. Using this system, we found that a particular type of mitochondrial uncouplers, lipophilic cations which possess a mild membrane-depolarizing activity, is especially efficient in the growth rescue. We have shown that two novel lipophylic cations of this type, dodecylmethyl diphenylamine (FS1) and diethyl (tetradecyl) phenyl ammonium bromide (Kor105) also do rescue the growth.

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Reduction in autophagy affects maintenance energy expenditure and survival in yeast

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Maintenance of cellular homeostasis is of vital importance for healthy aging. To fuel the processes underlying this maintenance, energy is needed, the so-called maintenance energy requirement. Among maintenance processes, autophagy plays a crucial role as it is involved in the turn-over and recycling of damaged cellular material, such as organelles or proteins. The contribution of autophagy to the maintenance energy requirement is however unknown. Taking advantage of the high degree of conservation of autophagy between humans and *Saccharomyces cerevisiae*, here this yeast is used as a model organism to study the impact of autophagy on the maintenance energy requirement. For this we combined the GFP-Atg8 cleavage assay with an advanced bioreactor cultivation set-up: yeast retentostat cultures. Using this approach, we show that autophagy is also highly active in chronologically aging, non-dividing, yet non-starved yeast cells. Deletion of the kinase ATG1, involved in macroautophagy, resulted in a 60% increase in the maintenance energy requirement, as well as a doubling of the specific death rate. Concomitantly, we observed a loss of respiratory capacity, this does however not fully explain the increases in maintenance requirement and specific death rate. Intriguingly, loss of Atg1 did not result in complete abolishment of GFP-Atg8 cleavage under these conditions and other forms of autophagy might hence be active. Overall, this illustrates the importance of autophagy on the energetics of aging cells and we present an alternative system for the widely-applied stationary phase cultures in yeast chronological aging studies.

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Metabolite aggregation: from basic biology to drug discovery

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The paramount role metabolites play across different branches of life, as well as their part in various disorders, has been investigated for decades. Yet, only recently it has been revealed by our group that some of these life-essential building blocks, including several amino acids and nucleobases, can form cytotoxic amyloid-like structures, similar to those associated with neurodegenerative disorders such as Alzheimer's disease. This novel paradigm offers new therapeutic and diagnostic opportunities for genetic metabolic disorders that are characterized by the accumulation of certain metabolites.
The power of yeast biology has been successfully applied for studying protein misfolding diseases and high-throughput drug screening. We have recently established the first *in vivo* yeast model of adenine self-assembly using a strain blocked in the enzymatic pathway downstream to adenine, which resulted in robust sensitivity to the metabolite. The validity of the system to study metabolite self-assembly was confirmed by staining with amyloid-specific dye and antibodies raised against the adenine assemblies. The addition of generic amyloid inhibitors rescued the toxicity and decreased staining thus validating its potential to serve as a drug screening platform. This powerful system is now used to gain insights into the cellular mechanisms underlying human pathologies and identify potential therapeutic leads. Finally, it could elucidate the metabolite homeostasis mechanisms ("metabolostasis") that maintain metabolites in a non-aggregated state under physiological conditions.

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Inactivation of the DNA damage response rescues organismal fitness in telomere-humanized yeast

Ms. Melania Jennifer D'Angiolo[1], Mr. Benjamin Barré[1], Mr. Eric Gilson[1], Mr. Gianni Liti[1]
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Telomeres are ribonucleoproteins that cap chromosome-ends and impede the activation of DNA-damage-response (DDR). Their length is maintained by telomerase using a RNA template. Telomeric sequences are conserved across the tree of life and consist of G-rich repeated units, but variation in length and sequence is present among distant taxa. Each species is likely fine-tuned for its own telomere properties, however their quantitative contribution to fitness remains largely unexplored. In the budding yeast *S. cerevisiae*, the telomerase RNA template is encoded by TLC1 and carries degenerated TG1-3 repeats. Previous studies showed that a 16-bp editing of TLC1 enables to reconfigure telomeres and generate yeasts with newly synthetised exact T2AG3 repeats, which show an intrinsic telomere dysfunction and a chronic activation of DDR. Since T2AG3 corresponds to the telomeric repeat found in humans (as well as many other organisms), these telomere-engineered yeasts were dubbed humanized yeasts. In this work, we evolved multiple lines of humanized and wild-type yeasts to characterize the effect of telomere variation on fitness. We sequentially combined two experimental evolution paradigms: first, we evolved cells through mutation accumulation lines (MALs) to minimize selection, to investigate the cellular and genomic effects during the fitness decay driven by telomere dysfunction. Next, we submitted MALs to adaptive evolution by multiple serial transfers (STs) of large population sizes, to map mutations that counteract the fitness decline. During MALs, humanized yeasts gradually slowed their growth and shortened chronological lifespan. Whole-genome-sequencing revealed that they had increased mutation rate and genome instability, with recurrent aneuploidies on chromosome XVI. After multiple STs, most humanized lines recovered a wild-type fitness, with independent occurrence of mutations in the MRX complex, a key effector of DDR. Overall, our results show that humanized telomeres increase mutation rate and cause a severe fitness decline which is rescued by the inactivation of DDR.

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Natural quercetin analogues modulate oxidative stress tolerance and increase lifespan extension of *S. cerevisiae*

Ms. Hira Zahoor[1], Ms. Kwanrutai Watchaputi[1], Ms. Janejira Hata[2], Mr. Apichart Suksamran[2], Ms. Nitnipa Soontorngun[1]

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Aging is a conserved phenomenon between yeast and human, making yeast a simple but powerful tool to examine anti-aging potential of bioactive substances. Here, flavonoid analogues of quercetin were examined using a model yeast *Saccharomyces cerevisiae* to uncover their antioxidant and anti-ageing properties and establish the potential connection to cellular adaptation to oxidative stress. Some quercetin analogues protected yeast cells against oxidative stress, induced by acetic acid or hydrogen peroxide, as shown by decreased cell sensitivity and reduction of intracellular reactive oxygen species. Structural differences of analogues suggested the importance of hydroxyl groups on the tested flavonoid molecules for the antioxidant activity. Using chronological assay, quercetin, morin and steppogenin could extend lifespan of wild-type *S. cerevisiae* by 15-25% as compared to the untreated cells. As oxidative stress is a key factor to ageing, acetic acid resistance was found to be associated with increased expression of a key growth signaling kinase TOR1 and two stress-responsive transcription factors MSN2/4. Addition of antioxidant morin could reduce their expression, suggesting a possible modulatory role of morin in controlling cell signaling and stress response. Therefore, yeast proves to be a versatile model organism to discover potentially rejuvenescent biomolecules from nature and gain insights into cross-talks between ageing and stress signaling pathways.

Session 8.2 Organelle dynamics and intracellular trafficking

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Actin-mediated endocytosis in budding yeast: regulation and force production mechanism

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Yeast cells assemble cortical actin patches to generate forces for production of endocytic vesicles. The pathway for clathrin-mediated endocytosis in budding yeast is mediated by some 50 proteins that are recruited to endocytic sites in a predictable order and with predictable timing. Studies will be described that investigate how actin assembly generates forces for endocytosis and how progress through this pathway is regulated. In a biochemical reconstitution system, actin assembles on supported lipid bilayers and pulls vesicles off the membrane. In live cell studies, progress through the endocytic pathway is dictated by endocytic cargo, which explains differences in assembly and dynamics of endocytic sites in buds versus mother cells. These observations have triggered a search for the proteins that regulate progress through the endocytic pathway.

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A biotinylation toolkit for high-throughput protein-protein interaction discovery

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Discovering functions for poorly characterized proteins remains the holy grail of cellular biology. A powerful strategy to shed light onto protein function is to uncover with whom a protein interacts, or its protein-protein interactions (PPIs). Immunoprecipitation (IP) followed by mass spectrometry (MS) is the gold standard in elucidating PPIs. However, IP-MS techniques mostly capture stable, long-lived interactions, such as those found between members of protein complexes. On the other hand, transient and dynamic interactions, like those between enzymes and substrates, are inherently unlikely to be captured by IP-MS, yet can be highly revealing to a protein's function or its substrate range.

One potential method to capture transient interactions is by use of proximity labeling enzymes, which conjugate a 'label' onto proteins in their vicinity. BioID and TurboID are two such enzymes engineered from the original biotin ligase, BirA. Unlike BirA, which can only biotinylate a specific peptide, these two 'promiscuous' ligases conjugate biotin onto any proximal lysine residue. Biotinylated proteins can subsequently be captured by streptavidin affinity-purification and identified by MS. While these methods are highly useful, they have mostly been used in small-scale, low-throughput experiments due to lack of systematic collections of proteins fused with these enzymes.

To propel the use of these tools to genome-wide experiments we constructed a yeast 'biotinylation toolkit' for high-throughput PPI discovery. Our toolkit includes four whole-genome libraries in which each *S. cerevisiae* protein is N-terminally tagged with either BirA, BioID or TurboID (in the latter case, with or without the capacity to downregulate the endogenous yeast biotin ligase Bpl1) as well as optimal protocols for their use. To allow for comparison between stable and transient interactions, an HA-tag for classical IP-MS is included in both BioID and TurboID libraries. I will present how these libraries can be used for functional discovery.

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Effects of DNA circle accumulation during yeast ageing

Mr. Yves Barral[1]

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Over the years budding yeast has turned in a powerful system for studying the ageing process at the cellular level. At the same time, since the ageing yeast mother cell generates young daughter cells, budding yeast emerged as a key model system for studying the mechanisms of cellular rejuvenation. Recently, our laboratory has addressed the role of lateral diffusion barriers that forms in the ER membranes at the mother-bud neck in the retention of ageing factors in the mother cell and the resetting of age in the daughter cells. These studies support the idea that there are two main types of ageing factors accumulating in yeast mother cells: protein aggregates and DNA circles emanating essentially by homologous recombination from the rDNA locus. In the last few years, we have focused on deciphering how DNA circles factors and the mechanisms by which they cause the fitness decay and the eventual death of ageing cells. Interestingly, we found that attachment of the DNA circles to nuclear pore complexes (NPCs), which is mediated by the SAGA complex and promotes the retention of the circles in the mother cell, causes the acetylation of the nucleoporin Nup60 and dissociation of the nuclear basket from NPCs. This in turns also promotes the dissociation of the cytoplasmic fibrils of NPCs. Preventing acetylation of Nup60 restores fibril stability and slows down aging. Together, our data indicate that NPC remodeling with age is due to regulatory events taking place upon DNA circle binding to NPCs and that this in turn affects specific aspects of the nucleo-cytoplasmic exchanges. We suggest that these ubiquitous ageing events contribute to the propagation of ageing from the nucleus to the cytoplasm in many eukaryotes.

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Peroxisomal membrane contact sites in the yeast *Hansenula polymorpha*

Ms. Ida van der Klei[1]

[1]*University of Groningen, Groningen, Netherlands*

Peroxisomes are cell organelles that play crucial roles in the function of eukaryotic cells. Their physiological role depends on the enzymes they contain. Common peroxisomal functions include fatty acid metabolism and hydrogen peroxide degradation.

Yeast are ideal model organisms to study peroxisomes. The proliferation and degradation of these organelles can easily be manipulated by changing growth conditions. Also, mutations that prevent normal peroxisome formation are not lethal in yeast.

For long it was thought that cell organelles function independent from other cellular compartments. However, recent studies showed that for the proper formation and function of organelles specific strong physical interactions with other organelles are required.

We used the yeast *Hansenula polymorpha* to study peroxisomal contact sites. We showed that peroxisomes form tight physical contacts with various membranes including mitochondria, the endoplasmic reticulum, the vacuole and plasma-membrane. The composition and function of these contacts will be discussed.

Fermenting futures: Exploring yeast biotechnology through an artistic lense

Art Showcase Event

Fermenting Futures: Exploring Yeast Biotechnology Through an Artistic Lens

We invite you to join us for the virtual launch of “Fermenting Futures” a new body of artworks developed for ICY 15 Meets 30 ICYGMB by internationally renowned artists Anna Dumitriu and Alex May. “Fermenting Futures” explores the significance of yeast biotechnology from a cultural, ethical and aesthetic perspective, engaging diverse audiences in the history and future of the field and its potential to offer environmental solutions.

In this event you can learn about the results of this fascinating art and science collaboration and the next steps, as well as hearing short talks from experts from both fields giving the historic and scientific background to the work and reflecting on its artistic impact.

The project builds on two research projects in the Institute of Microbiology and Microbial Biotechnology of the University of Natural Resources and Life Sciences in Vienna, which use genetic modification techniques and directed evolution. Created in collaboration with Professor Diethard Mattanovich, Professor Michael Sauer, Dr. Özge Ata and Dr. Martin Altvater at the Institute of Microbiology and Microbial Biotechnology of the University of Natural Resources and Life Sciences Vienna, Austria.

Schedule

August 24, 2021. All times in CEST

17:45 – 17:50 Introductions and background – Diethard Mattanovich

17:50 – 18:10 Anna Dumitriu and Alex May: Presentation of Fermenting Futures: Yeast BioArt Residency at BOKU and ACIB. Artists talk and a screening of a film of the installation

18:10 – 18:25 Florian Schneider: Developing the taste of modernity: breakthroughs in yeast technology in 19th century Vienna

18:25 – 18:35 Özge Ata: What makes yeast ferment? On evolution of fermentative metabolism, and synthetic biology to reroute the yeast metabolism

18:35 – 18:45 Martin Altvater: Changing yeast fermentation from alcohol to chemicals: how to make lactic acid and PLA with yeast

18:45 – 19:00 Ingeborg Reichle: Art in the Age of Environmental Crisis: Framing "Fermenting Futures" within Ecocritical Art History

19:00 – 19:15 Heike Sütter: Curatorial thoughts on Fermenting Futures: A Forthcoming Exhibition in Wiesbaden and an Art Historical Seminar at Goethe University

19:15 – 19:45 Q & A and Discussion Session
Chaired by Irini Papadimitriou, with Özge Ata, Anna Dumitriu, Wolfgang Giegler, Diethard Mattanovich, Alex May, Ingeborg Reichle, Sonja Schachinger, Florian Schneider, Heike Sütter.

Session 1.3 Yeast system biology

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The genomic making of yeast metabolic diversity

Mr. Chris Todd Hittinger[1]

[1]*University of Wisconsin-Madison, Madison, USA*

Over the last 400 million years, budding yeasts of the subphylum S*accharomycotina* have radiated to fill diverse niches in every continent and biome on the planet. By sequencing the genomes of and collecting high-throughput metabolic data for nearly all known species, the Y1000+ Project (http://y1000plus.org) has generated a draft Genotype-Phenotype Map of yeast biodiversity. Although many gene-trait correlations correspond well with mechanistic data from model systems, several gaps and discrepancies in the map suggest novel biology. The dataset is also rich in trait-trait correlations suggestive of ecological trait syndromes and gene-gene correlations that can be used to predict pathway membership. Many traits have been gained or lost multiple times during evolution, whereas other traits have only evolved rarely. Here, I will present several project highlights and illuminating examples of budding yeast genomic and metabolic evolution, as well as discuss prospects for filling in the map.

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Understanding a life – integration of time-resolved population and single cell data for a yeast cell from birth to division

Mrs. Edda Klipp[1]

[1]*Humboldt-Universität zu Berlin, Berlin, Germany*

With the progress of genome-wide experimental approaches we witness the establishment of more and more libraries of genome-wide data for proteins or RNA or metabolites, especially for specific cell lines or for well-studied model organisms such as *S. cerevisiae*. However, the separated consideration of metabolic networks or gene regulation networks does not tell us how these networks are integrated to allow a cell to grow, divide and respond to changing environments.

We use the yeast *Saccharomyces cerevisiae* as the model organism for eukaryotic cells allowing to comprehensively analyze regulatory networks and their integration with cellular physiology. We focus on processes during the lifetime of a single cell along one period of the cell division cycle and study the changes of metabolism, gene expression, or ion and nutrient transport during the growth of that cell.

We use a modular and iterative approach that allows for a systematic integration of cellular functions into a comprehensive model allowing to connect processes that are strongly interlinked in cellular life but measured separately. The modular concept also permits to zoom in and out if different aspects of regulation or dynamics come into focus.

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Energy efficiency and its implications on metabolic modes in yeast – a quantitative systems biology approach

Mr. Rahul Kumar, Ms. Nemailla Bonturi, **Mr. Petri-Jaan Lahtvee**
[1]Tallinn University of Technology, Tallinn, Estonia

Cells require energy in the form of ATP to sustain growth and proliferation. How metabolism regenerates ATP is impacted by the available resources and defines the performance of the host. Here, we investigated metabolism's dependency on nutrients, growth and stress factors in *Saccharomyces cerevisiae* using quantitative proteomics, phosphoproteomics, protein degradation rates and genome-scale metabolic modeling. We found carbon overflow to be growth independent but dependent on increased glucose utilization, which reduced proteome quantity in biomass. Proteome allocation to ribosomes was determinative for growth but not the metabolic modes, which differently generated ATP. Proteome abundance enabled more mitochondrion, a respiration mode requiring less NAD+ per ATP. However, proteome limitation induced resource allocation trade-off between ribosomes and mitochondrion, affecting ATP yields. With a systematic, large-scale study of protein degradation rates, we demonstrate how protein degradation is dependent on energy generation mode and hence have a significant effect on cellular energetics and the onset of overflow metabolism. Combined with the possibilities of synthetic biology to alter cellular metabolism, these findings have implications in bioprocess development but also in the fundamental understanding of metabolic diseases like cancer.

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A novel yeast hybrid modeling framework integrating Boolean and enzyme-constrained networks enables exploration of the interplay between signaling and metabolism

Ms. Linnea Österberg[1], Mr Iván Domenzain[1], Julia Münch[1], Jens Nielsen[1,2], Stefan Hohmann[1], Marija Cvijovic[1]
[1]Chalmers University of Technology, Gothenburg, Sweden, [2]BioInnovation Institute, Copenhagen, Denmark

The interplay between nutrient-induced signaling and metabolism plays an important role in maintaining homeostasis and its malfunction has been implicated in many different human diseases such as obesity, type 2 diabetes, cancer, and neurological disorders. Therefore, unraveling the role of nutrients as signaling molecules and metabolites together with their interconnectivity may provide a deeper understanding of how these conditions occur. Both signaling and metabolism have been extensively studied using various systems biology approaches. However, they are mainly studied individually and in addition, current models lack both the complexity of the dynamics and the effects of the crosstalk in the signaling system. To gain a better understanding of the interconnectivity between nutrient signaling and metabolism in yeast cells, we developed a hybrid model, combining a Boolean module, describing the main pathways of glucose and nitrogen signaling, and an enzyme-constrained model accounting for the central carbon metabolism of *Saccharomyces cerevisiae*, using a regulatory network as a link. The resulting hybrid model was able to capture a diverse utilization of isoenzymes and to our knowledge outperforms constraint-based models in the prediction of individual enzymes for both respiratory and mixed metabolism. The model showed that during fermentation, enzyme utilization has a major contribution in governing protein allocation, while in low glucose conditions robustness and control are prioritized. In addition, the model was capable of reproducing the regulatory effects that are associated with the Crabtree effect and glucose repression, as well as regulatory effects associated with lifespan increase during caloric restriction. Overall, we show that our hybrid model provides a comprehensive framework for the study of the non-trivial effects of the interplay between signaling and metabolism, suggesting connections between the Snf1 signaling pathways and processes that have been related to chronological lifespan of yeast cells.

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Systematic mapping of natural variants driving genetic context-dependent gene essentiality

Maykel Lopes[1], Jonas Barraud[1], Michael Wiederkehr[1], **Jolanda Van Leeuwen[1]**
[1]University Of Lausanne, Lausanne, Switzerland

Mutations often show phenotypic differences across genetically distinct individuals. In the most extreme case, a gene can be essential in one genetic background but have no effect on viability in another. Here, we investigated the frequency and underlying causes of differences in gene essentiality by examining 20 genetically diverse yeast strains. First, we generated a collection of 755 haploid query strains in the laboratory background S288c, each deleted for an essential gene, but viable because of the presence of the gene on a plasmid. We crossed this collection to a diverse set of wild yeast strains from various sources and tested for survival of haploid segregant progeny in the absence of the essential genes. We observed viable progeny in at least one cross for 30 essential genes (4%), suggesting that the wild strain contained variants that could bypass the requirement for the essential gene. Although some genes appeared to be essential in S288c only, the vast majority of genes were nonessential in only 1 or 2 genetic backgrounds. We did not observe a correlation between the genetic distance between strains and the number or identity of context-dependent essential genes. Finally, we are identifying and validating causal bypass suppressor variants using bulk segregant analysis and allele replacements. Ultimately, we aim to learn the general mechanisms that drive conditional essentiality and the frequency at which they occur during evolution. Understanding how the genetic background of an individual can affect the phenotype of a mutation of interest will provide insight on how genetic variance accumulates during evolution and affects genetic traits.

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Prediction of metabolic strategies in *Schizosaccharomyces pombe* based on optimal resource allocation

Mr. Pranas Grigaitis[1], Mrs. Eunice van Pelt-KleinJan[1], Mr. Douwe Grundel[1], Mr. Bas Teusink[1], Mr. Johan van Heerden[1]

[1]*Systems Biology Lab, Amsterdam Institute of Molecular and Life Sciences (AIMMS), Vrije Universiteit Amsterdam, Amsterdam, Netherlands*

Genome-scale metabolic models are computable knowledge bases containing information of all reactions that could take place in an organism, making them a powerful platform to investigate the metabolic potential of organisms. Scenarios of optimal resource allocation among cellular processes (such as overflow metabolism) can be captured in so-called proteome constrained (pc-) models by coupling metabolic reactions to expression and turnover of proteins, competing for limited biosynthetic resources and proteome space. Over the years, a well-curated genome-scale metabolic model has been developed for the popular model organism, the budding yeast, *Saccharomyces cerevisiae*. Together with the recently presented pcYeast model, this makes studies of its metabolism and resource allocation possible in silico and at genome-scale. However, few computational tools are available that facilitate the understanding of the physiology and resource allocation strategies of other yeast species.

Here we present the proteome-constrained model of the fission yeast *Schizosaccharomyces pombe*, pcPombe. As a working metabolic model was lacking, we first constructed a genome-scale model of *S. pombe* (pomGEM) by using the latest consensus genome-scale metabolic model of *S. cerevisiae* as a template. On the basis of pomGEM we developed pcPombe, and simulated glucose-limited growth across a range of growth rates, comparing the predicted fluxes to experimental measurements. Model predictions matched experimental data well, and for every simulation we identified active compartment-specific proteome constraints. In the model, different combinations of these constraints were limiting growth as the growth rate increased with increased glucose availability. Notably, the model suggests that the maximal protein capacity of mitochondria becomes limiting at μ>0.15/h, resulting in the initiation of fermentation, and the formation of ethanol that is experimentally observed. This illustrates how pc-models, in casu pcPombe, can be used to investigate the metabolic strategies of different species of yeasts.

Session 2.3 Engineering yeast for superior recombinant protein production

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Tools for construction of superior cell-factory hosts in genetically diverse yeast species

Mr. Uffe Mortensen[1], Ms. Zofia Jarczynska[1], Mr. Adrian Lopez[1], Mr. Niklas Andersen[1], Mr. Sebastian Hansen[1], Mr. José Ruiz[1], Mr. Tomas Strucko[1]

[1]*Technical University of Denmark, Lyngby, Denmark*

Yeasts have been widely used for construction of novel cell factories that can convert renewable biomass into valuable products ranging from biofuels to pharmaceuticals. The vast majority of proof-of-concept studies are based on bakers yeast *Saccharomyces cerevisiae* where an extensive genetic toolbox is in place. However, initial yields are often low often reflecting that the metabolism and cellular infrastructure of this yeast is not naturally geared for production of the new product and substantial genetic optimization is therefore required to increase the yields to levels that can bring the process to market. We envision that a faster approach towards market can be achieved by introducing a process into a range of diverse yeast species and then identify the best producer for further optimization. A bottleneck in this strategy is the lack of tools that allows genes to be easily integrated into the new hosts and tools that allow for subsequent genetic optimization.

We are currently developing a gene expression platform, DIVERSIFY, that facilitates quick and easy screening for production capabilities of any compound of interest in evolutionary distant yeast species. In DIVERSIFY, strains harbor a common multi-functional gene-targeting cassette in their genomes. The cassette contains standard gene-targeting sequences interspaced with I-SceI restriction site, dominant marker and chromogenic marker that enables easy screening for correct recombination. In addition, DNA double strand brake can be induced by the I-SceI meganuclase or by a CRISPR nuclease to promote gene targeting. To facilitate further engineering of DIVERSIFY strains, we develop genetic tools in yeasts that are not easy to manipulate. Here we present new CRISPR tools that allows robust introduction of gene expression cassettes, gene deletions and point mutations into the genome of the methylotrophic yeast *Komagataella phaffii* and the osmotolerant yeast *Debaryomyces hansenii*.

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Hydrolase expression in CBP yeast strains for production of ethanol from cellulosic and starchy feedstocks

Mr. Willem H. (Emile) van Zyl[1], Ms. Rosemary A. Cripwell[1], Mr. Riaan Den Haan[2], Ms. Shaunita H. Rose[1], Ms. Trudy Jansen[1], Ms. Marinda Viljoen-Bloom[1]

[1]University of Stellenbosch, Stellenbosch, South Africa, [2]University of Western Cape, Western Cape, South Africa

The yeast *Saccharomyces cerevisiae* has been used successfully as a cell factory to produce biofuels, green chemicals, and food products. When considering abundant polysaccharides such as starch and cellulose as feedstocks, the major limitation of *S. cerevisiae* is its inability to degrade these polymers to fermentable hexoses. Most studies consider the enzymatic hydrolysis of starch and cellulose and subsequent fermentation of the resulting sugar streams in separate steps for the production of bio-based products by *S. cerevisiae*. We are exploring an alternative consolidated bioprocessing (CBP) configuration using the single-step conversion of polysaccharides from cellulosic or starchy biomass to bioethanol.

The proof of concept for the one-step conversion of cellulosics and starch to ethanol has been demonstrated in our laboratory through the successful expression of the major cellulolytic and amylolytic activities in *S. cerevisiae*. Efficient hydrolysis of cellulose at minimum enzyme loadings can only be achieved through the synergistic actions of different cellulolytic activities produced by recombinant strains. We have shown that the production of different cellulases by *S. cerevisiae* can be enhanced by overexpressing native genes encoding for proteins that are involved in the secretion pathway or stress-relief response in yeast. Realizing that CBP yeasts for cellulosic conversion have to deal with additional stresses, such as inhibitor and temperature tolerances, we further explored native *S. cerevisiae* strains with superior phenotypes and their amenability as potential CBP hosts. Recent studies evaluating industrial amylolytic yeast strains revealed the potential negative impact of multiple recombinant genes on ethanol production. These findings emphasize the necessity to fine-tune gene expression to enable the CBP of raw starch utilization with little to no exogenous enzyme supplementation.

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Converting the cooking ingredient *Saccharomyces cerevisiae* into a next-generation therapeutic protein production system

Ms Mari Piirainen[1], Ms Laura Niemelä[1], **Mr. Alexander Frey[1]**
[1]Aalto University, Espoo, Finland

Baker's yeast is a cost-effective, low-complexity and safe production organism for therapeutic proteins. In the talk I will present how the evolutionary distant antibody producing plasma cells can be utilized as blueprint to redesign yeast for recombinant protein production. After introducing the general approach and its outcome, two aspects will be discussed in more details. (1) The Endoplasmic reticulum (ER) can be divided into two structurally distinct domains, the cisternal ER and the tubular ER network. The presentation will highlight how the size and morphology of the ER can be engineered to improve the protein folding process. The discussed approach is of broader interests as the molecular machinery controlling ER structure is highly conserved. (2) Moreover, the talk will discuss our approach to humanize N-glycosylation pathway. As antibodies are glycosylated proteins and these modifications are species specific, we developed over the last years an essential cell engineering technology that enables the production of antibodies with human-like protein modifications in yeast. Here the talk will highlight our latest results from a case study aiming at the production of a SARS-CoV-2 neutralizing antibody.

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Yarrowia lipolytica for the production of recombinant proteins: promoters, strains and processes.

Mr. Patrick Fickers[1], Mrs Young-Kyong Park[2], Mr Jean-Marc Nicaud[2], Mr Jin-Hua Mou[3], Mrs Carol Sze Ki Lin[3], **Ms. Imen Ben Tahar[1]**

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From erythritol metabolism, new efficient promoters, vectors and recipient strains have been developed recently for the production of recombinant proteins in *Y. lipolytica*. These synthetic promoters that derived from the native promoter of gene EYK1 encoding erythritol kinase may be used as constitutive or regulated depending on the recipient strain considered (i.e., wild-type or Δ*eyk1*). Using reporter proteins, they were found as efficient as the widely used pTEF and pLIP2 promoters. When the lipase CalB from *Candida antarctica* was used as a case study, lipase production titer was five-fold higher in process conditions as compared to that obtained with *Pichia pastoris* using pAOX1 expression-based system. Moreover, for *Y. lipolytica* the maximal lipase production titer was obtained in half the cultivation time. Finally, an innovative cell immobilisation system, namely in situ fibrous bed bioreactor (isFBB) was applied. As compared to both batch and fed-batch bioreactor processes, the lipase productivity (142 U/(mL.h)) in isFBB was 1.4-fold and 2.1-fold higher, respectively. This demonstrated that isFBB is an efficient technology for the production of recombinant enzymes in *Y. lipolytica*.

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Going beyond the limit: impact of increasing global translation activity on the productivity of recombinant secreted proteins in *Pichia pastoris*

Ms. Jennifer Staudacher[1,2], Ms. Corinna Rebnegger[1,2], Mr. Diethard Mattanovich[2], Ms. Brigitte Gasser[1,2]

[1]Christian Doppler Laboratory for Growth-decoupled Protein Production in Yeast, Vienna, Austria, [2]Department of Biotechnology, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria

Yeasts, especially *Pichia pastoris* (syn. *Komagataella phaffii*), are widely used organisms for commercial, heterologous protein production. *P. pastoris* is known for its high secretory efficiency and biomass yield, however specific productivities are low and tightly coupled to the growth rate. This highly impacts production processes, which are commonly not run on the maximum growth rate, thereby resulting in suboptimal productivities. To solve this, we evaluated *P. pastoris* microarray datasets. These showed a clear downregulation of protein translation related genes with decreasing growth rates, thus making modulating the translation machinery our focus.

By overexpression of selected differentially expressed translation factors, we could show a rate-limiting step to be translation initiation. Specifically, overexpression of factors associated with the closed-loop conformation, a structure that increases stability and rates of translation initiation before start codon scanning is initiated, showed the strongest effects. These increased titers of different heterologous proteins by up to 3-fold in fed-batch processes. Furthermore, global translation activity and total protein content were higher in the engineered cells. Translation factor overexpression therefore has a global effect on the cell. This work displays not only the interconnection of different protein synthesis steps but also the capacity *P. pastoris* has for protein production, and indicates that this host organism is not at its limit yet.

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Optimizing recombinant protein production by removal of a negative feedback mechanism induced by oxidative stress.

Ms. Veronica Gast[1], Mr. Mikael Molin[1], Ms. Verena Siewers[1]
[1]*Chalmers University of Technology, Göteborg, Sweden*

Many different yeast species are currently being used for the production of (pharmaceutical) proteins in industry. For a production process to be economically feasible, a major factor is the titer of product in the media. Therefore, an interesting research topic is to optimize protein production and secretion. When optimizing protein production most research is dedicated towards the secretory pathway.

In our research we decided to look at another part of the cell to increase the production and secretion of heterologous proteins. High protein production itself can be a burden and lead to oxidative stress in yeast. Besides the metabolic costs associated with oxidative stress we hypothesized the induction of a negative feedback loop that reduces translation and therefore protein production. To test our theory, we removed the kinase Gcn2, which would be at the center of this potential negative feedback loop. Upon removal of the kinase Gcn2 we observe a doubling of recombinant amylase titer, improved biomass yield and increased specific growth rate.

We studied the several underlying mechanisms of this feedback loop and found several surprising results. Our experiment indicates that upon removal of the kinase the intracellular oxidant level (H_2O_2) was reduced even though the protein production was increased. Alongside that observation we saw that there is increased expression of several oxidant scavengers and PDI1, a major foldase from the ER. Our data suggest that the kinase Gcn2 not only reduces translation but also the ability of yeast to adjust and manage the oxidative stress induced by recombinant protein production.

Session 5.3 Genome plasticity and complex genetic traits

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Saccharomyces Genome Resequencing Projects (SGRPs): the first decade

Mr. Gianni Liti[1]

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An understanding of natural variation is crucial to efforts in current biology and to decipher the dynamics of genome evolution. The budding yeast, *Saccharomyces cerevisiae*, has emerged as a leading system for population genomics studies due to its small, well-characterized genome and experimental tractability. In the past decade, we assembled a large collection of natural isolates of *S. cerevisiae* and its closest relative *S. paradoxus* strains and characterized them at the genomic and phenotypic levels. We applied different sequencing and computational approaches to investigate origin, evolution, secondary contacts, and domestication of the species. These data provide a comprehensive view of genomic diversity in budding yeast and expose pronounced population-level differences.

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Why is the yeast lag phase so long?

Mr. Kevin Verstrepen[1,2], Ms. Lieselotte Vermeersch[1,2], Mr. Lloyd Cool[1,2], Mr. Anton Gorkovskiy[1,2], Mr. Bram Cerulus[1,2], Ms. Gemma Perez-Samper[1,2], Mr. Abbas Jariani[1,2], Ms. Supinya Piampongsant[1,2]
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When faced with environmental changes, microbial cells enter a lag phase during which the cell cycle is arrested while cells adapt to the new situation. The discovery of this lag phase started the field of gene regulation, and ultimately led to the unraveling of underlying signaling pathways -and a Nobel Prize. Surprisingly, however, the factors that determine the exact duration of the lag phase remain largely elusive.
Naively, one would expect that cells adapt as quickly as possible. However, we show that the lag phase can last from several hours up to several days. Moreover, some cells within the same population take much longer than others, despite being genetically identical. In addition, the duration of the lag phase is also influenced by the past, with recent exposure to a given environment leading to a quicker adaptation when that environment returns. In contrast to what is generally assumed, genome-wide screens suggest that the length of the lag phase does not depend on the time it takes to induce specific genes related to uptake and metabolism of a specific carbon source. Instead, a more general switch between fermentation and respiration, the so-called Kluyver effect, seems to be the major bottleneck that determines lag duration. Interestingly, there is also a genetic component to lag duration, as some yeast strains show longer average lag times compared to others. QTL analysis suggests that natural variation in the uncharacterized gene YLR108C is at the heart of these strain-specific differences. Moreover, swapping YLR108C alleles shows that a short lag phase goes hand-in-hand with slower growth in stable environments.
Together, our results show that the lag phase is a complex phenomenon that is influenced by both genetic and epigenetic mechanisms that together coordinate the Kluyver effect to obtain optimal fitness in either stable or fluctuating environments.

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Mining and modelling the genome of yeast industrial hybrids

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Saccharomyces pastorianus is an industrial natural yeast evolved from different hybridisation events between the mesophilic *S. cerevisiae* and the cold-tolerant *S. eubayanus*. This complex aneuploid hybrid carries multiple copies of the parental alleles alongside specific hybrid genes and encodes for multiple protein isoforms which impart novel phenotypes, such as the strong ability to ferment at low temperature and under stressful conditions. Although many phenotypic traits of *S. pastorianus* are clearly established, the patterns of gene expression of this hybrid remain to be determined. First, to study the genome plasticity of this hybrid, we developed HybridMine, a new user-friendly, open-source tool for functional annotation of hybrid aneuploid genomes of any species by predicting parental alleles including paralogs. Next, we investigated the transcriptional signature of the different orthologous alleles in *S. pastorianus* CBS 1513 during temperature shifts in different culture media. We identified temperature-dependent media-independent genes and showed that 35% have their regulation dependent on extracellular leucine uptake, suggesting an interplay between leucine metabolism and temperature response. Moreover, the analysis of the expression of orthologous parental alleles unveiled that the majority of the genes express preferentially one parental allele over the other, and that *S. eubayanus*-like alleles are significantly over-represented among the genes involved in cold acclimatisation. The presence of functionally redundant parental alleles may impact on the nature of protein complexes established in the hybrid, where both parental alleles are competing. Our expression data indicate that the majority of the protein complexes established in the hybrid are likely to be either exclusively chimeric or uni-specific, and that the redundancy is discouraged, a scenario which fits well with the stoichiometric balance-hypothesis. This study offers a first overview of the transcriptional pattern of *S. pastorianus* and provide a rationalisation for its unique industrial traits at expression level.

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Insights into the cold-tolerant phenotype of *Saccharomyces arboricola* via restoration of fertility and quantitative genetics on multigenerational fertile interspecific hybrids

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Cold tolerance is a desired trait for fine-tuning the aroma profile of certain alcoholic beverages. Several *Saccharomyces* species have been adopted and domesticated to ferment at low temperatures. In the search for biodiversity, *Saccharomyces arboricola* was shown to have potential in cool fermentations in synthetic hybrids with *Saccharomyces cerevisiae* due to its cold tolerance; however, it has not yet been exploited for any biotechnological application. Although there are several studies on the cryophilic character of *Saccharomyces uvarum*, *Saccharomyces kurdriavzevii* and *Saccharomyces eubayanus*, including the impact of their mitochondrial DNA (mtDNA) inheritance in yeast hybrids, no data is present in the literature for this trait in *S. arboricola*. Recently, a new powerful quantitative genetics tool was demonstrated to assess such complex traits via restoring fertility in interspecific tetraploid *Saccharomyces* hybrids to allow continuous multigenerational breeding with additional focus on the nuclear-mitochondrial interactions.

In this study, we overcame sterility in *S. cerevisiae* x *S. arboricola* tetraploid hybrids and conducted quantitative trait loci (QTL) mapping using pooled F12 generation segregants with extreme cold-tolerance phenotypes to identify corresponding QTLs in *S. arboricola* with additional focus on the role of mtDNA inheritance. The tetraploids constructed were fertile and exhibited spore viability over 70% and a wide range of temperature tolerance between 4 and 37 oC regardless of the mtDNA origin. Growth was measured for 396 F12 segregants for each hybrid at 12 oC degrees. The extremes of the phenotype distribution were pooled to compare segregating SNP frequencies, identifying genomic regions associated with cold tolerance in the hybrids.

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A novel minichromosome is linked to superior bioethanol production in the yeast *Scheffersomyces stipitis*

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A fine-tuned balance between genome integrity and instability is essential for ensuring fitness while permitting adaptation. This is important for microorganisms that utilise genome plasticity as a strategy for rapid and reversible environmental adaptation.

The yeast *Scheffersomyces stipitis* holds an immense potential for second-generation biofuels, generated from lignocellulosic biomass. This is because *S. stipitis*, unlike *Saccharomyces cerevisiae*, ferments xylose, an abundant sugar in lignocellulose. *S. stipitis* use on an industrial scale is impaired by its inability to grow well in the presence of inhibitory compounds generated during the chemical pre-treatment required to extract sugars from lignobiomass. In vitro evolution experiments have allowed the isolation of better bioethanol-producing *S. stipitis* strains that are able to grow in the presence of inhibitory compounds.

In this study, we combined genome sequencing with classical molecular biology and genetics to identify the genetic mechanisms driving improved bioethanol production.

Our analyses demonstrated that improved bioethanol production is linked with the formation of a new mitotically semi-stable minichromosome that comprises of 87 Kb of Chromosome 5, centromeric sequence and telomeric ends. Our working hypothesis is that overexpression of the 25 minichromosome genes drives improved bioethanol production.

This is the first demonstration that large genomic changes drives improve bioethanol production.

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Loss of heterozygosity results in rapid but variable genome homogenization across yeast genetic backgrounds

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The dynamics and diversity of the appearance of genetic variants play an essential role in the evolution of the genome and the shaping of biodiversity. Recent population-wide genome sequencing surveys have highlighted the importance of loss-of-heterozygosity (LOH) events and have shown that they are a neglected part of the genetic diversity landscape. To assess the extent, variability, and spectrum, we explored the accumulation of LOH events in 169 heterozygous diploid *Saccharomyces cerevisiae* mutation accumulation lines across nine genetic backgrounds. In total, we detected a large set of 22,828 LOH events across distinct genetic backgrounds with a heterozygous level ranging from 0.1 to 1%. LOH events are very frequent with a rate consistently much higher than the mutation rate, showing their importance for genome evolution. We observed that the interstitial LOH (I-LOH) events, resulting in internal short LOH tracts, were much frequent (n = 19,660) than the terminal LOH (T-LOH) events, i.e., tracts extending to the end of the chromosome (n = 3,168). However, the spectrum, the rate, and the fraction of the genome under LOH vary across genetic backgrounds. Interestingly, we observed that the more the ancestors were heterozygous, the more they accumulated T-LOH events. In addition, frequent short I-LOH tracts are a signature of the lines derived from hybrids with low spore fertility. Finally, we found lines showing almost complete homozygotization during vegetative progression. Overall, our results highlight that the variable dynamics of the LOH accumulation across distinct genetic backgrounds might lead to rapid differential genome evolution during vegetative growth.

Session 7.2 Yeast model for human health research

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Tra1 and the response to stress: implications for human diseases

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Histone proteins package nuclear DNA into nucleosomes, the fundamental unit of chromatin. This chromatin structure regulates the accessibility of DNA to several cellular machineries and regulates processes, including transcription, the essential first step in the control of gene expression. Post-translational modification of the histone proteins influences chromatin structure. One such modification, the acetylation of lysine residues, relaxes the chromatin structure, facilitating the recruitment of the transcriptional machinery to target promoters. In the budding yeast *Saccharomyces cerevisiae*, Tra1 is an essential component of the SAGA and NuA4 acetyltransferase complexes that regulate gene expression through control of transcriptional activation and nucleosome acetylation. Recent studies have shown that Tra1 is essential for the cellular response to multiple stresses from protein misfolding to cell wall perturbation. Indeed, we found that Tra1 is crucial for the response to accumulation of toxic misfolded polyQ proteins in a yeast model of Huntington's Disease. We also recently undertook to characterize the role of Tra1 in the human pathogen *Candida albicans*. We found that Tra1 regulates the antifungal response and pathogenicity of C. albicans. Thus, tra1 emerges as a major regulator of cellular toxicity associated with multiple human diseases.

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Fission yeast model to study human hereditary disease and anti-cancer drug resistance

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Fission yeast shares many genetic and epigenetic controls with human cells. In this talk, I will demonstrate the usefulness of this model in (1) understanding the molecular function of methylenetetrahydrofolate reductase (MTHFR) gene, which is closely linked to hereditary aneuploidy diseases in human, particularly Down Syndrome. Studying of the fission yeast homolog of human MTHFR met11+ uncovered an epigenetic role in the maintenance of heterochromatin integrity that impacts meiotic chromosome segregation accuracy; (2) discovery of novel anti-cancer drug combinations. Employing chemogenomics approach in fission yeast uncovered a conserved genome-wide multidrug resistance gene network which contains sub-networks that response to different drugs. Further application of this information led to the identification of a doxorubicin/cisplatin/SAHA combination useful to attenuate human gastric cancer cells. This combination appears to cause cell death via a two-pronged mechanism: inducing DNA damage and concomitantly counteracting mTOR-regulated synthesis of DNA damage effector proteins, thus leading to persistence of DNA damage without repair capability to induce apoptosis in the cancer cells.

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Novel Warbicin family of glucose uptake inhibitors in yeast and human cells inhibits cancer cell proliferation

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Many cancer cells share with yeast a preference for fermentation over respiration. We isolated warbicin A as a compound restoring glucose growth of the yeast *tps1*Δ mutant, which undergoes apoptosis due to hyperactive glucose uptake and catabolism. Warbicin A and specific structural analogs inhibit glucose uptake by yeast Hxt and mammalian GLUT carriers with compound-specific kinetics. Warbicin compounds inhibit proliferation and trigger cell death in cancer cells in a dose-dependent manner. Specific concentrations did not evoke any major toxicity in mice but increase adipose tissue. Inhibition of yeast sugar uptake depends on sugar phosphorylation, suggesting transport-associated phosphorylation as a target. In vivo and in vitro evidence confirms physical interaction between yeast Hxt7 and hexokinase, while nuclear targeting of NLS-Hxk2 reduces *tps1*Δ glucose sensitivity. Based on chemical structure, we suggest that warbicin compounds replace inhibitory ATP in the cytosolic domain of glucose carriers, preventing its utilization by hexokinase in aberrant transport-associated phosphorylation, thereby reducing overactive glucose uptake and catabolism.

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S. cerevisiae - an invaluable tool for studying ATP synthase dysfunctions due to mutations in mitochondrial DNA

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Myopathies are a group of many diseases with a varied course caused by defects in mitochondrial oxidative phosphorylation system, OXPHOS. Owing to the many essential processes carried out by mitochondria and the complex cellular systems that support these processes, these diseases are diverse, pleiotropic, and challenging to study. The subunits of four of the five OXPHOS complexes are encoded in both genomes – nuclear and mitochondrial (mtDNA), therefore mutations in both genomes can lead to these diseases. In the case of mutations in the mitochondrial genome, the pathogenic nature is often difficult to determine due to the high variability of mtDNA and the fact that they are not frequent.
A lot of myopathies have been associated to defects in the ATP synthase - the fifth complex of OXPHOS catalyzing the last step in oxidative phosphorylation, ATP synthesis. Two subunits of ATP synthase are encoded in mtDNA: ATP6 and ATP8, both highly hydrophobic and engaged in the proton translocation through the ATP synthase proton channel: directly – ATP6 or indirectly – ATP8.
Much of our current understanding of mitochondrial function and dysfunction comes from studies in the baker's yeast *Saccharomyces cerevisiae*. Because of its good fermenting capacity, *S. cerevisiae* can survive mutations that inactivate OXPHOS and its mtDNA is amenable genetic manipulations. With the advent of structures of complete ATP synthases and biochemical data using yeast as a model we can begin to understand this molecular machine and its associated defects at the molecular level. We have characterized 19 mutations in MT-ATP6 gene, classified them as pathogenic or non-pathogenic. For the five most severe mutations, we have proposed a mechanism by which they disrupt enzyme functioning at the molecular level.

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Finding the targets of novel compounds using high-throughput chemical genomics

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Target identification of bioactive compounds is important for identifying tool compounds and drug leads but remains extremely challenging. Genome-scale chemical-genetic analysis tracks the effect of compounds on mutant fitness to identify novel compound-target pairs, providing an unbiased method for target identification. We developed a chemical-genetic pipeline for integrated target prediction in yeast. We screened DNA-barcoded mutant libraries in a pooled format to allow assembly of chemical-genetic profiles using next generation sequencing. Since the compounds of interest were limitedly available, all libraries were in a drug hypersensitive genetic background. We analyzed several compound collections, including a precious collection of natural products (RIKEN NPDepo), to generate 4,942 chemical-genetic profiles across four mutant libraries. Non-essential genes were covered by libraries of diagnostic and full genome haploid deletion mutants, while essential genes were covered by heterozygous diploid (HET), temperature sensitive (TS), and overexpression (MoBY-ORF) collections. Assuming that compound treatment mimics the target gene's deletion, haploid chemical-genetic profiles were compared to global genetic interaction data to predict target biological pathways. In contrast, HET compound-gene interactions directly predicted gene targets. Information from orthogonal screens was integrated to identify candidate target genes using a score that reflected signal specificity and functional overlap of predicted targets. Predictions were validated by whole genome analysis of drug resistant mutants, orthogonal screening using a human cell line CRISPR system which tests for conservation of the predicted target, and functional validation of targets.

Among the validated compound-target pairs were 1) RP-3-161, a natural product that targets Pik1 and inhibits *Mycobacterium tuberculosis* survival in macrophages, 2) NPD6433, which targets the yeast fatty acid synthase FAS1 and has potent antifungal activity, and 3) NPD5728 and T-711999, which inhibit charging of tRNA-Phe. In theory, our system serves as a model that can be applied to any single cell system, including other fungi and human cells.

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Targeting copper homeostasis improves functioning of *vps13*Δ yeast mutant cells, a model of VPS13-related diseases

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Ion homeostasis is crucial for organism functioning, and its alterations may cause diseases. For example, copper insufficiency and overload are associated with Menkes and Wilson's diseases, respectively, and iron imbalance is observed in Parkinson's and Alzheimer's diseases. To better understand human diseases, *Saccharomyces cerevisiae* yeast are used as a model organism. In our studies, we used the *vps13*Δ yeast strain as a model of rare neurological diseases caused by mutations in VPS13A-D genes. In this work, we show that overexpression of genes encoding copper transporters CTR1, CTR3, and CCC2, or the addition of copper salt to the medium, improved functioning of the *vps13*Δ mutant. We show that their mechanism of action, at least partially, depends on increasing iron content in the cells by the copper-dependent iron uptake system. Finally, we present that treatment with copper ionophores, disulfiram, elesclomol, and sodium pyrithione, also resulted in alleviation of the defects observed in *vps13*Δ cells. Our study points at copper and iron homeostasis as a potential therapeutic target for further investigation in higher eukaryotic models of VPS13-related diseases. Soczewka, P.; Tribouillard-Tanvier, D.; di Rago, J.-P.; Zoladek, T.; Kaminska, J. Int. J. Mol. Sci. 2021, 22, 2248. https://doi.org/10.3390/ijms22052248

Session 8.1 Lipids and membranes

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Roles and regulation of the fat-regulating phosphatidate phosphatase in lipid metabolism

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Phosphatidate phosphatase (PAP) is an evolutionarily conserved enzyme that plays a major role in lipid homeostasis by controlling the cellular levels of its substrate, phosphatidate, and its product, diacylglycerol. These lipids are essential intermediates for the synthesis of triacylglycerol and membrane phospholipids; they also function in lipid signaling, vesicular trafficking, lipid droplet formation, and phospholipid synthesis gene expression. The importance of PAP to lipid homeostasis and cell physiology is exemplified in yeast, mice, and humans by a plethora of cellular defects and lipid-based diseases associated with loss or overexpression of the enzyme activity. Our focus is on the mode of action and regulation of PAP in yeast, the organism from which the enzyme gene (PAH1) was discovered. PAP translocates from the cytosol (inactive form) to the nuclear/ER membrane (active form) through phosphorylation and dephosphorylation. Phosphorylation favors a cytosolic location, whereas the dephosphorylation favors a membrane location. The phosphorylation/dephosphorylation of PAP also affects its activity and susceptibility to degradation by the 20S proteasome. Structure-function studies are revealing important structural determinants in PAP that control the enzyme function in lipid metabolism.

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Tricalbin-mediated contact sites control phospholipid homeostasis to maintain plasma membrane integrity

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The evolutionarily conserved extended synaptotagmin (E-Syt) proteins are calcium-activated lipid transfer proteins that function at contacts between the endoplasmic reticulum and plasma membrane (ER-PM contacts). However, roles of the E-Syt family members in PM lipid organisation remain unclear.

Among the E-Syt family, the yeast tricalbin (Tcb) proteins are essential for PM integrity upon heat stress, but it is not known how they contribute to PM maintenance. Using quantitative lipidomics and microscopy, we find that the Tcb proteins regulate phosphatidylserine homeostasis at the PM. Moreover, upon heat-induced membrane stress, Tcb3 co-localises with the PM protein Sfk1 that is implicated in PM phospholipid asymmetry and integrity. The Tcb proteins and Sfk1 also promote the recruitment of Pkh1, a stress-activated protein kinase required for PM integrity. Phosphatidylserine has evolutionarily conserved roles in PM organisation, integrity, and repair. We suggest that phospholipid regulation is an ancient essential function of E-Syt family members in PM integrity.

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Membrane contact sites regulate vacuole morphology via sphingolipid metabolism

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Eukaryotic organelles function cooperatively by interacting with each other through the membrane contact sites (MCSs). MCSs play important roles in organelle biogenesis including signal transduction, ion exchange, lipid metabolism, transport, and morphology. However, the mechanisms of how MCSs regulate their intracellular processes are poorly understood. Here, we report that MCSs regulate vacuole morphology via sphingolipid metabolism in *Saccharomyces cerevisiae*.

Tricalbins (Tcb1, Tcb2, Tcb3) are integral endoplasmic reticulum (ER) membrane proteins involved in the formation of MCS between the ER and the plasma membrane or the ER and Golgi. We found that deletion of all tricalbins (Tcb1, Tcb2, Tcb3) caused vacuole fragmentation, and that the artificial MCS formation of the ER-plasma membrane or the ER-Golgi in the tricarbin-deleted mutant cells suppressed the phenotype, suggesting the involvement of MCSs in regulating vacuole morphology.

Since tricalbins are involved in non-vesicular transport of ceramide and the loss of function causes accumulation of phytosphingosine (PHS), which is a precursor of ceramide, we hypothesize that the accumulated PHS could trigger vacuole fragmentation. Indeed, we found that exogenously added PHS to wild-type cells caused vacuolar fragmentation.

Moreover, consistent with the hypothesis above, when overexpressed Rsb1, which is a transporter to translocate PHS from the cytoplasmic side toward the extracytoplasmic side of the plasma membranes, the vacuolar fragmentation in the tricalbin-deleted mutant cells was suppressed.

Finally, we investigated how PHS causes vacuolar fragmentation. ER is a place where PHS is synthesized and contacts the vacuoles via an MCS called nuclear vacuole junction (NVJ). Therefore, we examined whether NVJ is involved in vacuole fragmentation. We found that a disruption of NVJ formation suppressed the vacuole fragmentation caused by addition of PHS. Taken together, these results support the view that PHS accumulated in the ER is transported to the vacuole through NVJ and then triggers cause vacuole fragmentation.

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Uncovering the role of Eaf1 in the delicate balance of lipid droplet synthesis and membrane composition in *Saccharomyces cerevisiae*

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The yeast lysine acetyltransferase, NuA4, has been implicated in the regulation of various aspects of metabolism, including a poorly defined role in lipid homeostasis. Surprisingly we have discovered a new role for NuA4 in regulating phospholipid availability for organelle morphology. Upon deletion of EAF1, the main scaffolding subunit of NuA4, over 70% of *eaf1*Δ cells displayed nuclear flares or extension of the nuclear membrane, compared to only 7% in wild type (WT) cells. In addition to nuclear flares, the loss of the NuA4 complex resulted in defects in vacuole fusion, with over 60% of all *eaf1*Δ cells containing more than 10 vacuolar lobes, instead of an average of two to five vacuoles found in WT cells. The nuclear flares and vacuole fusion defects of *eaf1*Δ cells suggest a gross dysregulation of phospholipid production in the absence of NuA4.

How is NuA4 regulating phospholipid homeostasis? Recent studies have shown that the phosphatidic acid phosphohydrolase 1 (Pah1) is an acetylation target of NuA4. Sitting at the cross-roads between lipid droplet formation and membrane phospholipid production, Pah1 converts phosphatidic acid (PA) into diacylglycerol (DAG), which is then subsequently processed to form TAG and stored in lipid droplets. However, in the absence of Pah1 activity, excessive PA is converted to membrane phospholipids and similar to *eaf1*Δ cells, *pah1*Δ cells display nuclear flares and vacuole fusion defects. Here we present genetic and cell biology studies that show that the nuclear flare and vacuolar defects of *eaf1*Δ cells are due to mis-regulation of Pah1. This is accompanied by gross changes in subcellular pools of phospholipids, DAGs and lipid droplets in *eaf1*Δ cells, as detected through fluorescent lipid biosensors. Taken together, our work shows that NuA4 is critical in establishing the balance between lipid droplet formation and phospholipid availability for organelle and cell membranes.

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New insights into the Acetate Uptake Transporter (AceTr) Family: unveiling amino acid residues critical for specificity and activity

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Ato1, a member of the Acetate Uptake Transporter (AceTr) family, is the main acetate transporter in *Saccharomyces cerevisiae*. The analysis of conserved amino acid residues within AceTr family members combined with the study of Ato1 3D model based on SatP (Succinate-acetate-transporter Protein), was the rationale for selection of site-directed mutagenesis targets. The library of Ato1::GFP mutant alleles was functionally analyzed in the *S. cerevisiae* IMX1000 strain which shows residual growth in all carboxylic acids tested. A gain of function phenotype was found for mutations in the residues F98 and L219 located at the central constrictive site of the pore, enabling cells to grow on lactic and on succinic acid. This phenotype was associated with an increased transport activity for these substrates. A dominant negative acetic acid hypersensitivity was induced in *S. cerevisiae* cells expressing the E144A mutant, which was associated with an increased acetic acid uptake. By utilizing computer-assisted 3D-modelling tools we highlight structural features that explain the acquired traits found in the analyzed Ato1 mutants. Additionally, we achieved the proper expression of the *Escherichia coli* SatP, a homolog of Ato1, in *S. cerevisiae*. To our knowledge, this constitutes the first report of a fully functional bacterial plasma membrane transporter protein in yeast cells.

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Bypassing oxygen requirements for sterol synthesis in yeast

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Sterols are important constituents of cell membranes in most eukaryotes, where they contribute to cellular robustness. The conserved and elaborate sterol biosynthesis pathway is strongly oxygen dependent. De novo biosynthesis of one mol of the fungal sterol ergosterol requires 12 moles of oxygen. Anaerobic growth of the yeast *Saccharomyces cerevisiae* therefore strictly depends on supplementation of sterols to synthetic growth media. To eliminate this anaerobic auxotrophy, we set out to replace sterols with alternative polycyclic triterpenoids that can be synthesized in an oxygen independent manner in *S. cerevisiae*. For example, the pentacyclic triterpenoid tetrahymanol is produced by the fungi of the *Neocallimastigomycota* phylum, which use this molecule as membrane reinforcer instead of sterols to be able to survive in the anaerobic environment of the gut of large herbivores. Tetrahymanol is synthesized from squalene in a single, oxygen independent conversion. We demonstrate that expression of a squalene-tetrahymanol cyclase from the ciliate *Tetrahymena thermophila* in a sterol-synthesis deficient *S. cerevisiae* strain allows for synthesis of tetrahymanol and enables sustained sterol-free growth of this yeast under both aerobic and anaerobic conditions. In parallel, we reinvestigated an earlier observation that the yeast *Sch. japonicus* is capable of anaerobic growth in absence of an exogenous source of sterols. We identified the production of hopanoids, which are different pentacyclic triterpenoids, as the very likely basis for this ability. When a putative squalene-hopene cyclase from *Sch. japonicus* was expressed in *S. cerevisiae*, this resulted in the synthesis of various hopanoid molecules and improved anaerobic growth in absence of a sterol source. This research provides a basis for metabolic engineering strategies to eliminate the oxygen requirements associated with sterol synthesis in yeasts, as well as for a deeper investigation into the influence of membrane architecture on yeast physiology.

Plenary 3 Lecture

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Protein folding diseases: lessons learned from humanized yeast models

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The budding yeast *Saccharomyces cerevisiae* has contributed significantly to our current understanding of eukaryotic cell biology. It served as a tool and model to elucidate the molecular basis of a wide variety of cellular phenomena that are conserved in other organisms as well. Because of this conservation, the budding yeast became an attractive cellular and biological relevant model to investigate disease-associated proteins, even when the yeast genome does not encode for an apparent homologous counterpart. These so-called humanized yeast models hold great promise for the dissection of disease-related molecular processes and the discovery of novel medicinal compounds. A good example are the yeast models used to clarify the biochemical and cytotoxic properties of proteins linked to neurodegenerative disorders like Parkinson's, Huntington's and Alzheimer's disease, which are commonly classified as protein folding disorders. Studies with these yeast models not only provided fundamental insight on the interplay of protein quality control mechanisms and how failure of these systems result in cytotoxicity and eventually cell death, but also led to the identification of novel players in disease etiology and the validation of prognostic biomarkers that formed the basis for the development of improved diagnostic assays. In addition, these humanized yeast models offered an unsurpassed performance in phenotypic and chemo-genetic screenings aiming to select and study the mode-of-action of lead compounds with promising therapeutic activity.

Session 1.2 Signalling

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Regulation of a yeast monocarboxylate transporter by metabolic signals

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Plasma membrane (PM) nutrient transporters play essential roles in cell nutrition, signalling, and response to stress conditions or toxins. Their functional expression depends on complex and fine regulatory mechanisms, including endocytic internalization.

The yeast lactate transporter Jen1 has been used as model cargo to further dissect the mechanisms of internalization of nutrient transporters, when cells need to rapidly adapt to environmental changes. Jen1 ubiquitylation, by the ubiquitin ligase Rsp5, endocytosis and vacuolar degradation are regulated by two α-arrestins (Rod1 and Bul1), in response to distinct physiological constraints (1, 2, 3). Rod1-mediated endocytosis of Jen1 requires the presence of a rich carbon source, such as glucose, in a substrate transport independent manner (1,2). In addition, conformational modifications, associated to substrate transport, are likely to trigger Bul1-mediated endocytosis of Jen1, upon alkali stress (3).

Art-RSp5 complexes are regulated by metabolic signalling (reviewed in 4) which, in turn, regulates the steady state level of nutrient transporters at the plasma membrane. Understanding the physiological importance of tightly regulated endocytosis has the potential to maximize titer, rate and yield (TRY)-values of engineered cell factories in industrial biotechnological processes.

Recent data from our group will be presented supporting the emerging concept that cytosolic tails in eukaryotic PM transporters have acquired critical functional roles in trafficking, transport activity and regulated endocytosis. These studies can be exploited for expressing stable versions of nutrient transporters for downstream biochemical or biotechnological applications.

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Adaptation to osmostress by the Hog1 SAPK

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Exposure of cells to osmostress results in the activation of the Hog1/p38 family of stress-activated protein kinases (SAPKs). Activation of these highly conserved MAP kinases is required to generate a set of osmoadaptive responses essential for cell survival. To identify novel activities required for cell adaptation modulated by the MAPK, we performed a systematic identification of Hog1 targets using a biochemical approach. We found substrates related to gene expression regulation, which were involved in multiple steps of mRNA biogenesis. A single cell profiling of yeast exposed to stress showed the dependency of Hog1 for induction of stress-responsive genes and the increase on transcriptional heterogeneity that could indicate an increase landscape for cell adaptation mediated by the MAPK. In addition, we found novel functions of the MAPK such as the regulation of endocytosis and the control of protein degradation mediated by a novel modification of protein complex formation via TSA1 interaction. Altogether, our screening highlighted the relevance of this signaling pathway in the control of several aspects of the cell physiology to maximize cell survival in the presence of stress.

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Impact of tyrosine nitration on pyruvate decarboxylase activity and ethanol production in *Saccharomyces cerevisiae*

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Nitration of tyrosine residues, which is a chemical reaction that adds a nitro group to the 3-position carbon of the phenolic ring generating 3-nitrotyrosine (3NT), indicates an oxidative post-translational modification mediated by reactive nitrogen species (RNS). In an earlier study, nitrite inhibited alcoholic fermentation in the yeast *Saccharomyces cerevisiae* under an acidic condition. Acidification of nitrite results in the formation of RNS and would lead to tyrosine nitration. This study aims to investigate a connection between tyrosine nitration and the inhibitory effect of RNS on fermentation. As a result, acidified nitrite inhibited ethanol production of *S. cerevisiae* consistent with the previous work, while pyruvate was highly accumulated under the RNS condition. Subsequently, we measured the activities of enzymes involved in ethanol production, pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH). RNS stimulus did not affect ADH activity, but reduced PDC activity by about 50%. Among three isozymes of PDC in *S. cerevisiae* (Pdc1, Pdc5, and Pdc6), Pdc1 was further analyzed because it is a crucial isozyme responsible for PDC activity. Western blotting with α-3NT antibody and proteomic analysis with an LC-MS/MS verified the nitration of Pdc1 at positions of Tyr38, Tyr157, and Tyr344. In vitro experiments using the site-specific 3NT-incorporated recombinant Pdc1 revealed that nitration of Tyr157 and Tyr344 significantly reduced Pdc1 activity by 63.7% and 87.3%, respectively. Interestingly, we found that *S. cerevisiae* cells expressing the Tyr344Phe variant Pdc1, which is no longer converted into 3NT, produced higher ethanol than the wild-type cells under the RNS treatment conditions. These results indicate that tyrosine nitration at Tyr344 in Pdc1 decreased ethanol production by reducing its enzymatic activity.

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Characterizing a role for NuA4 in the regulation of ergosterol in yeast

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NuA4 is an essential lysine acetyltransferase (KAT) in yeast and is homologous to Tip60, a disease relevant protein complex in mammalian cells. As our lab and others have recently identified a novel role for NuA4 in lipid regulation, we performed a global lipidomic analysis. We determined that two mutants of NuA4, a temperature sensitive mutant of the catalytic subunit (*esa1-ts*) and deletion of the non-essential scaffolding subunit (*eaf1*Δ), have an increase in the relative abundance of ergosteryl esters, the yeast equivalent to cholesterol esters. Following this discovery, we have also found that the NuA4 mutants have altered sensitivity to many compounds which target ergosterol synthesis and ergosterol in the membrane. This suggests that ergosterol regulation in NuA4 mutants may be altered. We are working to measure ergosterol directly in these mutants as well as determine how NuA4 may affect ergosterol regulation in the cell. Specifically, we are using the deletion mutant array, a collection of yeast strains with each gene deleted, to look for genes that reverse the sensitivity of NuA4 mutants to ergosterol targeting compounds such as Amphotericin B and Nystatin. Overall, this study aims to characterize changes in ergosterol regulation in yeast NuA4 mutants. Given the exceptional conservation of KAT function across eukaryotes, this may provide insight into understanding the biological roles of Tip60 in lipid regulation and the cellular consequences of its deregulation in disease.

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The impact of intracellular metabolite levels on xylose sensing in recombinant *Saccharomyces cerevisiae*

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Agricultural wastes and other lignocellulosic substrates represent an attractive alternative to edible feedstock for the sustainable production of bulk chemicals such as ethanol. Although the industry-favored yeast *S. cerevisiae*, chosen for its robust and reliable fermentation, is able to utilize the hexose fraction of these substrates, large quantities of pentose sugars remain untouched. Since pentose sugars, particularly xylose, can constitute up to 40% of the total sugar content, *S. cerevisiae* has been engineered to enable xylose utilization. However, xylose is mostly consumed after glucose depletion and, even then, only at a much lower rate. This has primarily been attributed to a competition for transport. But it has also been shown that xylose is not recognized as a fermentable carbon source, which hints towards larger issues at the sugar signaling level [1].

We previously showed that *S. cerevisiae* capable of xylose utilization exhibits a sugar signaling state similar to that of low glucose concentrations when exposed to high xylose concentrations [2]. In this presentation, we will share the results of our follow-up investigation where we aim to determine whether the sugar signal seen on xylose arises from changes in the level of metabolic intermediates formed during the catabolism of xylose, or from the internalized xylose itself. The study combines flow cytometry with metabolomics to identify correlations between specific metabolic intermediates and fluorescent signals triggered by the induction of GFP-biosensors linked to the Rgt2p/Snf3p, SNF1/Mig1p, and cAMP/PKA glucose signaling pathways.

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Wsc1 and MAP kinase proteins suppress pexophagy in the methylotrophic yeast *Komagataella phaffii*

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In the methylotrophic yeast *Komagataella phaffii* (*Pichia pastoris*), the plasma membrane protein KpWsc1 senses environmental methanol concentrations, and transmits its information to activate the genes for methanol metabolism. When the medium contains high concentration of methanol, peroxisomes containing major methanol-utilizing enzymes are induced for methanol metabolism. On the other hand, when the methanol concentration becomes low during the methanol culture, peroxisomes are degraded by pexophagy. KpAtg30 is a scaffold protein necessary for pexophagy, which is controlled by its phosphorylation. However, the regulatory pathway from KpWsc1 and detailed molecular mechanism of pexophagy were not known.
In this study, we revealed that KpWsc1 negatively regulates pexophagy in the presence of > 0.15% methanol. The phosphorylation level of Atg30 was increased by depletion of KpMpk1 (MAPK protein downstream of KpWsc1), KpRlm1 (transcription factor involved in cell wall synthesis), KpMsg5 and KpPtp2a (phosphatases) together with that of Wsc1. We concluded that the single methanol sensor KpWsc1 conducts the regulation of peroxisome synthesis and degradation according to the methanol concentration.

Session 3.1 Synthetic genomes and genome editing

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Reinventing the genome of *Saccharomyces cerevisiae* as a novel framework for engineering the next generation industrial strains

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The International Synthetic Yeast Genome Project (Sc2.0) represents an exciting foray into eukaryotic genome re-engineering and the establishment of a novel framework for the development of bespoke industrial yeast strains. In 2019, the Sc2.0 consortium announced the completion of the first draft set of 16 synthetic chromosomes in 'BY' lineage strains directly derived from a well-researched laboratory-adapted strain of *Saccharomyces cerevisiae*, S288c. These chemically-synthesised chromosomes are currently being 'debugged' and consolidated in a single yeast cell. The synthetic chromosomes were designed to include site-specific recombination sequences that would enable subsequent in vitro evolution of multiple variations of the synthetic strain generated. This opens up a whole new dimension – not just one synthetic genome, but whole populations of synthetic genomes will be available for analysis and future study. Such synthetic strains could accelerate the annotation of *S. cerevisiae's* many genes of unknown function, and answer profound questions about fundamental properties of chromosomes, genome organisation, gene content, function of RNA splicing, the extent to which small RNAs play a role in yeast biology, and questions relating to genome structure and evolution. As we are gaining new insights into the intricacies of what makes these synthetic laboratory versions of *S. cerevisiae* tick, we will also learn how to extrapolate that knowledge to the whole gamut of environmental isolates and industrial strains of *S. cerevisiae*, each with their distinctive phenotypes that provide an advantage in a specific environmental niche or industry. Deeper insight into the role of these strain-specific genes can be gained by synthesising and analysing 75 open reading frames, which exist across the breadth of strain-specific ORF diversity of the *S. cerevisiae* pan-genome, but which are absent from the S288c derivative strains. This will provide the means to generate semi-synthetic strains to a variety of industrial settings, such as winemaking.

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Chromosome engineering for the construction of modular genomes in *Saccharomyces cerevisiae*

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To be successful, the biobased economy requires all powerful microbial cell factories that can perform optimally in harsh industrial environments. The construction of such microbes requires extensive genome engineering, both to add new functionalities and to rewire existing ones. While 'plug-and-play' designer genomes might become a reality in the future, extensive genome engineering remains a challenge, even in the highly tractable and popular yeast *Saccharomyces cerevisiae*.

This presentation introduces our recent advances in chromosome engineering, meant to enable large scale, modular remodelling of pathways and functions in *S. cerevisiae*. More particularly, we demonstrate how synthetic chromosomes, assembled in vivo from a large number of DNA parts, can be used as orthogonal expression platforms to rewire native cellular processes and implement new functionalities. Using supernumerary 'test chromosomes' mostly composed of DNA non-coding in yeast, we addressed fundamental questions regarding chromosome design, assembly efficiency and fidelity as well as stability and functionality. The potential of modular, specialized chromosomes was then demonstrated by the assembly in vivo, in two transformation steps, of neochromosomes carrying 20 native and 21 heterologous genes, designed for the de novo production of anthocyanins, native to plants, from glucose. This work paves the way for future microbial cell factories with modular genomes in which core metabolic networks, localized on satellite, specialized NeoChrs can be swapped for alternative configurations and serve as landing pads for addition of functionalities.

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Synthetic chromosome consolidation for Sc2.0 and CRISPR-based mapping of single and combinatorial bugs

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A major milestone has been achieved in the Sc2.0 project, as all the synthetic chromosomes have been synthesized. One key challenge is combining them into a single yeast strain. Here, we describe the consolidation of multiple synthetic chromosomes using the endoreduplication intercross method to generate a novel strain with 6.5 synthetic chromosomes. To expedite the consolidation, we employed a second method, chromoduction, to incorporate the largest chromosome (synIV), thereby consolidating half of the Sc2.0 genome.

To identify and correct genetic “bugs” that arose from synthetic chromosome assembly and consolidation, we developed a method known as CRISPR Directed Biallelic URA3-assisted Genome Scan, or CRISPR D-BUGS. We successfully repaired bugs identified in single synthetic chromosomes, including synI and synII, and a combinatorial bug mapped to synIII and synX, revealing an unexpected connection between inositol metabolism and tRNA abundance.

We expect to build the final Sc2.0 strain with all 16 synthetic chromosomes in the near future. This organism will be the first eukaryote with its genome redesigned and synthesized, serving as a platform for systematic eukaryotic genome study and will use in industrial applications.

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gEL DNA: a cloning- and polymerase chain reaction–free method for CRISPR-based multiplexed genome editing

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Even for the genetically accessible yeast *Saccharomyces cerevisiae*, the CRISPR-Cas DNA editing technology has strongly accelerated and facilitated strain construction. Several methods have been validated for fast and highly efficient single editing events, and diverse approaches for multiplex genome editing have been described in the literature by means of SpCas9 or FnCas12a endonucleases and their associated guide RNAs (gRNAs). The gRNAs used to guide the Cas endonuclease to the editing site are typically expressed from plasmids using native Pol II or Pol III RNA polymerases. These gRNA expression plasmids require laborious, time-consuming cloning steps, which hampers their implementation for academic and applied purposes. In this study, we explore the potential of expressing gRNA from linear DNA fragments using the T7 RNA polymerase (T7RNAP) for single and multiplex genome editing in *Saccharomyces cerevisiae*. Using FnCas12a, this work demonstrates that transforming short, linear DNA fragments encoding gRNAs in yeast strains expressing T7RNAP promotes highly efficient single and duplex DNA editing. These DNA fragments can be custom ordered, which makes this approach highly suitable for high-throughput strain construction. This work expands the CRISPR toolbox for large-scale strain construction programs in *S. cerevisiae* and promises to be relevant for other less genetically accessible yeast species.

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Development of genetic modification tools for *Hanseniaspora uvarum*

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The apiculate yeast *Hanseniaspora uvarum* is a yeast species frequently found in the context of vineyards and wineries and also at the beginning of spontaneous fermentations of grape must. Even though it has long been considered a spoilage yeast, it has increasingly become the focus of research in recent years, as shown by the publication of several studies on whole-genome sequencing. Tools such as genetic manipulation for more detailed research into the aroma metabolism pathways of this yeast species have been lacking until now. We show the development of a first attempt to genetically modify this yeast. For this purpose, the HuATF1 gene, which encodes a putative alcohol acetyltransferase that plays a major role in acetate ester formation, was removed using a disruption cassette. A synthetic marker gene composed of the TEF promoter from *H. uvarum* and a hygromycin resistance open reading frame flanked by 1000 bp flanking homology regions to the target gene was used for transformation. By increasing the antibiotic concentration, we succeeded in obtaining transformants in which both alleles of the ATF1 gene were completely deleted. A phenotypic characterisation carried out by fermentation in Müller-Thurgau must showed a significantly lower production of acetate esters, especially ethyl acetate. This first attempt at developing gene modification tools paves the way for further functional gene analysis of this yeast.

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Mapping an environmental suppression network of essential genes in yeast

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Genes are typically classified as essential based on particular laboratory control conditions. Nevertheless, the phenotypic effect of the lack of an essential gene can be modified by environmental factors, implying that a proportion of the genes catalogued as essential may be dispensable under certain conditions.

To systematically assess the fraction of environment-dependent essential genes, we screened complete deletion alleles of 873 genes (≃80% of all essential genes in yeast) under 21 environments targeting a variety of fundamental biological pathways and processes including the maintenance of genome integrity, RNA and protein expression, and osmotic, oxidative and reductive stress response pathways.

Our preliminary analyses suggest that 1 to 11% of the essential genes are not required for viability in a specific environment, revealing interesting gene candidates with a strong environment-specific suppression effect. For example, we found that specific glycolytic enzymes were no longer essential in the presence of sorbitol as carbon source, and that several proteins with a role in releasing nuclear export proteins from ribosomal subunits were not required for viability at elevated temperatures. Together with further analyses and validations our aim is to provide a novel network of high-confidence environmental suppression interactions in yeast.

Session 4.1 Yeast for food, fermentation, and processing

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Applications of yeasts in tomorrow's sustainable food production

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With an enhanced focus on the interrelationship between climate change, food systems and food security there is an increasing need to understand the role of foodborne microorganisms in food processing. Fermented products account for up to 40% of the food and beverages produced globally. Both conventional and non-conventional yeasts play a significant role in many of these including bread, cheese, kefir, wine, beer as well as meat products such as sausages and ham. In addition, many indigenous food and beverages rely on yeasts for their production. Compared to e.g., lactic acid bacteria, which are often used in the production of fermented foods, yeasts offer many advantages, as they are generally more robust, have a more diverse enzyme profile and have better inhibitory effects against spoilage molds. Yeasts are not only important for the organoleptic quality of fermented products, they also have an impact on the shelf life and nutritional value, in some cases even providing host-beneficial effects. Diversity in ecology and technological traits is seen at both inter- and intraspecies level, and most fermented food products offer a multicultural microbial society where synergistic and antagonistic interactions take place at various level. Detailed knowledge on the microbiota and its interactions is therefore required for improvement of product quality and production of sustainable food products of consistent quality. The presentation will give an update on current knowledge on yeasts occurring in fermented food supported by scientific results on species diversity, technological properties and ability to act as bio-preservative agents.

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Contribution of selected yeasts to enhance the coffee flavor

Mrs. Rosane Schwan

Coffee is one of the most essential traded commodities of high economic impact in producing and consuming countries. In recent years, microbial activity during coffee fermentation has attracted much interest in the coffee industry. This process generates an array of chemical changes essential for developing complex flavors in the beverage sensory profile. The main microbial groups include bacteria, yeast, and filamentous fungi during natural coffee fermentation, in which yeast dominates the process. Different yeast species were inoculated as starter cultures during Coffea arabica fermentation. *Saccharomyces cerevisiae*, *Candida parapsilosis*, *Torulaspora delbrueckii*, *Pichia kluyveri*, *Pichia anomala*, *Meyerozyma caribbica*, and *Hanseniaspora uvarum* were used as potential starter cultures in coffee processing. The yeast has a complementary role associated with coffee quality and contributes to the efficient removal of the coffee bean mucilage layer, promoting the synthesis of yeast-specific volatile constituents. These starter cultures' presence influenced some volatile compounds, such as 2-phenylethanol, 4-vinylguaiacol, and 4- vinylphenol produced by *Pichia* strains. In pulped natural coffee, 2-methyl-2-pentenal was produced by *T. delbrueckii* and formic acid, 2- methyl-1,3-butadiene, 3,4-dimethyl-2-pentanone, and pantolactone by *S. cerevisiae*. In natural coffee, *T. delbrueckii* produced 2-phenyl-2-butenal, 5-amino-1-naphthol, 3,4-dimethyl-2-pentanone, and 1-hydroxy- 2-propanone. However, coffee is a complex matrix and contains many volatile compounds, so it is unlikely that a specific chemical compound can determine the beverage's flavor/aroma. Yeast inoculation resulted in different sensory descriptors and differences in the other attributes evaluated. The spice flavor was noticed in all treatments inoculated with *C. parapsilosis*, and nuts/almond notes were inoculated with *S. cerevisiae*. The combination of *Saccharomyces* and non-*Saccharomyces* yeasts was the most indicated starter culture for increasing the sensorial profile of coffee evaluated by Q-Graders. Higher complexity in flavor, higher frequency of high sweetness, caramelized sugar flavor, nuts, floral and fruity attributes occurred on average in all varieties when inoculated with specific yeast.

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Genomic features, aroma profiles and immune modulatory activity of *Debaryomyces hansenii* complex strains isolated from Korean soybean fermented food

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Fermented soybean products have been getting the spotlight in the international market due to their nutritive value and many health benefits. During soybean fermentation, yeasts play salient roles in the production of diverse flavor compounds that are important to the quality of the soybean products. In this study, we analyzed the genome structure, flavor profiles, and immune modulation activity of halophilic yeast *Debaryomyces hansenii* KD2 and C0-11-Y2, which were isolated from Korean traditional fermented soybean products, called "Jang". The ploidy analysis by FACS and whole genome sequencing revealed that the genome of KD2 strain is haploid with the size of approx. 13 Mb, whereas that of C0-11-Y2 strain appeared to be diploid with the size of 26 Mb. Interestingly, the phylogenetic analysis based on the intron sequences indicated that the C0-11-Y2 is generated by hybridization between an ancestor strain of *D. hansenii* and a strain of *D. tyrocola*. Both *D. hansenii* complex strains produced various flavor components of butter, caramel, and cheese and showed high bioconversion activity from ferulic acids to 4-vinylguaiacol, but with distinctive profiles. Along with the viability in the presence of bile salt and at low pH, both *D. hansenii* strains induced higher level of IL-10, an anti-inflammatory cytokine, compared to the established probiotic yeast *Saccharomyces* boulardii. Besides, the jang strains of *D. hansenii* were shown to be avirulent without oral acute toxicity in systemic infection using mice models. Altogether, our results strongly indicated that the *D. hansenii* jang strains show their high potential as fermentation starter and as probiotic candidates to improve Korean traditional Jang products with high quality and functionalities.

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Fermenting with sourdough or commercial yeast leads to different bread nutritional and organoleptic quality

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Fermented food is taking up the room nowadays with among them, bread as staple fermented food. Through history, two major ways of bread making have emerged and differ now from their fermenting agent: sourdough and commercial yeast. Sourdough contains a microbiota mostly composed of lactic acid bacteria and yeasts, the yeasts being mainly of the *Saccharomyces* and *Kazachstania* genus. Commercial yeasts are usually composed of a single strain of *Saccharomyces cerevisiae*, produced at industrial scale. The development of illnesses related to food intolerances and the attraction of artisanal practices respectful of biodiversity has led to a renewed interest in sourdough bread making. However, so far, scientific knowledge on the effect of the fermenting agent on the nutritional quality and diversity of breads remains limited.

We conducted a participatory experiment in a bakery where we made breads with eight different fermenting agents and two different wheat varieties flours. Three sourdoughs with either K*azachstania bulderi* as dominant yeast species or *S. cerevisiae* and *Kazachstania unispora* as co-dominant yeast species or *S. cerevisiae* and *Torulaspora delbrueckii* as co-dominant yeast species, three commercial yeasts and two mixes of sourdough and commercial yeast were used.

Except for bread minerals contents which mostly depended on wheat variety, bread quality mostly depended on the fermenting agent. Interestingly, we found that the proportion of insoluble proteins was significantly lower in sourdough breads than in commercial yeast breads. Sourdough breads also had a lower sugar content and organic acids content. These differences were mostly driven by a lower amount of maltose and malate. Aroma profiles of sourdough differed from each other and from yeast breads while those of yeast breads were very similar to each other. These results highlight the interest of sourdough bread and the role of fermenting microbiota in bread nutritional and organoleptic quality.

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Drosophila malerkotliana's gut, a trawl for varied yeast species having ethanol fermentation and biocontrol capabilities.

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Drosophila species feed on yeasts throughout their lifecycle and non-motile yeasts depend on Drosophila to vector them to new, sugar-rich substrates. The gut associated yeast community from non- melanogaster Drosophila species has been under explored. In the present study, we investigated the *D. malerkotliana's* gut associated yeast community and their potential applications in ethanol fermentation and biocontrol of fungal phytopathogens. The flies were collected, while they were feeding on a stinkhorn mushroom and their species identity was confirmed by COI gene sequencing. We isolated 36 yeasts from the gut of these wild caught flies and post MSP-PCR screening, 22 yeasts were identified by sequence analysis of the D1/D2 domain. These yeasts belonged to 6 different genera and 13 different species viz. *Pichia kudriavzevii*, *P. fermentans*, *P. kluyveri*, *P. occidentalis*, *Hanseniaspora thailandica*, *H. lindneri*, *H. opuntiae*, *H.uvarum*, *Suhomyces drosophilae*, *Candida sorboxylosa*, *C. phyllophila*, *Kazachstania exigua*, and *Wickerhamomyces anomalus*. It is known that the *Drosophila* species are attracted to fermented fruits, wherein the fermentation causing organism is usually yeast. Therefore, we also tested these gut associated yeasts for their ability to produce ethanol. Many yeast isolates showed high ethanol production in the range of 6.0 to 6.8 g l-1 with the fermentation efficiency of 57.4 – 64.9% at 30ºC using 2% glucose. The maximum ethanol titer was achieved by *H. lindneri*, 6.8 g l-1 with 64.9% fermentation efficiency. We also assessed the antagonistic activity of the selected yeasts against two potent phytopathogenic fungi viz. *Colletotrichum gloeosporioides* and *Fusarium oxysporum* and found that few yeast species exhibited potent antagonistic activity. Our study indicate that the gut of non- melanogaster Drosophila species could also be a potential niche for diverse yeast species with applications in food and wine fermentation and biological control of fungal phytopathogens.

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Influence of phytosterols and ergosterols on wine alcoholic fermentation for *Saccharomyces cerevisiae* strains

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Sterols are a class of the eukaryotic lipidome that is essential for the maintenance of the cell membrane integrity and their good functionality. During alcoholic fermentation, they ensure yeast growth, metabolism and viability, as well as resistance to osmotic stress and ethanol inhibition. Two sterol sources can support yeasts to adapt to fermentation stress conditions: ergosterol, produced by yeast in aerobic conditions, and phytosterols, plant sterols found in grape musts imported by yeasts in anaerobiosis. Little is known about the physiological impact of the assimilation of phytosterols in comparison to ergosterol and the influence of sterol nature on fermentation kinetics parameters. Moreover, studies done until today analyzed a limited number of yeasts strains. For this reason, the aim of this work is to compare the fermentation performances of 27 *Saccharomyces cerevisiae* strains with phytosterols and ergosterol on two conditions: sterol stress (sterol starvation) and osmotic stress (the most common stress during fermentation due to high concentrations of sugars).

Experiments were performed in 300 mL fermenters without oxygen and monitored in order to obtain kinetics parameters. Cell count and cell viability were measured around 80% of fermentation progress. Central carbon metabolism (CCM) metabolites were quantified at the end of fermentation.

Principal Component Analysis revealed the huge phenotype diversity of strains in this study. Analysis of variance indicated that both the strain and the nature of sterol explained the differences on yeast fermentation performances. Strains with a high viability at the end of the fermentation finished fermenting earlier. Finally, ergosterol allowed a better completion of fermentation in both stress conditions tested.

These results highlighted the role of sterols in wine alcoholic fermentation to ensure yeast growth and avoid sluggish or stuck fermentations. Interestingly, sterol nature affected yeast viability, biomass, kinetics parameters and biosynthesis of CCM metabolites.

Session 6.1 Mitotic cell cycle

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Tight control of Rad51 recombinase level ensures proper DNA content after cell division

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When DNA repair pathway is not available, DNA lesions lead to survival limitation. DNA double-strand breaks become especially deleterious when the error-free repair pathway via homologous recombination is not available. In such case, cells take the risk of using the error-prone recombination pathways in order to repair the DNA breaks, resume cell cycle, and continue growth. This comes at the high expense of reduced survival or at least lower comfort of life as a consequence of losing fragments of their chromosomes or genome rearrangements. One of major players involved in recombinational repair of DNA damage is recombinase Rad51, a protein responsible for presynaptic complex formation.

Previously, we noticed that the level of Rad51 is strongly increased when illegitimate recombination is engaged and although the regulation of turnover of this protein is not known yet, we expect that an excessively high level of Rad51 may lead to genome instability. Here we show that Rad51 level is actively regulated in response to genotoxic stress and is degraded via ubiquitin-dependent proteolysis. The ubiquitination of Rad51 appears as a complex task, involving multiple ubiquitin-conjugating enzymes (E2) and ubiquitin ligases (E3) from various families, including SUMO-targeted ubiquitin ligases (STUbLs). We demonstrated that the regulation of the Rad51 level is SUMO-dependent in an intricate manner. The level of Rad51 is increased in strains lacking functional SUMO E3 ligases, such as *siz1*Δ *siz2*Δ and *mms21-1*, or in *smt3allR* strain carrying SUMO variant unable to produce SUMO chains, or in *slx8*Δ strain lacking one of known STUbLs. All these data suggest SUMO-dependent Rad51 degradation. In contrast, a strain lacking both Slx8 and another STUbL, Rad18, accumulates ubiquitinated forms of Rad51. Interestingly, we also found that posttranslational modifications influenced the ability of Rad51 to form oligomeric forms.

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The vacuole drives the morphology of the nucleus and the ribosomal DNA loop upon mitotic arrests

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The ribosomal DNA array (rDNA) of *Saccharomyces cerevisiae* has served as a model to address chromosome organization. In cells arrested before anaphase onset (mid-M), the rDNA acquires a highly structured organization referred to as the rDNA loop, whose length can double the cell diameter. Previous works established that complexes such as condensin and cohesin are essential to attain this structure. Here, we will report that the rDNA loop adopts distinct bar-like morphologies that arise as spatial adaptations to changes in the nuclear morphology triggered during the mid-M arrests. Interestingly, the formation of the rDNA loop results in the concomitant appearance of a space under the loop (SUL) which is devoid of DNA, ribosomal RNA (rRNA), nucleolar proteins, and even freely circulating nucleoplasmic proteins, yet colocalizes almost perfectly with the vacuole. Following the rDNA and its flanking regions, we propose that the rDNA-associated nuclear envelope (NE) reshapes into a flare that evolves into either a handle or a spoon to accommodate the vacuole, with the nucleus eventually becoming bi-lobed. We will finally show that the formation and maintenance of all these changes require new membrane synthesis, an active TORC1 and an inactive Cdc14, whereas microtubules hinder the flare-to-handle transition [1].

[1] https://www.biorxiv.org/content/10.1101/2021.06.23.449658v1

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The spatiotemporal proteome of the yeast cell division cycle

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The successful execution of the cell cycle program relies on timely coordinated changes in protein localization and concentration. Recently, cell population-level approaches were used to monitor changes in protein concentration during the yeast cell cycle. However, the systematic assessment of protein localization dynamics as a function of cell cycle progression remains challenging. We have established an experimental and computational pipeline which allowed us to monitor the combined changes in subcellular localization and concentration of ≈3900 proteins in single live cells, during the division cycle of budding yeast. We report that more than a quarter of the assessed yeast proteome exhibits cell cycle-related periodicity. However, proteins tend to change in either concentration or localization, but most often not in both during the cell cycle. By mapping with high-resolution the location of individual proteins in different cell cycle phases, we show that protein movements span more that 15 distinct subcellular localization classes and involve mostly physically large compartments and sites of polarized growth. Often, proteins involved in compartment-specific localization movements are also enriched for specific bioprocesses. By combining our protein localization and concentration measurements, we show that intracompartmental changes in protein localization can mediate changes in local protein concentration in a cell cycle-specific way, in the absence of changes in total protein levels. By integrating concentration measurements of all proteins, we show that the total proteome content follows a global trend during the cell cycle, with most proteins displaying differential scaling with cell size during G1 and peaking in concentration around cell cycle commitment. Finally, by complementing our proteome data with cell cycle-resolved transcriptome and translational efficiency measurements, we provide insights into the underlying mechanisms that determine the periodicity in proteome concentration. Collectively, we present a high-resolution, proteome-level spatiotemporal map of the yeast cell division cycle.

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Mitotic exit may be regulated by a SIN-like signalling pathway in the budding yeast *Ogataea polymorpha*

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In the budding yeast, since the site of cytokinesis (bud neck) is determined before spindle formation, the cells must orient the spindle relative to the cell polarity axis and delay cell cycle progression until the spindle is correctly oriented. Precise regulation of the spindle pole body-associated signalling pathway, Mitotic exit network (MEN), ensures that mitotic exit and cytokinesis occur only after one set of chromosomes has passed through the bud neck and entered the daughter cell body. Except for Cdc15 kinase the amino acid sequences of all MEN and SPOC components are well conserved in *Ogataea polymorpha*. Among *O. polymorpha* kinases that showed similarities to ScCdc15, two had no other obvious homologues in *S. cerevisiae* and these were named as OpHCD1 and OpHCD2 for homologue candidate of ScCdc15. Since the deletion of either gene resulted in lethality under standard growth conditions, conditional mutants were constructed by either introducing the ATP analog sensitive mutation for OpHCD1 or, for OpHCD2, using the modified versions of the auxin-degron system, that were recently developed in *S. cerevisiae*,. Both mutants exhibited significant delay in late anaphase, suggesting that these genes may have roles in mitotic exit. Similar to ScCdc15, both Hcd1-GFP and Hcd2-GFP associated with the SPB in late anaphase. However, these SPB associations were asymmetric with strict restriction to the SPB in the mother cell. SPB localisation was affected by both the state of the upstream Tem1 GTPase homologue and by depletion of the mitotic Polo-like kinase, further supporting their having an MEN role in *O. polymorpha*. These results suggest that MEN in *O. polymorpha* may resemble the Septation Initiation Network in *Schizosacchromyces pombe*, which is an equivalent signaling pathway to MEN, but has two kinases, Sid1 and Cdc7, at the position of Cdc15 in the pathway.

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Purine auxotrophic starvation evokes quiescence like phenotype in the budding yeast

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Purine nucleotide metabolism is well conserved across eukaryotic cells. Interestingly, purine auxotrophy is a typical trait of intracellular parasites, like toxoplasma and leishmania. Also many *Saccharomyces cerevisiae* laboratory strains are purine auxotrophs. Budding yeast can serve as a model to illustrate phenotypic features that are brought about when transition from prototroph to purine auxotroph occurrs.

Budding yeast effectively stops metabolism and initiates quiescence phenotype in the case of depletion of carbon or nitrogen sources. We explored effects brought by purine starvation in cellular and in the global transcriptome level.

We observed, that purine starved cells stop their cycle in G1/G0 state, become tolerant to severe environmental stresses. Also, intracellular RNA concentration decreases and massive downregulation of ribosomal biosynthesis genes occurs. At the same time, purine starvation phenotype develops via expression of specific genes, distinct from non-starving. Rim15p and its downstream effectors are involved in upregulation of stress responsive genes during purine starvation. Yet, how purine depletion is signalled to Rim15p is not known.

We think that purine auxotrophic starvation induces phenotype which in many aspects mimics “natural” nitrogen or carbon starvations, thus resembling stationary phase or quiescent cells. Our results demonstrate that organised metabolic response is initiated not only via “natural starvations”, but also when starving for metabolic intermediates, like purines. Moreover, our findings are in line with observations in other eukaryotic purine auxotrophs, which in the case of purine starvation arrest their cell cycle and increase resilience against many environmental stresses.

Acknowledgements
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Control of sphingolipid homeostasis through cell cycle checkpoints

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G1/S transition in yeast is the first and most known checkpoint and it is crucial for the initiation of mitotic division, integrating a wide network of cellular processes. Here we show that one of the key G1/S regulators, the SBF complex (Swi4/Swi6 Binding Factor) is required for myriocin resistance, an inhibitor of sphingolipid synthesis. This phenotype is not shared with its downstream target mutants (CLN1/CLN2) or MBF complex, its partner in G1/S control. Based on data mining results, we selected putative Swi4p targets related to sphingolipid metabolism and studied their gene transcription as well as metabolite levels during cell cycle progression. Genes which encode key enzymes for the synthesis of long chain bases (LCBs) and ceramides were periodically expressed during the mitotic cell cycle, having a peak at G1/S, and required SWI4 for full activation at this stage. In addition, HPLC-MS/MS data indicated that *swi4*Δ cells have decreased levels of sphingolipids during progression of the cell cycle, particularly, dihydrosphingosine (DHS), C24-phytoceramides and C24-inositolphosphoryl ceramide (IPC) while it had increased levels of mannosylinositol phosphorylceramide (MIPC). Furthermore, we demonstrated that both inhibition of de novo sphingolipid synthesis by myriocin or SWI4 deletion caused partial arrest at the G2/M phase. Importantly, our lipidomic data demonstrated that the sphingolipid profile of WT cells treated with myriocin resembled that of *swi4*Δ cells, with lower levels of DHS, IPC and higher levels of MIPC. Taken together, these results show that SBF complex plays an essential role in the regulation of sphingolipid homeostasis, which reflects in the correct progression through the G2/M phase of the cell cycle.

Workshop W1: Yeast Bioprocess Scale up & Scale down

Virtual Workshop

Yeast Bioprocess Scale up & Scale down

Schedule
August 25, 2021 All times in CEST

15:40	Welcome – Ralf Takors
15:40-15:55	Alvin Nienow: Organism/Mixing Synergy for Optimum Scale-down/Scale-up: Yeast in Brewing as an Example
15:55-16:10	Matthias Reuss: Euler-Lagrangian simulations: A proper tool for investigating the impact of mixing on synchronization of autonomous simulations in yeast populations
16:10-16:20	Discussion I
16:20-16:40	Ralf Takors: Regulation dynamics of *S. cerevisiae* when being exposed to large-scale conditions: Peculiarities of experimental observations and modelling approach
16:40-16:45	Steven Minden: Scale-up/scale-down device IBVT, U Stuttgart
16:45-17:05	Frank Delvigne
17:05-17:10	Movie: Microfluidic device, U Liege
17:10-17:20	Discussion II

Plenary 4 Lecture

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Functional amino acid engineering in yeast: proline metabolism and its application to brewing

Mr. Hiroshi Takagi[1]

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In yeast, amino acid metabolism varies under different growth environments by regulating anabolic and catabolic processes. The elucidation of metabolic regulatory mechanisms and physiological roles is important for understanding life phenomenon. In terms of application, control of amino acid composition and content contributes to an improvement in productivity and add the value of alcoholic beverages. I will introduce two topics on proline (Pro) that is used not only for a nitrogen source but also for a stress protectant in *Saccharomyces cerevisiae*.

Pro is poorly utilized by *S. cerevisiae*, leading to a nitrogen deficiency and Pro accumulation during brewing. We recently found that arginine (Arg), not ammonium ion, inhibits Pro utilization by specifically inducing the endocytosis of the high-affinity Pro transporter Put4. Moreover, it was shown that the Arg-induced endocytosis of Put4 is mediated by the ubiquitin ligase Rsp5 and the α-arrestin protein Art3. Our results may involve an important mechanism for Arg-mediated inhibition of Pro utilization in *S. cerevisiae*. The development of yeast strains that can efficiently assimilate the abundant Pro in wort and grape must during the fermentation processes could be promising for the improvement of beer and wine quality.

To improve the ethanol productivity of Japanese sake, we isolated Pro-accumulating mutants derived from sake yeast. One of them produced greater amounts of Pro and ornithine (Orn) compared to the parent. Orn is a non-proteinogenic amino acid that improves various negative physical and emotional states. We also identified a homo-allelic mutation in the ARG5,6 gene encoding the Thr340Ile variant N-acetylglutamate kinase in the mutant. Enzyme activity of the variant was insensitive to feedback inhibition by Arg, leading to Orn accumulation. Sake and sake cake brewed with the mutant contained higher Orn than those brewed with the parent. These results contribute to the development of yeast strains for Orn overproduction.

Session 3.2 Tools, parts and chassis for yeast synthetic biology

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Synthetic yeast genomes beyond Sc2.0 – Synthetic Clusters

Mr. Tom Ellis[1]

[1]*Imperial College London, London, United Kingdom*

The international project to construct a synthetic version of the yeast genome (Sc2.0) has been one of the highest visibility research projects for both the yeast community and the synthetic biology in recent years. As this grand project draws to a close, Sc2.0 partners like our team at Imperial College London, are now beginning to use the tools and knowledge of synthetic yeast genome assembly to ask new questions of yeast biology and genomics, and develop new biotechnologies. In this talk, I will provide examples on how our team are now using synthetic genomics to examine and exploit Synthetic Clusters, where sets of genes that encode a common function are relocated from their native genomic loci into new synthetic defragmented or refactored clusters in the chromosomes. We are using this synthetic cluster method to fine-tune pheromone sensing, to explore the minimal gene set for the cell cycle, and to control and direct aromatic amino acid biosynthesis. We are now building new tools for inducible heterochromatin-silencing of clusters and multiplex nanopore sequencing of different cluster designs to be able to accelerate this radical new approach.

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Development and application of new tools to engineer *Kluyveromyces marxianus* for use in industrial biotechnology

Mr Arun Rajkumar, Mr Joel Akinola, Mr Darren Fenton, Ms Lorena Donzella, Ms MJ Sousa, Mr JM Daran, Mr Pavel Baranov, **Mr. John Morrissey**[1]

[1]*University College Cork, Cork, Ireland*

Synthetic biology and other technologies are major forces that will drive the democratisation of yeast species for biotechnology. Prior to the development of these newer methods, it was only possible to genetically engineer a small number of yeast species, and, in reality, only *Saccharomyces cerevisiae* was fully amenable to extensive reprogramming. Now, it should be possible to broaden the portfolio of host organisms to take advantage of yeast diversity and the intrinsic characteristics of different species. This is not necessarily straightforward, however, as the development of reengineered cell factory yeasts requires both synthetic biology tools and improved knowledge of the underlying organismal biology. Over the past number of years, we have been tacking these issues with *Kluyveromyces marxianus*, a yeast that is well known because of its association with fermented dairy products and its intrinsic thermotolerance. Our synthetic strategy comprises two parts: host strain engineering and in vitro assembly of pathways. Using relatively standard CRISPR-Cas9 approaches in amenable strains, it is now routine to perform gene inactivation via NHEJ (non-homologous end joining repair) or targeted deletions of introduction of heterologous DNA with HDR (homology-dependent repair). Construction of heterologous pathways is carried out in vitro using Golden Gate technology and the YTK standard that is widely adopted in the yeast community. We also generated and validated a collection of promoters, terminators and other DNA "parts" for flexible pathway assembly. To advance knowledge of *K. marxianus* biology, we developed a ribosome profiling platform, improved genome annotations, and comprehensively surveyed and studied sugar transporters. In this presentation, the various tools and insights gained will be summarised and their potential will be illustrated with a case study showing how we engineered *K. marxianus* for de novo production of aromatic molecules from 2G carbon sources.

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Multidimensional single-cell benchmarking of inducible promoters for precise dynamic control in budding yeast

Mr. Vojislav Gligorovski[1], Mr. Ahmad Sadeghi[1], Mr. Sahand Jamal Rahi[1]
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For quantitative systems and synthetic biology, simultaneous readout of multiple cellular processes as well as precise, independent control over different genes' activities are essential. In contrast to readout systems such as fluorescent proteins, control systems such as inducible transcription-factor-promoter systems have only been characterized in an ad hoc fashion, impeding precise system-level manipulations of biological systems and reliable modeling.

To guide future choices of inducible systems, we built and benchmarked comprehensive single-copy library of inducible promoters controlling fluorescent protein expression in budding yeast that includes GAL1pr, GALLpr MET3pr, CUP1pr, PHO5pr, estradiol-inducible, tetracycline-inducible and different variants of light inducible promoters. We present key kinetic parameters, scaling of noise levels, impacts on growth, and, crucially, the fundamental leakiness of each system. This multidimensional benchmarking additionally uncovers unexpected disadvantages of widely used tools, e.g., nonmonotonic activity of the MET3 and GALL promoters, slow off kinetics of the doxycycline-induced tetOpr system, small dynamic range of CUP1pr, and high variability of PHO5pr. Evaluating the ARG3 promoter for potential use as a new inducible system, we discovered that it functions as an OR gate induced by the lack of arginine or presence of methionine. To demonstrate the ability to finely control genetic circuits, we experimentally tuned the time between cell cycle Start and mitotic entry in budding yeast, artificially simulating near-wild-type timing.

Our results define the compromises that need to be made for quantitative experiments in systems and synthetic biology.

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A Cas3 base-editor for confined mutagenesis in *S. cerevisiae*

Ms. Anna Zimmermann[1]

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Directed evolution is a common strategy to obtain superior industrial microbes. Because the relatively low supply of beneficial mutations limits the efficiency of directed evolution, various strategies are used to increase the occurrence of mutations. These include mutagens such as UV and EMS, which increase the mutation rate throughout the genome, as well as more directed approaches like error-prone PCR and repeated transformation with variable oligonucleotides. However, each of these strategies comes with important limitations. Increasing the global mutation rate implies the risk of detrimental hitchhiker mutations that reduce the strain's performance, while more directed approaches often only target one or few very specific loci, for example encoding an active site.

Here, we report the development of a Cas3-based strategy to increase the rate of random mutations within specifiable, large genomic regions, without affecting the rest of the genome. The tool combines CRISPR-guided targeting and inducible base-editors that feature a cytidine deaminase fused to Cas3, the signature enzyme of class 1 type I-E CRISPR-Cas systems. The helicase activity of Cas3 unwinds large stretches of dsDNA (10-100 kb) into ssDNA, providing substrate for the cytidine deaminase. The large action radius of Cas3 allows the deaminase to mutate extended regions of DNA, a feat that is not possible with other base-editing tools. Initial results show that our Cas3 base-editor is able to significantly increase cytidine deaminations within at least 12 kb downstream of the target site. The average spread of introduced mutations ranges from ~800 to ~1000 bp. Whole genome sequencing reveals a greater than 50-fold increase in cytidine deaminations within the targeted window compared to rest of the genome. The extended reach of our Cas3 base-editor as well as its ability to specifically act on user defined regions have potential use for the optimization of entire heterologous pathways in *S. cerevisiae*.

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Yarrowia lipolytica as a biotechnological workhorse beyond lipid production

Mr. Rodrigo Ledesma Amaro[1]

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In the last years, *Yarrowia lipolytica* has become an organism of choice for the bioproduction of lipids and lipid derived compounds. The increasing interest in this organism by both academia and industry has boosted the creation of synthetic biology tools that have turned this yeast from “non-conventional” to “conventional”. In this session, we will be sharing our *Yarrowia* toolbox for highly efficient engineering, which combines CRISPR, Golden Gate, Recombineering and a large library of characterized promoters, including inducible ones.

Beyond the promise of *Yarrowia* to produce lipids, lipids derived compounds, and organic acids, the industrial features of this yeast (grows at high cell densities, consumes a variety of convenient substrates, tolerate contaminants, and more) have made researchers start looking into the production of other high value metabolites. Some of our works towards the production of terpenes and flavonoids will be presented here.

Finally, the discussion will open also to other of our projects carried out in non-*Yarrowia* organisms.

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Development of a dedicated Golden Gate assembly platform for *Rhodotorula toruloides* and metabolic engineering for improving carotenoid production

Ms. Nemailla Bonturi[1,2], Ms. Marina Julio Pinheiro[2,3], Ms Eka Rusadze[2], Mr. Tobias Eichinger[2], Paola Monteiro de Oliveira[1,2], Mr. Everson Alves Miranda[3], Mr. Rodrigo Ledesma-Amaro[4,5], Mr. Petri Jaan Lahtvee[1,2]

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Microbial cell factories are essential for the sustainable production of chemicals and materials. The majority of bioprocesses with microbial cell are focused on genetically modified *Saccharomyces cerevisiae*. However, non-*Saccharomyces species* or non-conventional yeasts are interesting biotech workhorses as they can naturally convert a wide range of substrates into industrially relevant building blocks by native metabolic routes. Furthermore, they often have a higher tolerance to inhibitors in lignocellulosic hydrolysates which might result in a more efficient biorefinery. *Rhodotorula toruloides* is an oleaginous and carotenogenic nonconventional yeast capable of efficiently utilizing lignocellulosic hydrolysates as a substrate. However, there is a lack of reliable synthetic biology and metabolic engineering tools for this microorganism. In this study, the Golden Gate assembly platform was adapted to develop an efficient assembly system for *R. toruloides*. The DNA fragments were assembled with predesigned 4-nt overhangs, building three transcriptional units, a selection marker, and insertional units for genome targeting. The platform was validated by overexpressing the carotenoid production pathway. The total carotenoids increased by 210%. The dedicated GGA platform fills a gap in the advanced genome engineering toolkit of *R. toruloides*, enabling the efficient design of complex metabolic pathways.

Session 4.2 Yeast for fermented beverages

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The wine yeast ecosystem: physiological and molecular responses to single and multiple competing yeast species

Mr. Florian Franz Bauer[1], Ms Cleo Conacher[1], Ms Natasha Luyt[1], Ms Mathabatha Evodia Setati[1], Ms Debra Rossouw[1]

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Several yeast species are commonly and globally found in natural grape juice fermentation. Many of these species are significant contributors to the fermentation process and the character of the final product. *Saccharomyces cerevisiae*, the generic "wine yeast", in fact only dominates the final stages of the process. Considering the long history of such human controlled alcoholic fermentation, their annual predictable occurrence and the large yearly volumes, such fermentations represent a significant anthropogenic and global ecological niche, and a model to study evolutionarily relevant ecological interactions between microorganisms. We have previously reported that such ecological interactions are a primary driver of population dynamics within this system. Here we present data on the impact of individual species and of specific environmental parameters on the ecology of binary and multi-species systems, and identify specific molecular responses to such biotic stresses. The data in particular suggest that the transcriptomic response to interactions in this system are in part the result of specific interactions between two species, but also reflect higher order responses to the combined presence of multiple species. Data using flow cytometry and membrane bioreactors furthermore show that the physical presence of species is an important trigger of specific transcriptional responses, and that cell wall proteins, including those encoded by the FLO gene family, play a major part in this response. Taken together, the data suggest that yeast species have evolved specific molecular responses to the presence of other yeast species, and that these responses are mediated through both large-scale metabolic reprogramming and through specific adjustment of cell wall properties.

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Origins of maltotriose assimilation in *Saccharomyces pastorianus*

Mr. Jean-Marc Daran[1]

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The lager brewing yeast *Saccharomyces pastorianus*, an interspecies hybrid of *S. eubayanus* and *S. cerevisiae*, ferments maltotriose, maltose, sucrose, glucose and fructose in wort to ethanol, carbon dioxide and flavour compounds. Complete and timely conversion of maltotriose by industrial *S. pastorianus* strains is a key requirement for process intensification. As sequences and structures of *S. pastorianus* genomes are being resolved, molecular mechanisms and evolutionary origins of several industrially relevant phenotypes such as maltotriose consumption remain unknown. In this presentation, we will present evidence that the *S. pastorianus* maltotriose phenotype could result from regulatory interaction between *S. cerevisiae* maltose transcription activator and the promoter of SeAGT1 and from in vivo recombination between different maltose-transporter genes.
We experimentally confirmed the heterotic nature of the phenotype, and thus these results provide experimental evidence of the evolutionary origin of an essential phenotype of lager brewing strains.

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Sequencing of Canadian *Saccharomyces cerevisiae* wine strains reveals four subpopulations with similarity to North American Oak, Sake, and Commercial strains.

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Vineyards in wine regions around the world are reservoirs of yeast with oenological potential. *Saccharomyces cerevisiae*, carries out fermentation of grape sugars to ethanol and generates flavour and aroma compounds in wine. We have isolated and genetically screened thousands of *S. cerevisiae* strains from spontaneous fermentations of grapes from the Okanagan Valley (OV) wine region in British Columbia, Canada. Wineries place a high value on identifying yeast native to their region to develop a region-specific (terroir) wine program. The genomes of 75 OV wine strains were sequenced using Illumina paired end reads and compared to representative genomes from global wild and domesticated strains. Phylogenetic analysis based on biallelic single nucleotide polymorphism (SNP) data show that OV strains cluster into four clades: Commercial wine, OV, North American Oak and Sake. The non-commercial clades demonstrate decreased heterozygosity and an increase in genetic diversity, signatures that these strains are wild and not domesticated. We also analyzed gene copy number variations (CNVs) in OV strains to find evidence of domestication and wine making traits. Strains in the commercial and OV wine clades have gene CNV reflective of wine making traits such as tolerance to environmental stress, carbohydrate metabolism and nutrient requirements. North American oak strains demonstrate higher diversity in gene content as evidenced by elevated CNV in genes absent from commercial and OV clades, and this may reflect their generalist niche in the wild. While Mediterranean Oak *S. cerevisiae* appear to be the progenitor of domesticated commercial wine yeast strains, North American Oak strain genotypes have previously been identified from North American vineyard grapes. To the best of our knowledge, however, this is the first study to isolate North American *S. cerevisiae* strains with sequence similarity to a North American oak strain from spontaneous wine fermentations.

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Dominance of *S. cerevisiae* over *S. kudriavzevii* in alcoholic fermentation: modeling physiology, fermentation and microbial interactions

Mr Artai Rodríguez-Moimenta[1], Mr David Henriques[1], Mr Javier Alonso-del-Real[1], Ms Amparo Querol[1], **Ms. Eva Balsa-Canto[1]**

[1]Csic, Vigo, Spain

The ability of *Saccharomyces cerevisiae* to outcompete other microorganisms during wine fermentation has been typically attributed to its high fermentative power and capacity to resist the harsh environmental conditions. However, recent studies have pointed out other mechanisms that might contribute to the *S. cerevisiae* leadership. Those mechanisms include cell-to-cell contact, nutrient competition, or the secretion of antimicrobial metabolites (killing effect).

This work explores the role of these mechanisms in mixed wine fermentations of *S. cerevisiae* and *S. kudriavzevii* using a model-based approach. We proposed various models accounting for ethanol inhibition, cell-to-cell contact, nutrient competition, killing effect and processing temperature. Next, we calibrated and validated the candidate models using a multi-experiment data fitting approach, including 14 single and mixed fermentations at different temperatures. In each experiment, cell counts, glucose, fructose, ethanol, glycerol, acetic acid or CO2 were measured through time. Finally, we compared all models using sound statistical measures of goodness-of-fit.

The best model successfully explains the physiology, fermentation and microbial interactions in the considered mixed cultures. The model reveals that ethanol inhibition, nitrogen competition and killing effects contribute to the success of *S. cerevisiae* at high, close-to-optimal temperatures. Even when *S. cerevisiae* accelerates YAN uptake in the presence of *S. kudriavzevii*, this factor alone cannot explain exclusion, even if a cell-to-cell contact mechanism is incorporated into the model. The killing effect is determinant for *S. cerevisiae* to exclude *S. kudriavzevii*. Remarkably, the longer lag-phase experienced by *S. cerevisiae* and the lower fluxes of nitrogen and hexoses at cold fermentations suffice to guarantee the coexistence of both species at low temperatures.

The model is general enough to be used for the automatic design of sequential fermentations. We have formulated and solved several multiobjective optimization problems and shown how the sequence differs depending on the final aim.

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Identification of the molecular bases underlying the fermentation differences between natural isolates of *Saccharomyces eubayanus* in beer must

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Since its isolation in Patagonia Argentina in 2011 and identification as one of the lager yeast parents, *Saccharomyces eubayanus* has gained relevance due to its importance in the fermentation of lager beer. Since then, the number of isolated strains has increased, showing a wide genetic and phenotypic diversity. Interestingly, although these isolates originate from wild environments, they can grow and ferment beer must, a promising feature for using new strains in brewing. In this sense, the use of *S. eubayanus* in brewing requires an understanding of the molecular mechanisms that underlie the fermentative differences generated from its natural genetic diversity. In this context, in order to identify these molecular mechanisms, a differential gene expression analysis (DEG) was performed in three strains of *S. eubayanus*, of which two had similar fermentative capacities (CBS12357T and CL467.1), and one had a low fermentation capacity (QC18). Strains with better fermentation capacity showed similar expression patterns compared with the QC18 strain. For example, CBS12357T and CL467.1 overexpressed genes related to maltose metabolism, fatty acid metabolism, and cell membrane components, while the QC18 strain showed overexpression of genes related to methionine metabolism. Furthermore, we identified overrepresentation of Transcription Factors (TFs) regulating DEG, highlighting TFs belonging to the Hap2p/3p/4p/5p complex, which regulates the expression of genes related to respiration and the diauxic shift between carbon sources. Diauxic shift assays from glucose to maltose in the QC18 strain showed a greater lag phase than strains with higher fermentation capacity. Interestingly, the QC18Δ*hap5* mutant improves the adaptation of glucose to maltose. These results may indicate that a lower fermentation capacity in the QC18 strain could be due to the lower expression of regulatory genes involved in the transition from glucose to maltose.

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A dynamic genome-scale model shows different redox balancing among cryotolerant yeast species in fermentation.

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Saccharomyces cerevisiae is the dominant yeast for biotechnology applications. It has been used to produce fermented foods and beverages for millennia and in last years to make biofuel, glycerol, biopharmaceutical proteins, or secondary metabolites, such as aroma or bioflavours. While many research efforts focus on engineering *S. cerevisiae* strains for particular applications, yeasts constitute over 1,500 species with great potential for biotechnology, and alternative physiological capabilities of lesser-known yeasts are currently not fully exploited.

Recent studies revealed that *S. uvarum* and *S. kudriavzevii* strains show interesting physiological properties relevant to wine producers. These cryotolerant species produce more glycerol and less ethanol than *S. cerevisiae* wine strains, and the aroma profiles are also different.

This work uses a recently developed multi-phase multi-objective dynamic genome-scale approach to investigate the origins of the phenotypic divergence between these yeast species. The model explains the dynamics of the consumption of carbon and nitrogen (organic and inorganic) sources and the production of primary and secondary metabolites.

We calibrated the model using data from more than 40 external metabolites, including alcohols and higher alcohols, esters, etc., and biomass information for the different strains under consideration. The model explained the experimental data for all strains successfully and brought novel insights into how these achieve redox balance during wine fermentation. In particular, we hypothesize cryotolerant yeast strains can use the GABA shunt as an alternative NADPH source and store reductive power (necessary to subdue the oxidative stress under cold conditions) in lipids or other polymers. Additionally, intracellular flux predictions are compatible with recent experimental work showing that most carbon skeletons used to form higher alcohols (i.e., isoamyl alcohol, isobutanol and 2-phenylethanol) are synthesized de novo.

Multi-phase and multi-objective dynamic genome-scale models can comprehensively picture the main metabolic steps inside the cell during batch cultures.

Session 6.2 Mating, meiosis, sporulation

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Origin and evolution of yeast's natural genome engineering system, mating-type switching

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Budding yeasts show a vast diversity of life cycles and mating systems. Some species are primarily haploid, but others are primarily diploid. Some species are homothallic (self-fertile), but others are heterothallic. Homothallism can be primary (where any cell can mate with any other cell) or secondary (where mating is always between MATa and MATα cells, but cells can switch their mating type). In some species, zygotes can divide mitotically to produce stable diploid cultures, but in other species the diploid state is unstable and zygotes enter meiosis as soon as they are formed. We are exploring the evolutionary transitions between these different lifestyles, and the effect of lifestyle on genome organization. We find that there have been multiple transitions from heterothallism to homothallism during budding yeast evolution, with few transitions in the opposite direction. Mating-type switching originated numerous times independently, on different branches of the phylogenetic tree. Switching most commonly occurs by a flip/flop mechanism with two MAT loci (one active, one silent) on an invertible section of chromosome, as in *Ogataea polymorpha*. In the clade containing *S. cerevisiae*, this mechanism evolved further by adding a second silenced locus (HML/HMR). This clade also gained a specific endonuclease, HO, to cut the MAT locus. HO endonuclease evolved from a new type of mobile genetic element related to inteins, whose original cleavage target was the glycolytic gene FBA1.

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Trajectory and uniqueness of mutational signatures in yeast mutators

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The acquisition of mutations plays critical roles in adaptation, evolution and tumorigenesis. Massive genome sequencing has allowed extraction of specific features of many mutational landscapes, but it remains difficult to retrospectively determine the mechanistic origin(s), selective forces, and trajectories of transient or persistent mutations and genome rearrangements. Overall, we conducted a prospective reciprocal approach to inactivate >20 single and multiple mutant genes involved in distinct genome maintenance processes and characterize de novo mutations in hybrid diploid *Saccharomyces cerevisiae* mutation accumulation lines. This approach revealed the diversity, complexity, and ultimate uniqueness of mutational landscapes, differently composed of base substitutions, small insertions/deletions, structural variants, loss-of-heterozygosity (LOH) events and/or ploidy variations. Overall, these “mutomes” and their trajectories revealed upon single-cell bottleneck lineages provide a mechanistic framework to understand the origin and dynamics of genome variations that transiently arise or accumulate during yeast clonal evolution. Several landscapes parallel the repertoire of mutational signatures in human cancers while others are either novel or composites of sub-signatures resulting from distinct DNA damage lesions.

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Spo11 produces chromosomal fragments as an alternative way to start recombination

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Meiotic recombination is initiated by programmed DNA double-strand breaks (DSBs) introduced by Spo11, a relative of topoisomerases. We discovered novel Spo11-induced lesions, chromosomal gaps from coordinated pairs of DSBs. Isolation and genome-wide mapping of the resulting fragments with single base-pair precision revealed that Spo11 prefers to cut sequences with similarity to a DNA-bending motif. This indicates that DNA is likely bent during cleavage in vitro. Moreover, fragment lengths have a periodicity close to (10.4n + 3) base pairs, which indicates that Spo11 favours cleavage on the same face of underwound, likely negatively supercoiled, DNA. Consistent with this, double DSB signals overlap topoisomerase II-binding sites, pointing to a role for topological stress and DNA crossings in break formation and stimulates a novel model for the formation of DSBs and double DSBs. Double DSB gaps, ca 20% of all initiation events, can account for a set of hitherto unexplained gene conversion events. Possible evolutionary and pathological implications of these newly discovered lesions will be discussed.

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Sexual reproduction and hybrid formation in *Komagataella* species

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The yeast genus *Komagataella* consists of seven methylotrophic species. *K. phaffii* and *K. pastoris* (also known as *Pichia pastoris*) are by far the most studied, as they are important hosts for the biotechnological production of recombinant proteins and chemicals. All known *Komagataella* isolates are haploid and propagate mostly by mitotic cell division. Mating and sporulation can be induced by nitrogen starvation, although the relevance of sexual reproduction and the conditions triggering mating in nature are still unclear.

Komagataella strains are homothallic and switch their mating type by a chromosomal inversion mechanism, which limits the investigation of mating-type-specific behaviour. Deletion of a homologous region required for mating-type switching enabled the generation of heterothallic strains of the *K. phaffii* type strain CBS2612. These strains were used to elucidate MAT gene function and mating-type-specific gene expression in haploid a- and α-type strains. Another important aspect of yeast mating is partner recognition by pheromone signalling. Using transcriptomics data analysis, two a-factor pheromone genes and a gene coding for an α-factor protease could be identified. Pheromone sensitivity assays of signalling mutants and wild type cells were used to characterize the function of the newly identified genes and showed that high concentrations of mating pheromones are required for activating the mating response pathway in *K. phaffi*i.

According to the biological species concept, individual species are separated by reproductive isolation. It has been shown for other yeasts, that reproductive isolation mostly happens at the stage of meiosis, while the formation of hybrids between closely related species is usually possible. Mating experiments with a number of *Komagataella* isolates confirmed that interspecies hybrids can easily be obtained under laboratory conditions. Further analysis of the sporulation products of different hybrids will give a more detailed insight into the degree of reproductive isolation between *Komagataella* species.

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Efficient breeding of industrial brewing yeast strains using CRISPR/Cas9-aided mating-type switching

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Yeast breeding is a powerful tool for developing and improving brewing yeast in a number of industry-relevant respects. However, breeding of industrial brewing yeast can be challenging, as strains are typically sterile and have large complex genomes. To facilitate breeding, we used the CRISPR/Cas9 system to generate double-stranded breaks in the MAT locus, generating transformants with a single specified mating type. The single mating type remained stable even after loss of the Cas9 plasmid, despite the strains being homothallic, and these strains could be readily mated with other brewing yeast transformants of opposite mating type. As a proof of concept, we applied this technology to generate yeast hybrids with an aim to increase beta-lyase activity for fermentation of beer with enhanced hop flavour. First, a genetic and phenotypic pre-screening of 38 strains was carried out in order to identify potential parent strains with high beta-lyase activity. Mating-competent transformants of eight parent strains were generated, and these were used to generate over 60 hybrids that were screened for beta-lyase activity. Selected POF+ hybrids were further sporulated to generate meiotic segregants with high beta-lyase activity, efficient wort fermentation and lack of POF; all traits that are desirable in strains for the fermentation of modern hop-forward beers. Our study demonstrates the power of combining the CRISPR/Cas9 system with classic yeast breeding to facilitate development and diversification of brewing yeast. Beer yeasts with enhanced beta-lyase activity could help brewers heighten the flavour of popular beer styles such as “hazy” IPA, and/or reduce the cost impact of modern IPA hopping rates.

Workshop W2: Plant biomass substrates

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Towards valorization of pectin-rich agro-industrial residues: engineering of *Saccharomyces cerevisiae* for efficient co-utilization of D-galacturonic acid and glycerol

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Pectin-rich plant biomass residues represent underutilized feedstocks for industrial biotechnology. The consumption of the oxidized sugar D-galacturonic acid (D-GalUA) is one major challenge and the reduced compound glycerol has been considered an attractive co-substrate for the establishment of a redox-neutral ethanol fermentation process. We engineered the yeast *S. cerevisiae* to i) efficiently connect the fungal D-GalUA pathway with the central carbon catabolism and ii) provide the possibility of co-feeding glycerol. The constructed strain is able to consume D-GalUA with a maximum specific rate of 0.23 g/gCDW/h in synthetic minimal medium containing D-GalUA and glycerol. An isotope-labelling study using ^{13}C glycerol confirmed co-fermentation of D-GalUA and glycerol to ethanol. D-GalUA and glycerol co-consumption was also demonstrated in crude substrates (mixture of sugar beet pulp hydrolysate and crude glycerol). The current study therefore holds great promise for future co-conversion of side streams from different industries into biofuels and bio-based chemicals.

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Development of an industrial xylanolytic *Saccharomyces cerevisiae* strain using CRISPR/Cas9 technology for multiple-copy gene integration and high enzymatic activity

Mr. João Heitor Manfrão Netto[1], Mr. Jonas Ravn[1], Mr. Iván Ciklic[2], Mr. Venkat Konasani[1], Lisbeth Olsson[1], Cecilia Geijer[1]

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There is a worldwide growing interest to utilize lignocellulosic biomass as raw material for production of fuels, chemicals, and materials. Lignocellulose is composed of three different carbon polymers: cellulose, hemicellulose, and lignin. Hemicellulose is mainly composed of xylan, which can comprise up to 35% of the total dry weight of plants. Xylan is converted to fermentable D-xylose primarily through the biomass pretreatment step, where also various inhibitory compounds are formed that are detrimental for the subsequent steps of enzymatic hydrolysis and yeast fermentation. The aim of this study is to construct industrial strains of *S. cerevisiae* that can efficiently degrade and ferment xylan for production of chemicals and materials. For this purpose, we have developed a CRISPR/Cas9-based technology that enables markerless genome integration of multiple copies of genes encoding an endoxylanase (XYN2) from *Trichoderma reesei* and a β-xylosidase XYLA from *Aspergillus oryzae*. The genes have been fused to the SED1 signal peptide for optimal enzyme secretion. The gene cassettes will now be integrated in the genomes of two industrial xylose-fermenting strains, followed by a high-throughput screening procedure to identify transformants with superior xylanolytic activities. Lastly, the potential of the resulting strains will be explored to produce chemicals and materials describing a feasible strategy to utilize the plant biomass as raw material.

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The flexibility of *Scheffersomyces stipitis* to valorize residual biomasses for vitamin B9 production

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Lignocellulose is the major structural component of woody and non-woody plants, representing a major potential source of renewable organic matter. Lignocellulose is primarily composed by two carbohydrate polymers, cellulose and hemicellulose and by lignin (an aromatic polymer). These complex polymers contain different sugar monomers and phenolic precursors, harboring an enormous biotechnological value, since they can potentially be converted into different value-added products.
The present work aims at the valorization of residual agricultural biomasses into folates by a yeast microbial cell factory.
Folate, also known as folic acid, is a B-complex vitamin currently produced by chemical synthesis mainly in the form of folic acid, which is suboptimal in terms of bioactivity. It is therefore desirable to develop novel economical and sustainable strategies towards replacing the chemical production from fossil sources with folate produced using residues from agriculture.
We evaluated the production of folate in shake flasks starting from three different residual biomasses, sugar beet pulp (SBP), sugar beet molasses (SBM), and unfermented grape marcs (UGM), exploiting the yeast *Scheffersomyces stipitis*, naturally able to produce folates, under different fermentation parameters.
S. stipitis was able to metabolize these biomasses, while producing interesting amounts of folate, if compared to the well-known yeast cell factory *Saccharomyces cerevisiae*. These results provide a solid starting point for setting up bioreactor fermentations: attention will be devoted at maximizing the production while fully exploiting residual biomasses, in order to obtain data for techno-economic analysis.

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Secretory expression of cellobiohydrolase-I in *Saccharomyces cerevisiae* improves 2G Ethanol production from saccharified pretreated waste paper and cardboard fiber pulp

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Waste paper and cardboard (WPC) fiber derived from the Organic Fraction of Municipal Solid Waste (OFMSW) is a very interesting second-generation (2G) feedstock for a sugar platform biorefinery contributing to a circular Bio-economy. The glucan and xylan fraction (65-70%) of WPC can be converted to sugars by enzymatic saccharification. In this study, the WPC fiber was obtained from an industrial waste sorting factory. The fiber was first pretreated with acid to remove the residual ash present. A low dosage of cellulase enzyme cocktail Cellic CTEC3 (2.25g/100g dry fiber) was used to saccharify the fiber into fermentable sugars resulting in a total sugar content of 10.4%(w/v). Cellobiohydrolase-I (CBH-I) was identified as the most limiting cellulase enzyme during Simultaneous Saccharification and Fermentation (SSCF) of WPC pulp. Therefore, to further improve the production of 2G bioethanol from OFMSW, we engineered a xylose-fermenting industrial yeast strain, BMD44, with secreted CBH-I, obtaining the strain BMD44-CBHI.

The CBH-I gene from *Talaromyces emersonii* was codon optimized and genomically integrated in BMD44 using CRISPR-Cas9 technology. Four copies of the CBH-I were inserted and the integration confirmed by PCR. The secretory expression of the protein was detected by SDS-PAGE, revealing a molecular mass of 64 KDa, indicating that the protein is glycosylated. Specific activity of the secreted CBH-I protein was determined with PASC as substrate and resulted in 58mU/mg secreted protein. The BMD44 and BMD44-CBH-I strains were further evaluated with pre-saccharified WPC fiber pulp containing 25% (w/v) solids. Under SSCF conditions, the engineered BMD44-CBH-I strain produced 49 g/L Ethanol compared to 44g/L produced by the parent strain BMD44. The CBH-I secreting yeast demonstrated a higher Ethanol titer, yield and productivity due to its higher saccharification efficiency of the pulp.

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Transcriptional control of lignocellulosic sugar utilization in yeasts.

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Saccharomyces cerevisiae preferentially uses glucose as a carbon source for growth and energy generation. Following glucose depletion, it can utilize a wide variety of other carbons including nonfermentable compounds and alternative sugars. A shift to a poorer carbon source results in dramatic reprogramming of gene expression of genes in various metabolic and related pathways. This review study is aimed at describing the recent progress made toward understanding the mechanism of transcriptional control of genes responsible for utilization of alternative carbon sources with focusing on lignocellulosic sugars. A central transcription factors which become activated under low glucose levels have been identified, including the transcriptional activators namely Cat8, Sip4, Adr1, Rds2, Hap4 and recently Znf1. Their interactions and roles appear to influence fermentation productivity and cell response to different stresses. This inevitably affects production of cellular metabolites and other valuable products of yeasts such as biofuels or biochemicals including xylitol when xylose is used as a substrate. Better understanding of yeast carbon and sugar metabolism will enable effective engineering of yeast strains not only *Saccharomyces* species but also the non-conventional yeasts as a successful cell factory or a platform for lignocellulosic bioconversion.

Plenary 5 Lecture

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Antifungal tolerance: spanning the genetic and phenotypic diversity of Candida albicans

Ms. Judith Berman[1]

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Mortality from invasive fungal diseases approaches 50%, despite the use of available antifungal drug. The rare appearance of antifungal drug resistance (e.g., <2% of *C. albicans* isolates are drug resistant) cannot explain these treatment failures. Antifungal tolerance is a poorly understood property that is expressed to different degrees in different susceptible (non-resistant) isolates, yet is not measured routinely in the clinic or in most research studies. We are studying how tolerance differs between isolates, what biological mechanisms drive it and how it affects only some cells within a single isolate. We also are interested in how to inhibit it so as to improve treatment outcomes. The cell biological properties of genetic and non-genetic contributions to *C. albicans* antifungal tolerance along with a large scale 'OMICS approach that we have embarked upon to address the mechanistic issues at the species, isolate and single-cell levels.

Workshop W3: Wild Brewing - History and Present

Virtual Workshop

Wild brewing: history and present

We invite you to join us for the "Wild Brewing" virtual workshop offered as a part of the ICY 15 Meets 30 ICYGMB.

As a celebration of its 10th anniversary, Diego Libkind, Chris Hittinger and José Sampaio will give insights behind the landmark paper detailing the discovery of the wild yeast *S. eubayanus*, the genetic parent of the modern lager brewing strain. The co-discoverers will further discuss some of the exciting new research that has followed in the areas of bioprospecting and hybridization of wild yeast strains for industrial applications.

Some years later much of the *S. eubayanus* story had been elucidated, however a question still remained: can this wild yeast strain produce quality beer? This challenge was taken up in partnership with Heineken Brewing Company with the development of the "H41 Wild Lager", released in 2018. Willem van Waesberghe and Viktor Boer of Heineken will detail the evolution of this beer from a brewing concept to commercial beer and give insights on the potential of wild yeast in brewing from a large-scale perspective. Finally, there will be a virtual walk-through of the brewing of the ICY15 Vienna Lager beer, produced in the Brew Lab facility of the FH Vienna Campus by Michael Maurer, Özge Ata and Charles Moritz.

Following the presentations there will be a panel discussion where the audience will have the opportunity to ask questions of the speakers. As a unique aspect of this workshop, this session will be open to a wider audience including those from the brewing industry, allowing for broader input and exchange of ideas.

Grab a beer and join us for a dive into this historic yeast discovery and a discussion of the broader prospects of brewing with wild yeasts in a casual and collaborative setting. We look forward to your attendance and engagement at the event, cheers!

Schedule

August 26, 2021. All times in CEST

17:45 – 17:50: Introductions and background – Diethard Mattanovich

17:50 – 18:20: Diego Libkind, Chris Hittinger, and José Sampaio

18:20 – 18:50: Willem van Waesberghe and Viktor Boer

18:50 – 19:00: Charles Moritz

19:00 – 19:30: Q & A and Open Discussion Session
Chaired by Diego Libkind, with Chris Hittinger, José Sampaio, Willem van Waesberghe, Viktor Boer, Özge Ata, and Charles Moritz.

Session 3.3 Synthetic biology applications

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Coupling the CRISPR/Cas9 system to rDNA-NTS-based multiple integration for marker recycling in recombinant yeasts

Mrs. Hyun Ah Kang[1]

[1]*Chung-Ang University, Seoul, South Korea*

For construction of yeast cell factories for producing metabolites or proteins of commercial value, multiple integration of the expression cassettes of heterologous genes into host genome is required. Furthermore, recycling of multiple integrated selection markers would be necessary to introduce subsequently multiple biosynthetic genes for implementation of heterologous metabolic pathways or for co-expression of several subunit proteins. We had previously developed a new set of multiple integration cassettes by exploiting the non-transcribed spacer (NTS) of ribosomal DNA (rDNA) in combination with defective selection markers in *Saccharomyces cerevisiae*. The 5' and 3'-fragments of rDNA-NTS2 were used as flanking sequences for the expression cassettes carrying a set of auxotrophic selection markers with truncated promoters of different lengths. The integration numbers of NTS-based expression cassettes showed a proportional increase with the extent of decreased expression of the auxotrophic markers, up to ~30 copies. In a following research, we evaluated Cre-lox and CRISPR-Cas9 as a tool for recycling of multiple-integrated selection markers. The simultaneous pop-out of the expression cassettes, along with the URA3 markers hampered the use of Cre-lox as a marker-recycling tool in the recombinant *S. cerevisiae* strains carrying multiple integrated selection genes. To overcome this problem, we constructed a set of CRISPR-Cas9 vectors to contain gRNA targeting auxotrophic marker genes, such as URA3, HIS3, LEU2, and TRP1. We showed that introducing stop codons into selection marker genes by the CRISPR-Cas9/gRNA vectors with the donor DNA fragments is an efficient strategy to recycle multiple-integrated selection markers while maintaining the multiple-integrated expression cassettes. The rDNA-NTS integrative cassettes coupled with CRISPR/Cas9 are expected as useful genetic tools to construct synthetic yeast cells carrying optimal copies of a desired expression cassette by sequential rounds of gene manipulation.

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Development of engineering tools for methylotrophic yeast *Pichia pastoris* and their applications to small antibody secretory production

Mr. Akihiko Kondo[1,2], Mr. Yoichiro Ito[1,2], Mr. Yasuyuki Nakamura[1,2], Mr. Daisuke Sasaki[1,2], Mr. Jun Ishii[1,2]
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The methylotrophic yeast *Komagataella phaffii*, commonly known as *Pichia pastoris*, is a suitable host species for the production of proteins. A number of enzymes and therapeutic proteins have been produced in *P. pastoris*. Several of these proteins had been commercially available for many years, and some have been approved by the US Food and Drug Administration (FDA) for clinical use. This species allows high-cell-density cultivation, eukaryotic posttranslational modification, and potentially high-level secretory protein production as well as genetic manipulation. Genetic engineering tools continue to be developed for use in this organism, accelerating strain development. Publication of the detailed genome sequence of *P. pastoris* has facilitated the application of a wide range of techniques in this microbe. In combination with recent progress in genetic engineering and synthetic biology, an advanced genetic toolbox for strain development in *P. pastoris* has accumulated; however, its variety and number are still not enough compared to the budding yeast *Saccharomyces cerevisiae*. To overcome this limitation, we developed several engineering tools for this organism such as a stable, autonomously replicating plasmid vector, a terminator catalogue, a targeted homologous recombination method and a genome-wide screening strategy. Using the established tools, we applied them to the strain development of *P. pastoris* for protein productions including small antibody fragments.

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Microbial assimilation of CO_2 into value-added chemicals by *Komagataella phaffii*

Ms. Özge Ata[1,2], Ms. Lisa Lutz[2], Mr. Michael Baumschabl[2], Mr. Diethard Mattanovich[1,2]
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Climate crisis is arguably the biggest challenge humankind is facing these days. One clear cause is the atmospheric CO_2 level: It has increased from 0.26% to 0.42% after the industrial revolution. Indeed, more CO_2 is emitted due to human activity than it can be captured by plants and microorganisms, causing an imbalance in the carbon cycle. Towards a more sustainable future, we need to restore this balance.
To address this problem, we aim to enable microbial assimilation of CO_2 as a carbon sink by converting it into value-added, bio-based polymers. Recently, we developed a synthetic autotrophic *Komagataella phaffii* (*Pichia pastoris*) that can grow solely on CO_2 for biomass formation while using methanol to harvest energy (Gassler et al. 2020). In this work, we further engineered this CO_2 fixing *K. phaffii* and use it as a platform to produce value-added organic molecules. Using synthetic biology tools and CRISPR-Cas9, we generated an autotrophic *K. phaffii* strain that can produce itaconic acid by fixing CO_2. Balancing the synthetic itaconic acid metabolism, identifying and engineering targets in the central carbon pathway and optimizing the process parameters resulted in a final titer of 1.8 g/L itaconic acid in shake flasks. 13C-labelling experiments were conducted to confirm the incorporation of the captured CO_2 into itaconic acid. We also investigated the itaconic acid production performance of the CO_2-fixing *K. phaffii* strain in lab-scale bioreactors. In the light of our results, we show that the synthetic autotrophic yeast *K. phaffii* can be a platform for microbial conversion of CO_2 into chemicals and help us to fight against the climate change.

Gassler, T., et al (2020). The industrial yeast *Pichia pastoris* is converted from a heterotroph into an autotroph capable of growth on CO2. Nature biotechnology, 38(2), 210–216.

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Engineering a second-generation *Saccharomyces cerevisiae* for 2,3-butanediol production

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2,3-butanediol (2,3-BDO) is an important platform compound for various applications in the chemical, cosmetics, and biofuel industry. *Saccharomyces cerevisiae* is a promising metabolic platform to produce pyruvate derivatives, such as 2,3-BDO, 3-hydroxypropionic acid, and lactic acid when ethanol production is abolished by deleting the PDC genes. In this work, all the PDC genes (PDC1,5,6) were separately deleted by integration of a 2,3-BDO pathway cassette, containing AlsS, AlsD and bdhA. The resulting PDC(-) strain showed fast glucose consumption rate (6.31 g/(L.h)) without growth defect in 20% YPD medium, producing 57.17 g/L 2,3-BDO. The reason could be that the AlsS gene product has some similar functionality with the PDC gene products, supplying some C2 for growth. On the other hand, a high concentration of glycerol (69.80 g/L) was produced to re-oxidize the surplus NADH from 2,3-BDO synthesis. The water-forming NADH oxidase (NoxE) was then integrated in HGS7 to facilitate the oxidation of NADH, which dramatically decreased the glycerol production from 69.80 g/L to 21.76 g/L. The genes encoding NAD-dependent glycerol-3-phosphate dehydrogenase (GPD1,2) were then deleted to eliminate the glycerol production. The *gpd*Δ strain (HGS15) has no glycerol production but has an osmotic problem when ferment in high glucose concentration (20%) likely because of the lack of glycerol to resist the osmotic pressure. To solve this problem, the GPD1 ORF with a weak promoter (CYC1) was additionally expressed, resulting in strain HGS28. This caused lower production of glycerol (9.44g/L) with 78.31 g/L 2,3-BDO and 6.73 g/L acetoin after 59h in 20% YPD medium. In the further work, the strain will be optimized to lower glycerol production further with more copies of the NoxE gene to increase the 2,3-BDO production rate.

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Implementing re-configurable biological computation with distributed multicellular consortia

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The use of synthetic biological circuits to deal with numerous biological challenges has been proposed in several works, but their actual implementation is still remote. One of the major problems encountered in building biological circuits is the complexity of the cellular engineering needed to achieve complex circuits and the lack of building general-purpose biological systems. The generation of re-programmable circuits can enhance the possibility to increase circuit flexibility and scalability of complex cell-based computing devices. In this work, we present a new architecture to produce reprogrammable biological circuits to create of a variety of different functions with minimal cell engineering. We demonstrate that it is possible to create several circuits only using a small set of engineered cells that can be externally reprogrammed to implement simple logics in front of definite inputs. In this manner, a device composed of these cells, depending on its program, can develop different circuits without the need of further cell engineering or rearrangements. The reprogrammability of biological circuits is an intrinsic capacity that is not given in electronics and should be exploited to encourage the use of Biocomputing as a tool to solve complex biological problems.

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Engineering a gut biosensor for the detection of colitis using the probiotic yeast strain *Saccharomyces boulardii*

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Inflammatory bowel disease presents a diagnostic challenge in that periodically monitoring inflammation within the intestine is difficult without highly invasive methods. A more convenient method to detect intestinal inflammation would be helpful to confirm clinical diagnoses and to evaluate the efficacy of therapeutic approaches. There is increased interest in using probiotics for *in situ* detection of intestinal diseases. These living microorganisms have the advantage of being able to travel through (or reside in) the intestine providing an opportunity to design genetically encoded biosensors to detect disease biomarkers. Here we describe progress towards an intestinal inflammation biosensor in *Saccharomyces boulardii*, a probiotic yeast that is genetically similar to the well-studied *Saccharomyces cerevisiae* (making it relatively straightforward to genetically modify), but with an improved tolerance towards the harsh conditions of the gastrointestinal tract (making it more suitable for intestinal biosensing). We recently developed an improved oxidative stress sensing biosensor within *Saccharomyces boulardii* that has a very strong and sensitive response towards reactive oxygen species- a common marker of inflammation. Based on this sensor, we are developing a probiotic yeast reporter that could be ingested by a patient, recovered in a stool sample, plated, and visually inspected to see if it had encountered inflammation. To this end, we modified a Cre/lox system to switch the characteristic colony colour of *Saccharomyces boulardii* from white to red upon detection of reactive oxygen species through the excision of a genomically integrated lox-flanked ADE2 cassette; the final output is then the fraction of plated red and white colonies. Here, we present results on the optimization and tuning of the oxidative stress sensor, along with preliminary results from a DSS mouse model of colitis. We believe that the results of our work could prove useful for the development of other probiotic yeast-based biosensors.

Session 4.3 Aroma and fragrance production

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Tailoring yeast for sustainable manufacturing of aromas and more

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Biology is unparalleled in its molecular diversity and wide application space. One application of biological natural products is delightful aromas and fragrances, which are traditionally extracted from plants and animals. These traditional sources are unable to support a projected doubling of worldwide demand for aroma chemicals in the next 10-12 years. Industrial fermentation of tailored yeast strains can readily fill this gap. Optimization of microbial production of any molecular target requires repeated iterations of the design-build-test-analyze engineering cycle. At Amyris, advanced tools for strain engineering, high throughput screening, analytics, and bioinformatics have been developed to rapidly accelerate the engineering cycle and reduce the number of necessary iterations. This presentation will describe the next-generation strain development pipeline that enables Amyris scientists to rapidly convert naïve yeast strains into manufacturing strains for multiple aroma chemicals.

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Differences in wine aroma synthesis among *Saccharomyces* species: metabolic and comparative genomics approach

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Alternative *Saccharomyces* yeasts, such as *S. kudriavzevii* or *S. uvarum*, can help to solve the new challenges of the wine industry. These species exhibit good fermentative capabilities at low temperatures, wines with lower alcohol and higher glycerol amounts and good aromatic profiles. To improve the knowledge among these species a comparative metabolomic analysis during winemaking where performed. The main metabolic differences arose on lipid composition into the stationary phase, divergent patterns of production of short-chain fatty acids and erythritol and also differences in aromatic amino acid biosynthesis and sulphur amino acid metabolism, including the glutathione pathway (1,2).
Analysing the differences in the aroma synthesis, *S. uvarum* strains were characterized by higher production of 2-phenylethyl acetate, geraniol and branched-chain esters, providing floral and fruity notes to wines. S. kudriavzevii strains showed a great ability to release up to 47-fold more of 4-methyl-4-mercaptopentan-2-one (4MMP) than *S. cerevisiae*. Finally, some wild *S. cerevisiae* isolates showed a very particular aroma profile due to the highest production of ethyl 4-methylvalerate (lactic and fruity notes), g-octalactone (coconut notes) and furfurylthiol (roasted coffee notes).
By a comparative genomic analysis of the genes involved in aroma synthesis among the genome sequences of *S. cerevisiae*, *S. uvarum* and *S. kudriavzevii*, we observed important differences at the nucleotide level in some genes involved in the aroma synthesis like ARO10 (encoding a phenylpyruvate decarboxylase) (3), ATF1 and ATF2 (coding for alcohol acetyltransferases 1 and 2, respectively) (4) or a genes under positive selection in *S. kudriavzevii* like ARO4; it encodes a 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase that catalyzes the first step in aromatic amino acid biosynthesis.

(1) Minebois et al. (2020) Food Microbiol. 2020 90:103484
(2) Minebois et al. (2020) Environ Microbiol. 2020 22(9):3700-3721
(3) Stribny et al., (2016) Microb Cell Fact. 2016 12;15:51
(4) Stribny et al., (2016) Front Microbiol. 2016 7;7:897

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Comparative genomics unveils fundamental traits on the biology and physiology of bio-flavouring species of the *Saccharomycodeacea* family

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The *Saccharomycodeacea* family, sister to the extensively studied *Saccharomycetacea* family, comprises several species with a recognized potential as bio-flavouring agents out of which those belonging to the *Hanseniaspora* and *Saccharomycodes* genus stand out. While *Hanseniaspora* are generally described to positively influence wine/beer aroma, the contribution of *S. ludwigii* (the better studied *Saccharomycodes* species) for aroma is far less clear, with positive contributions being described in the production of ciders or craft beers, but being a feared agent in wine making due to its capacity to produce the off-flavours ethyl acetate, acetaldehyde or acetoin. The scarce knowledge gathered on the biology and physiology of *Saccharomycodeacea* species rendered difficult to understand this different behaviour complicating their rational exploration and, consequently, restraining a full disclosure of their bio-flavouring potential. Recently, our groups have disclosed the first fully annotated genome sequences of *H. guilliermondii* and *S. ludwigii* strains. While *Hanseniaspora* species lack functional neoglucogenesis, glyoxylate cycle and thiamine biosynthetic pathways, *S. ludwigii* had all these pathways complete (in fact, significant expansion of thiamine-biosynthesis was observed) and it was even found to encode genes for catabolism of N-acetylglucosamine, a unique feature within *Saccharomycodeacea* species and rare among non-dimorphic yeasts. Comparison of the FLAVOROMA of these species unveiled a set of *Hanseniaspora*-specific alcohol acetyl-transferases, while *S. ludwigii* was found to be equipped with three putative bacterial-like acetoin reductases. Further results obtained in this comparative genomics analysis, specially focusing environmental stress response and emphasising the adaptive responses to ethanol and to SO_2, considering the remarkably high tolerance of *S. ludwigii* to this largely explored beverages' preservative.

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Dynamics of benzenoids and isoprenoids production during wine fermentation with *Hanseniaspora vineae* and *Saccharomyces cerevisiae* yeasts

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In the last decade mixed cultures in wine fermentation have begun to be considered as an alternative to traditional inoculation using pure selected *Saccharomyces cerevisiae* strains. Moreover, the non-*Saccharomyces* yeast *Hanseniaspora vineae* is a species with clear oenological traits that have been used successfully in winemaking under mixed cultures, giving desirable aromas to the final wine.
Different aroma production during wine fermentation depends on the inoculation strategy used. It can be the result of the sum of compounds produced by each of the microorganisms involved in the process, but usually is due to metabolic changes caused by strain interactions.
Yeast interactions can be positive or negative regarding to the effects that the strains involved cause each other, for example altering their growth and fermentation performance. Some aromatic molecules have been described as potential mediators in yeast interaction. Among them, some positive flavour compounds such as benzenoids and isoprenoids are considered chemical signals able to modify the metabolism of microorganisms.
Therefore, comparing the dynamics of production of these compounds throughout wine fermentation, using different inoculation strategies, it would be possible to determine the effect of possible yeast interaction.
Pure *H. vineae* 12/196AF and *S. cerevisiae* TS28 cultures and their mixed cultures, using sequential inoculation and co-inoculation, were performed to compare benzenoids and isopropanoids profiles of the four treatments during the process. A synthetic must with 100mg/L of YAN was fermented for 1, 4, 7 and 10 days following volatile compounds formation by gas chromatography-mass spectrometry.
Significant differences were found comparing the aroma of wines fermented with different inoculation treatments. Some compounds were detected along the whole process and accumulated such as benzyl alcohol, 2-phenylethylacetate, farnesol, tyrosol, tryptophol and tryptophol acetate. Other compounds, detected during the process but not significantly at the final wines, were linalool, geraniol, indol, nerolidol and 4-vinylguaiacol.

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QTL mapping reveals genome regions possibly involved in the variations of H_2S production in presence of SO_2 during alcoholic fermentation.

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During alcoholic fermentation, *Saccharomyces cerevisiae* requires S-containing amino acids, that it produces through the sulfur assimilation pathway (SAP). In this pathway, *S. cerevisiae* reduces sulfate into sulfur dioxide (SO_2) and sulfide (H_2S), whose overproduction is a major issue in winemaking due to its negative impact on wine aroma. The amount of H_2S produced is very strain-specific and depends as well on the SO_2 concentration, which is often added in winemaking. First, we set up a quantitative method to measure the H_2S produced during alcoholic fermentation and evaluated this production for 34 strains isolated from wine, flor or oak. Large differences between strains were detected, and strains carrying a specific allele of SKP2 (SKP2-JN10), an allelic variant related to low H_2S and SO_2 excretion (1), were identified as low producers. However, when exposed to SO_2, several strains carrying the SKP2-JN10 allele produce H_2S again. In order to decipher the genetic bases of the H_2S production in the presence of SO_2, we used a Quantitative Trait Locus (QTL) mapping strategy using a Bulk Segregant Analysis (BSA). We crossed a moderate H_2S producer strain carrying SKP2-JN10 allele, with a strain that produced very little H_2S in the presence of SO_2. A progeny of 96 of segregants was obtained and phenotyped for sulfide production. The comparison of the genome of a pool of segregants producing high H_2S with the pool of non-producers pointed out to several genome regions possibly involved in the variations of H_2S production. We are studying the role of a number of candidate genes and we expect that this approach will provide novel targets for the breeding wine yeast starters.

1 - Noble et al. 2015 Microbiol cell factory 14:68

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Yeast species versus fermentation conditions: what has the bigger impact on flavour of Scotch Whisky?

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Water, barley, yeast, and maturation in an oak cask: these are the ingredients for one of Scotland's most important products: Scotch Whisky. The yeast converts the sugars from malted barley into alcohol, but it also produces a variety of flavourful secondary metabolites. Depending on yeast strain and fermentation conditions, different congeners (flavour-active volatile chemicals) are produced. The Scotch Whisky industry only sources a limited range of commercial yeast strains all belonging to *Saccharomyces cerevisiae*. Brewing and oenology research has shown a clear link between yeast diversity, fermentation conditions and the flavour of beer and wine. The influence of yeast in influencing the organoleptic properties of distilled spirits is a poorly researched topic. The present study investigated the influence of a standard distilling yeast: *S. cerevisiae* against the fission yeast, *Schizosaccharomyces pombe*, to produce flavoursome Scotch Whisky distillates. The fermentation time, wort gravity, temperature and yeast pitching rate were investigated by a Design of Experiment (DoE) approach. Fermentation progress was followed by monitoring carbon dioxide evolution kinetics and at the end of the fermentation, residual carbohydrates, pH, yeast cell density and viability were assessed. The fermented wash was distilled, and the resultant "low wines" were assessed by Gas Chromatography-Mass Spectrometry to measure the relative change in abundance of 202 congeners. When comparing model whisky fermentations of *S. cerevisiae* and *Schiz. pombe*, major differences in the congener profiles of subsequent distillates were observed from chemical analyses. Smaller differences in congener production were linked to the fermentation conditions. However, the fermentation conditions were substantial to reach a complete fermentation. This research has implications for whisky distillers seeking to create more flavoursome spirits by selecting non-conventional yeasts and by modulating the fermentation conditions to maximise product yield.

Session 6.3 Environmental communication and stress

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Robustness is a key property for yeast bioprocesses and strain development

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Yeast is broadly exploited for industrial use, and strains are constantly improved to meet the requirements to produce the targeted product with high yield, productivity and titer. Successful strains have consistent performance also in presence of different perturbations, i.e., their performance is robust. With a research focus on microbial robustness, the concept as such will be discussed and contrasted to tolerance toward specific stresses. Furthermore, tools to quantitatively assess and understand microbial robustness will be presented.

Two concrete examples of research approaches towards robust processes and strains will be given. First the concept of short-term adaptation will be discussed. Here, we have demonstrated that short-term adaptation of yeast during propagation will lead to improved performance during lignocellulosic fermentations. Cellular events that lead to the improved performance include both stress responses and increased metabolic capacity of vitamin production. The second concept that will be discussed involve membrane engineering and the influence of weak acid stress on cellular metabolism. Examples will be given of the use of membrane engineering to modulate cell membrane composition and diffusion of weak acids. Our strategies examine adaptive responses in fitness to stressful conditions as a starting point for identifying and engineering molecular traits behind microbial robustness.

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The coordinated translation of functionally related mRNAs in factories: roles in metabolic regulation and inheritance

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The fate of mRNA can be co-ordinately regulated in cells. For instance, under many stress conditions there is a rapid inhibition of translation initiation, followed by a relocalisation of mRNA for storage and/or degradation to either stress granules (SGs) or RNA processing bodies (P-bodies). Recently, we have shown that even under non-stress conditions certain ubiquitously expressed and heavily translated mRNAs are localised. We have defined classes of granule including CoFe (Core Fermentation) granules that contain the majority of the glycolytic mRNAs and Translation Factor (TF) granules that contain many translation factor mRNAs. In contrast to P-bodies and SGs, where translationally repressed mRNAs are housed, we find that at the CoFe and TF sites, mRNAs are actively translated, and this translation is required for their correct localisation. Following the translational inhibition associated with stress, the CoFe and TF granules rapidly coalesce to provide a platform for P-body/ SG formation. We postulate that the CoFe and TF granules play roles in highly efficient and co-ordinated translation, allowing cells to manage and harmonize the production of components from the same protein complex and/or metabolic pathway. In addition, for the TF granules, we identify a mechanism by which the protein synthesis machinery is inherited by the daughter cell and targeted to regions of active growth. Such a feedforward mechanism would ensure adequate provision of the translation machinery where it is to be needed most over a coming cellular growth cycle.

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Minority potassium-uptake system Trk2 has crucial role in yeast survival of glucose-induced cell death

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The existence of programmed cell death in *Saccharomyces cerevisiae* has been reported for many years. Glucose induces the death of *S. cerevisiae* in the absence of additional nutrients within a few hours, and the absence of active potassium uptake makes cells highly sensitive to this process. *S. cerevisiae* cells possess two transporters, Trk1 and Trk2, which ensure a high intracellular concentration of potassium, necessary for many physiological processes. Trk1 is the major system responsible for potassium acquisition in growing and dividing cells. The contribution of Trk2 to potassium uptake in growing cells is almost negligible, but Trk2 becomes crucial for stationary cells for their survival of some stresses e.g., anhydrobiosis. As a new finding, we show that both Trk systems contribute to the relative thermotolerance of *S. cerevisiae* BY4741. Our results also demonstrate that Trk2 is much more important for the cell survival of glucose-induced cell death than Trk1, and that stationary cells deficient in active potassium uptake lose their ATP stocks more rapidly than cells with functional Trk systems. This is probably due to the upregulated activity of plasma-membrane Pma1 H+-ATPase, and consequently, it is the reason why these cells die earlier than cells with functional active potassium uptake.

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Strain level variation in yeast extracellular vesicles

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Extracellular vesicle (EV) is the generic term for particles delimited by a lipid bilayer that are naturally released from cells. EVs are released by cells of all domains of life; they are heterogenous in size (from few up to 1000 nm in diameter), and their function differs depending on their cell of origin, biogenesis mechanism and cargo. In fungi, they represent a way to interact with other organisms through the transport of nucleic acids, proteins and polysaccharides. Although their biogenesis pathways have not been fully clarified yet, composition and functions of fungal EVs have already been observed in different physiological and pathological contexts. The EVs functions which have been identified so far include modulation of the host immune system. One possible hypothesis for this modulation is through the interaction between host immune cells and the small RNAs transported by fungal EVs, which are known to have immunomodulatory potential. This study aims to assess variety in production levels and immunological effects of EVs released by yeast strains from different species and origin. In particular, strains of *Saccharomyces cerevisiae*, *Pichia fermentans* and *Kluyveromyces marxianus*, isolated either from Tuscany grapes or fermented milk of the Yaghnob Valley in Tajikistan, have been screened for their ability to produce high levels of EVs in different growth conditions. Then, immunomodulation potential of extracted EVs have been studied through immune assays after internalization from human dendritic cells. Results showed a significative reduction of antigen presentation ability of dendritic cells which have been treated with EVs from a *S. cerevisiae* and a *P. fermentans* strains isolated from fermented milk. In conclusion, this study suggest that different yeast strains produce EVs with different effects, and that a possible mechanism of beneficial effects of fermented beverages is based on the release of EVs from their yeast strains.

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Life in the bioink: Physiology and energetics of *Saccharomyces cerevisiae* embedded in polymerized Pluronic F127-DMA, a "smart" thermoresponsive hydrogel

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"Smart" materials are substances designed to be responsive to environmental stimuli, e.g., to pH, light, or changes in temperature. One such thermoresponsive polymer is Pluronic F127, a biologically compatible reversely-gelling material, i.e., liquid at near 0°C but hydrogel at room temperature. Because of this property, it can be mixed with cells on ice and then 3D-printed at room temperature, thus serving as a convenient bioink matrix. Ends of Pluronic F127 molecules can also be chemically modified to support the fixation of hydrogel into one permanent shape, no matter the temperature. However, beyond rudimentary observations of biocompatibility, it remains unclear how cells cope with conditions inside such a substance. To investigate this issue, we immobilized yeast *S. cerevisiae* in Pluronic F127-dimethacylate (F127-DMA), molded the produced hydrogel, fixed its shape through photopolymerization, and incubated it in either water or yeast synthetic medium. To investigate physiology and energetics at a single-cell level, we applied a genetically encoded GFP-based ratiometric biosensor which allows for the measurement of physiologically relevant concentrations of intracellular ATP. Our results confirm the biocompatibility of F127-DMA but also show it promotes the development of very heterogeneous cell populations that reflect nutrient and oxygen gradients that exist in the hydrogel. Thus, cells near the edge of the hydrogel proliferate quickly and even form microcolonies. In contrast, those removed from the edge divide infrequently and are seemingly dormant, demonstrating that in bioink and 3D printing, the location does matter.

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Systematic identification of genes whose overexpression functions adaptively in various environments reveals insufficiencies of yeast in laboratory

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Adaptation to the environment is a fundamental characteristic of life, but the mechanism behind it remains unclear. We have developed a method to systematically identify “Genes whose Overexpression Functions Adaptively: GOFAs”, by combining a long-read sequencer, ONT nanopore sequencer and a genome-wide multicopy library, gTOW6000. In this presentation, we present the results of our search for GOFAs under various stresses and show that a comprehensive search for GOFAs reveals what is lacking in laboratory yeast.

For example, under high NaCl concentration, genes related to calcium homeostasis such as ECM27 and GDT1 were obtained as GOFAs. When exposed to high concentrations of NaCl, the cytoplasmic calcium concentration of the overexpression strains of these genes increased compared to the control. When calcium chloride was added to the high-salt medium, the growth rate of the yeast was restored.

We also found that the inhibitory effect of calcium on salt stress was more pronounced in non-laboratory yeasts and was observed in yeasts other than *S. cerevisiae*, such as *S. paradoxus*, *S. bayanus* and *S. mikatae*. This suggested that sodium tolerance requires an adequate amount of calcium, which is lacking in the laboratory environment.

As another example, under heat shock stress, CTR1, a transporter of monovalent copper ions, was obtained as GOFAs. When copper sulfate was added to the medium, the survival rate under heat shock increased. This suggests that copper ions are required for proper heat stress resistance and deficient in the laboratory environment.

The systematic search for GOFAs to elucidate the insufficiencies of yeast in the laboratory can lead to a deeper understanding of yeast physiology under stress and natural growth conditions.

Session 8.3 Chromosome structure and function

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Chromosome organisation in meiosis

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Cohesin organises the genome by forming intra-chromosomal loops and inter-sister chromatid linkages. During gamete formation by meiosis, chromosomes undergo extensive reshaping to support crossover recombination and establish distinctly regulatable domains of sister chromatid cohesion. We show that restriction of chromatin loop size and cohesion maintenance by Eco1 acetyltransferase specializes meiotic chromosomes. Acetylation of cohesin's Smc3 subunit during, but independent of meiotic DNA replication is critical for prophase exit in meiotic DNA break-competent cells. Eco1 antagonises cohesin release by Wpl1, which we show is essential for centromeric cohesion and co-segregation of sister chromatids in meiosis I. In addition, a Wpl1-independent activity of Eco1 sets chromatin boundaries to position loops and build cohesion, thereby allowing sequential homolog and sister chromatid segregation in meiosis I and II. Our findings reveal how local chromosome structuring through Eco1-dependent cohesin regulation directs genome transmission into gametes.

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Regulation of meiotic prophase events by the AAA+ ATPase Pch2

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During meiotic prophase, several events such as recombination and chromosome synapsis are influenced by the AAA+ ATPase Pch2. In budding yeast, cells that lack the AAA+ ATPase Pch2 show an impaired cell cycle arrest in response to synapsis defects. However, unperturbed *pch2*Δ cells are delayed in meiotic prophase, suggesting paradoxical roles for Pch2 in cell cycle progression. Here, we provide insight into the checkpoint roles of Pch2 and its connection to Hop1, a HORMA domain-containing client protein. Contrary to current understanding, we find that Pch2 (together with Hop1) is crucial for checkpoint function in response to both recombination and synapsis defects, thus revealing a shared meiotic checkpoint cascade. Meiotic checkpoint responses are transduced by DNA break-dependent phosphorylation of Hop1. Based on our data and on the described effect of Pch2 on HORMA topology, we propose that Pch2 promotes checkpoint proficiency by catalyzing the availability of signaling-competent Hop1. Conversely, we demonstrate that Pch2 can act as a checkpoint silencer, also in the face of persistent DNA repair defects. We establish a framework in which Pch2 and Hop1 form a homeostatic module that governs general meiotic checkpoint function. We show that this module can-depending on the cellular context-fuel or extinguish meiotic checkpoint function, which explains the contradictory roles of Pch2 in cell cycle control. Within the meiotic prophase checkpoint, the Pch2-Hop1 module thus operates analogous to the Pch2/TRIP13- Mad2 module in the spindle assembly checkpoint that monitors chromosome segregation. I will present our combined work investigating the function and regulation of the AAA+ ATPase, with a focus on its effect of its direct downstream substrate, the HORMA domain-containing RHM complex.

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Ufd4 ubiquitin ligase interacts with cohesin complex and promotes sister chromatid cohesion

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Cohesin is a multiprotein, ring-like complex crucial for sister chromatid cohesion and proper chromosome segregation. It also mediates chromosome condensation, genome organization through chromatin looping and DNA damage repair. In an effort to further our understanding of cohesin function we performed proteomic screen that identified Ufd4 ubiquitin ligase as a potential cohesin interactor. Using proximity ligation assay as well as coimmunoprecipitation assay we confirmed that Ufd4 physically interacts with cohesin. Moreover, we showed that Ufd4 binds to cohesin throughout the cell cycle starting with late G1/early S phase when Scc1 cohesin subunit becomes resynthesized. Next, to investigate if Ufd4 influences protein abundance of cohesin subunits we performed cycloheximide chase analysis. Our results indicate that lack of Ufd4 does not affect protein levels of cohesin and its regulators. Next, we examined whether UFD4 deletion impacts cohesin binding to chromatin. We found that disruption of UFD4 results in mildly reduced cohesin levels at centromeres as well as chromosome arms and rDNA. Interestingly, lack of Ufd4 is accompanied by moderate premature sister chromatid separation but not by defect in chromosome condensation. Taken together, our results suggest that Ufd4 may be an auxiliary factor that promotes cohesin binding to chromatin and thus efficient sister chromatin cohesion. Further research will be required to reveal how Ufd4 influences cohesin functions.

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The telomeric 5' end nucleotide is regulated in the budding yeast *Naumovozyma castellii*

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Telomeres are essential for maintaining chromosomal integrity, by forming protective chromatin structures at the end of linear chromosomes. The telomeric DNA sequences are composed of short tandem repeats and are bound by an array of proteins. In the budding yeast *Naumovozyma castellii*, Rap1 binds the telomeric double-stranded (ds) DNA and Cdc13 binds the single-stranded (ss) 3' overhang, leading to further recruitment of several other telomere proteins. The junction between the ds and ss telomeric DNA, the ds-ss junction, is fundamental in the protection and maintenance of the telomeric structure, as it dictates the interactions between Rap1 and Cdc13.

To investigate the ds-ss junction in *N. castellii*, we developed a PCR-based method, termed Permutation-Specific Telomere PCR (PST-PCR), for determination of the 5' end terminal nucleotide within the 8 bp telomeric repeat. We examined logarithmically growing cells of two wild-type *N. castellii* strains. Out of the eight different possible permutations, we observed that telomeres mainly terminate in either of two specific 5' end permutations of the repeat, both corresponding to a terminal adenine nucleotide. Strikingly, two permutations are completely absent at the 5' end, indicating that not all ds-ss junction structures would allow the establishment of the protective telomere chromatin cap structure. Using in vitro DNA end protection assays, we determined that binding of Rap1 and Cdc13 at the most abundant ds-ss junction ensures the protection of both 5' ends and 3' overhangs from exonucleolytic degradation. Our results provide mechanistic insights into telomere protection, indicating that the telomeric 5' ends are regulated to encompass distinct permutations of the telomeric sequence, and reveal that Rap1 and Cdc13 have complementary protective roles.

253

Unique function of the N-terminal domain of Sld3 in *Schizosaccharomyces pombe*

Mrs. Shiho Ogawa[1], Ms. Sae Ninomiya[1], **Mr. Seiji Tanaka[1]**
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The yeast Sld3 protein is essential for the initiation of DNA replication. During DNA replication, two bidirectional replication forks are established at one replication origin, and at each replication fork, a replicative helicase must unwind the double-stranded DNA. Therefore, to generate bidirectional replication forks at an origin, two replicative helicases are loaded at each replication origin. Because Sld3 has an essential role in the activation of the replicative helicase, two copies of Sld3 are also thought to work at each origin during DNA replication initiation for efficient helicase activation. In the budding yeast *Saccharomyces cerevisiae*, two copies of Sld7 can bridge two copies of Sld3 via its self-interaction domain and this may be the case in mammalian cells. However, in the fission yeast *Schizosaccharomyces pombe*, no Sld7 orthologue has been identified so far. We have tried to identify the Sld7 in *S. pombe*, and this resulted in fail so far. Instead, we have found that *S. pombe* Sld3 (SpSld3) contains a self-interaction domain in its N-terminal portion.
N-terminal 123 amino acids of SpSld3 was sufficient for the self-interaction and the sequence of this domain is only conserved among *Schizosaccharomyces* species and no similar sequences were found in other species. Deletion of this domain showed little effect on cell growth, however, N-terminal point mutants that harbor mutations in this domain that abolish/diminish the self-interaction inhibited cell growth. Interestingly, such mutants also showed abolished/diminished interaction with Cut5. Sld3-Cut5 interaction is essential for the helicase activation and the C-terminal domain of SpSld3, which is conserved in eukaryotes, is responsible for the interaction. Therefore, our results suggest that dimer formation of SpSld3 via its N-terminal domain has a novel role in the initiation of DNA replication in *S. pombe*.

Plenary 6 Lecture

401

Engineering yeast to brew medicines via an integrated systems approach

Mrs. Christina Smolke[1]

[1]*Antheia & Stanford University*

Plants are a rich source of medicinal compounds. However, the discovery, synthesis, and supply chains for plant-based medicines remain ad hoc, biased, and tedious. While microbial biosynthesis presents compelling alternatives to traditional approaches based on extraction from natural plant hosts, many challenges exist in the reconstruction of plant specialized metabolic pathways in microbial hosts. We have developed approaches to address the challenges that arise in the reconstruction of complex plant biosynthetic pathways in microorganisms. We have recently applied these strategies to develop yeast production platforms for important classes of plant alkaloids, including the tropane alkaloids.

Tropane alkaloids from nightshade plants are neurotransmitter inhibitors used for treating neuromuscular disorders and are classified as essential medicines by the World Health Organization. We engineered baker's yeast to produce natural and non-natural medicinal tropane alkaloids starting from simple sugars and amino acids. We combined functional genomics to identify missing pathway enzymes, protein engineering to enable functional acyltransferase expression via trafficking to the vacuole, and strain optimization to improve titers. We further demonstrated that strategies to address metabolite transport limitations can further increase tropane alkaloid production. Our integrated system positions >20 proteins adapted from yeast, bacteria, plants, and animals across six sub-cellular locations to recapitulate the spatial organization of tropane alkaloid biosynthesis in plants. Microbial biosynthesis platforms can facilitate discovery of novel plant natural product derivatives as novel therapeutics for neurological disease and, once scaled, enable robust and agile supply of these essential medicines.

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POSTERS PRESENTATIONS

Room A – Augarten

Poster prizes were kindly granted by the Federation of European Microbiological Societies' (FEMS) journal *FEMS Yeast Research* and by *Microbiology*, a broad-scope microbiology journal published by the Microbiology Society.

A01

Recycling industrial food wastes by using oleaginous yeasts *Rhodosporidium azoricum* and *Trichosporon oleaginosus* for biomass and lipid production.

Ms. Silvia Donzella[1], **Ms. Immacolata Serra[2]**, Mr. Andrea Fumagalli[1], Mrs. Concetta Compagno[1]
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*these authors contributed equally to this work
^corresponding author

Microbial lipids have been emerging as a sustainable alternative to vegetable oils and animal fat to produce not only biodiesel but other industrial relevant chemicals. To develop fermentative processes, the use of waste for microbial growth can represent a way for upgrading low value feedstock to high value products, addressing one of the main goals of circular economy, the reduction of waste by recycle. Two oleaginous yeasts, *Rhodosporidium azoricum* and *Trichosporon oleaginosus*, were used in our study to set a lipid production process starting from pumpkin peel waste.
First, we found the best conditions of enzyme concentration and time of hydrolysis suited for the pumpkin peel treatment, obtaining a sugars mixture mainly consisting of sucrose, glucose and fructose. After the study of sugar utilization in mineral media, batch fermentations were performed using pumpkin peel hydrolysate, which was found to support yeast growth and to induce lipid accumulation without any addition.
Lastly, a two-stage process was carried out in 2L bioreactor using syrup waste from candy fruits manufacture as feed. By this strategy, after 90 hours we produced yeast biomass containing 55% of lipids, that represents a concentration of 24 g/L, and corresponding to a lipid productivity of 0,26 g/L/h and lipid yield of 0,29.
This proof-of-concept study has shown the feasibility to produce yeast biomass and lipids in an economical way using a medium derived from renewable and low-cost feedstocks, demonstrating a possible process of upgrading low value industrial food wastes to high value products.

A02

Cardoon roots valorization by combined microbial biotransformation for lactic acid production

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Cardoon, *Cynara cardunculus L.* from *Asteraceae* family, is a Mediterranean perennial plant, growing naturally in harsh habitat characterized by high temperature, salinity and drought. Interest in valorizing this crop has been growing in recent years. In particular, inulin obtained from the roots can be used as substrate in industrial fermentation processes for the production of different chemicals, therefore with a wide range of applications. In this context, our work is based on the use of cardoon roots hydrolysates as fermentation substrate to obtain a microbial sustainable process for lactic acid (LA) production, known to be an attractive chemical platform with different uses, in particular as monomer for polylactic acid (PLA) production.
In order to release fructose from inulin we exploited the inulinase-producing yeast *Kluyveromyces marxianus*, a promising industrial host for the production of several compounds because of its thermotolerance, high growth rate, and broad substrate spectrum, but poor in respect to organic acid tolerance. For the production of LA from fructose, we exploited two engineered yeasts already characterized for high titer and yield of production and for robustness towards organic acid, respectively: *Saccharomyces cerevisiae* (strain m850 [1]) and *Zygosaccharomyces parabailii* (strain Z2 *ΔLeu+LDH* [2]). The natural ability to hydrolyze inulin was used to set-up and compare two bioprocesses based on cardoon roots: 1) SSF (Simultaneous Saccharification and Fermentation), where the supernatant of *K. marxianus* cultivation was used in media formulation and *S. cerevisiae* or *Z. parabailii* were inoculated for converting the sugar in LA; 2) CBP (Consolidated BioProcess), in which *K. marxianus* was inoculated together with one or the other LA producing yeasts, in different ratio. Our results will be presented and commented, together with future perspectives.

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A03

Rational design of branched-chain amino acid aminotransferases in the yeast *Saccharomyces cerevisiae* to improve branched-chain higher alcohol production

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Branched-chain higher alcohols (BCHAs), including isobutanol, isoamyl alcohol, and active amyl alcohol are beneficial compounds for several industries. In the yeast *Saccharomyces cerevisiae*, BCHAs can be synthesized from the branched-chain amino acids (BCAAs) via the Ehrlich degradation pathway. Metabolism of BCAAs in *S. cerevisiae* is tightly regulated, such as a feedback inhibition mechanism and nitrogen catabolite repression (NCR). Branched-chain amino acid aminotransferases (BCATs) catalyze a bi-directional transamination reaction between α-keto acids (BCKAs) and BCAAs, and have an integral role in the BCAA metabolism. BCATs are encoded by the BAT1 and BAT2 genes in *S. cerevisiae*, which are localized in mitochondria and cytosol, respectively. Although it is apparent that the BCATs are important for BCAAs metabolism and BCHAs production, no investigation has been conducted to clarify the relationship between the primary structures and physiological functions of BCATs in the cell. In this study, we aimed to improve BCHAs productivity by engineering of BCATs. By computational simulation, we obtained six candidates for BCATs variants: Gly316Ser, Gly316Trp, and Ala318Gln for Bat2 (corresponding with Gly333Ser, Gly333Trp, and Ala335Gln for Bat1, respectively). As a result, most of the variants showed a growth defect phenotype in minimal medium. Intracellular BCAAs contents in the variants were not much change, but isobutanol productivities of Gly333Trp (Bat1) and Gly316Ser (Bat2) were greatly increased to 18.5-folded and 17.0-folded, respectively compared to the original wild-type. To confirm the alteration of enzymatic activity on the BCAT variants, we are assaying their enzymatic activity by using the recombinant purified enzymes.

A04

Attachment patterns and metabolism of *Saccharomyces cerevisiae* cells on the surfaces of substrates with strictly defined surface topography

Mr. Yuri Dekhtyar[1], Mrs. Galina Hrustalova[2], Mr. Alexander Rapoport[2], Ms. Linda Rozenfelde[2], Mrs. Lidija Saulite[2], **Mr. Hermanis Sorokins[1]**, Ms. Sabine Teifurova[1]
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Naturally occurring microscale surface topography is composed of many different types of features (singular topographic unit, i.e., a peak or valley) with differing roughness feature parameters (feature height, shape, etc.). When studying the effect of microscale surface topography on cell attachment it is not obvious which kind of topography would be suited for influencing a target cells propensity for attachment. The use of surfaces with strictly defined microscale features could help understand which kind of surface topography is preferential for the attachment of target cells. Surfaces with strictly defined microscale features for the study of the effects that certain roughness feature parameters can have on the cells can be fabricated using microelectromechanical system (MEMS) manufacturing approaches. In this work we demonstrate the attachment of *Saccharomyces cerevisiae* cells to surfaces with strictly defined microscale features fabricated using MEMS manufacturing approaches. The effects that topography feature shape and size and the distance in between individual features have on the attachment and metabolism of the cells are described.
This research is funded by the Latvian Council of Science, project “Engineered surface platform for immobilization of microorganisms”, project No. lzp-2018/ 1-0460.

A05

Production of tryptophan derivatives in non-*Saccharomyces yeasts*

Mrs. Ileana Vigentini[1], Mr. Michele Dei Cas[2], Mrs. Sara Vitalini[3], Mr. Marcello Iriti[3], Mrs. Rita Paroni[2], Mr. Roberto Foschino[1]

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In recent years, investigations regarding the beneficial effects of fermented foods on human health have increased considerably. This effect is often associated with the metabolism of yeasts that can represent a relevant reservoir of functional molecules, TRP derivatives (dTRPs) above all. dTRPs are generated from the kynurenine (KYN) and melatonin (MEL) pathways and they can show antioxidant properties or work as neuro-hormons and/or gastro-protective metabolites. The present study investigated the KYN and MEL metabolisms in TRP-enriched media inoculated with *Torulaspora delbrueckii* and *Zygosaccharomyces bailii* yeast species.
As far the KYN route, *T. delbrueckii* accumulated about 0.27 and 0.11 mg/L of KYN and kynurenic acid (KYNA), respectively. Other potential bioactive compounds were released; in particular, 0.0014 mg/L of anthranilic acid was detected in the supernatants. On the other hands, *Z. bailii* released KYN and derivatives at higher amounts; while KYN concentration was comparable to the one measured in *T. delbrueckii* cultures, KYNA reached about 1 mg/L. Anthranilic acid was accumulated at 0.07 mg/L, together with 3-idroxyantranylic acid and 3-idroxykynurenine (0.01-0.02 mg/L).
Regarding the MEL biosynthesis pathway, the investigated species produced MEL isomers (MIs), at 4.8 min (MI1) and 5.7 min (MI2) retention times, although with different kinetics. Although MEL was never detected, *Z. bailii* resulted the best producing yeast of MIs (0.6 mg/L MI1 and 0.2 µg/L MI2). For the first time a tryptophan ethyl ester (TEE) isomer was identified at 4.8 min retention time in both yeast cultures; it accumulated at 0.02 and 0.37 mg/L in *T. delbrueckii* and *Z. bailii* supernatants, respectively.
In conclusion, considering the high interest in DTRP in the panorama of functional foods, the results provide the basis for in-depth studies on the synthesis of bioactive compounds by non-*Saccharomyces yeasts* and their metabolic role.

A06

Xylose metabolism in *Komagataella phaffii* (*Pichia pastoris*)

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Pichia pastoris is a widely used methylotrophic yeast in the pharmaceutical and biotechnological industries, especially in the production of proteins. It was reclassified to the new genus *Komagataella* and the previous *P. pastoris* was separated into two species: *K. phaffii* and *K. pastoris*. These yeasts are most commonly grown to high cell densities using glucose, glycerol, ethanol, and methanol aerobically as carbon sources. The current understanding is that *Komagataella* strains do not assimilate xylose as a carbon source, although Li et al reported slow growth of non-engineered *K. phaffii* GS115 cells. Xylose is a pentose sugar, second most abundant in nature, and highly abundant in lignocellulosic biomass. It is considered a substrate of interest to produce value-added products.

In this work, a screening for *Komagataella* strains capable of grow on xylose was performed. Growth on xylose was identified in minimal media for *K. phaffii* X33, K. pastoris CBS 704, and K. populi CBS 1236, with xylitol as a by-product. Physiological characterization was done in minimal media supplemented with 2% xylose as the only carbon source. After ten days of cultivation, strains consumed 95%, 47%, and 61% of the available xylose, respectively. To characterize xylose assimilation, cells were grown in 13C-labeled xylose. Elemental Analysis - Isotope Ratio Mass Spectrometry (EA-IRMS) analysis demonstrated that the yeast was capable to assimilate xylose in the cell biomass. The incorporation of 13C was also analyzed in the pentose phosphate pathway and glycolysis metabolites. Transcript levels of the three genes of the putative oxidative xylose pathway (GRE3, SOR1, and XKS1) were analyzed by quantitative PCR for *K. phaffi*i and K. populi. Overall, the pathway is strongly upregulated in minimal media containing xylose as a carbon source when compared to glucose.

A07

Bioconversion of high-toxic glycolaldehyde by engineered *S. cerevisiae*.

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The yeast *S. cerevisiae* is a well-characterized industrial workhouse for the production of fuels, chemicals, and polymers from renewable substrates. Chemical toxicity of biomass-derived substrates poses a significant challenge to deploying *S. cerevisiae* for industrial biotechnology applications, and chemicals with an aldehyde functional group pose significant toxicity on yeast. Glycolaldehdye is ubiquitous short aliphatic, α-oxoaldehyde, commonly found in the biomass-derived substrates. It forms through retro-aldol condensation of sugars, and concentration ranges from 0.1-850 mM. We discovered that glycolaldehyde is a key inhibitor of *S. cerevisiae* fermentation, and uncovered the molecular mechanisms of the toxicity of glycolaldehyde on yeast via comprehensive omics analysis. Next, we developed the in vivo detoxification pathway by reducing glycolaldehyde to less toxic ethylene glycol.1 We further engineered the redox cofactor utilization pathways, and protein quality control machinery, including SUMO-dependent ubiquitination to further improve the tolerance of yeast to glycolaldehyde. Indeed, we established the two-tier molecular toxicity tolerance mechanism against glycolaldehyde, namely detoxification and cell protection.2 The uncovered novel pathways were integrated into the engineered xylose-utilizing strain, and demonstrated the efficient xylose metabolism in the presence of complex inhibitors-containing plant hydrolysate.3 The discovered metabolic routes aid to re-wire the pentose phosphate pathway for co-production of ethylene glycol and ethanol via engineered *S. cerevisiae* strain. An in-depth understanding of glycolaldehyde metabolism and cell defense mechanisms enables the development of industrial relevant biocatalysts for the valorization of high toxic aldehyde-containing unconventional substrates streams such as industrial wastewater streams and plastic.4, 5

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A08

High-level production of lysine in the yeast *Saccharomyces cerevisiae* by rational design of homocitrate synthase

Mr. Shota Isogai[1], Mr. Tomonori Matsushita[1], Mr. Hiroyuki Imanishi[1], Mr. Jirasin Koonthongkaew[1], Mr. Yoichi Toyokawa[1], Mr. Akira Nishimura[1], Mr. Xiao Yi[2], Mr. Romas Kazlauskas[2], Mr. Hiroshi Takagi[1]

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Homocitrate synthase (HCS) catalyzes the aldol condensation of 2-oxoglutarate (2-OG) and acetyl coenzyme A (AcCoA) to form homocitrate, which is the first enzyme of the lysine biosynthetic pathway in the yeast *Saccharomyces cerevisiae*. The HCS activity is tightly regulated via feedback inhibition by the end product lysine. Here, we designed a feedback inhibition-insensitive HCS of *S. cerevisiae* (ScLys20) for high-level production of lysine in yeast cells. In silico docking of the substrate 2-OG and the inhibitor lysine to ScLys20 predicted that the substitution of serine to glutamate at position 385 would be more suitable for desensitization of the lysine feedback inhibition than the substitution from serine to phenylalanine in the already-known variant Ser385Phe. Enzymatic analysis revealed that the Ser385Glu variant is far more insensitive to feedback inhibition than the Ser385Phe variant. We also found that the lysine content in yeast cells expressing the Ser385Glu variant was 4.62-fold and 1.47-fold higher than that of cells expressing the wild-type HCS and Ser385Phe variant, respectively, due to the extreme desensitization to feedback inhibition. In this study, we obtained highly feedback inhibition-insensitive HCS using in silico docking and enzymatic analysis. Our results indicate that the rational engineering of HCS for feedback-inhibition desensitization by lysine and could be useful for constructing new yeast strains with higher lysine productivity.

A09

Identification of major phosphofructokinase genes for D-glucose consumption in *Komagataella phaffi* CBS7435

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Komagataella phaffi (*Pichia pastoris*) is one of the most used host for recombinant protein production. P. pastoris as recombinant protein production host also provide advantages of higher eukaryotic expression system, for example posttranslational modification, protein secretion, protein processing, and protein folding. One of the most important reactions in glycolysis is the phosphorylation of fructose-6-phosphate to fructose-1,6-bisphosphate. There are 3 known genes involved for the phosphorylation of fructose-6-phosphate in P. pastoris: PFK1 (α subunit), PFK2 (β subunit), and PFK300 (γ subunit). In this study, we analyzed the effect of a single deletion of each gene on D-glucose consumption and growth. Deletions were conducted using CRISPR-Cas9 system and the Golden Gate assembly. The growth characteristics of the deletion strains were analyzed using shake flask experiment in a defined medium with 5 g/L D-glucose as the sole carbon source. In line with previous studies, the deletion of the γ subunit did not alter the D-glucose consumption and the growth significantly. However, both the deletion of α and β subunit led to a significant reduce of the growth and D-glucose consumption profile in a defined medium with 5 g/L D-glucose. Deletion of β subunit led to more reduce growth and D-glucose consumption compared to the deletion of α subunit.

A10

“Metabolostasis” of aggregation-prone metabolites: the possible role of Atr1 in adenine transport in yeast

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Adenine is one of the life-essential building blocks that compose DNA. Recent evidence from our lab proved that this ubiquitous molecule, as well as other nucleobases and amino acids, could self-assemble and form cytotoxic amyloid-like structures. These ‘metabolite amyloids’ show significant biophysical and chemical similarity to their proteinaceous counterparts, which have been associated with various human disorders including Alzheimer’s disease, Parkinson’s disease, and type II diabetes. These results provide a novel etiology to Inborn Error of Metabolism disorders, suggesting that metabolite aggregation might be the molecular cause of these diseases. While such metabolite nanostructures have been avidly studied in vitro, little is known about the biological consequences of metabolite self-assembly, as well as of the intracellular homeostatic mechanisms that keep aggregation-prone metabolites from aggregating. Aiming to tackle these fundamental questions, we have recently established a novel in vivo model for adenine accumulation and self-assembly in yeast by blocking the enzymatic pathway downstream of adenine. This manipulation led to the formation of amyloid-like structures and severe growth inhibition upon adenine feeding. Here, we carried out a systematic genome-wide screen to identify genes that rescue the notable adenine sensitivity of the salvage mutant upon overexpression. Using the Synthetic Genetic Array (SGA) methodology we have identified Atr1, a transporter of the evolutionary conserved major facilitator superfamily, as a major suppressor of the phenotype. Interestingly, Atr1 was previously annotated as an efflux pump of 3-aminotriazole, a synthetic toxin that shows notable chemical similarity to adenine. Our results suggest a new and crucial function of Atr1 as an adenine transporter and provide proof-of-concept for the essential role of quality-control mechanisms that maintain metabolite homeostasis – “metabolostasis”.

A11

Comparative analysis of xylose consumption rate of different native pentose-consuming yeasts

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Conversion of all sugars present at lignocellulosic biomass would increase production and reduce cost of second-generation ethanol. *Saccharomyces cerevisiae* is the main yeast used for alcohol production worldwide, but it cannot produce ethanol from xylose, the second most abundant sugar in nature. In general, naturally xylose-fermenting yeasts are capable of fermenting xylose only when the oxygen flow is tightly regulated. Because fermentative conditions such as media composition, cell density and oxygen availability are usually different, a comparative assessment among xylose-consuming yeasts based on literature data becomes difficult. Thus, a systematic comparison of yeasts performance might help to elucidate important steps involved in xylose transport and metabolism.

The aim of this work was to compare the performance of native pentose-consuming yeasts under oxygen-limited and anaerobic conditions. The biomass growth, substrate consumption and products accumulation of *Scheffersomyces stipitis, Spathaspora passalidarum, Pachysolen tannophilus, Ogataea siamensis, Kluyveromyces marxianus, Meyerozyma guilliermondii, Yamadazyma sp., Candida sp., Naganishia sp. and Rhodotorula sp.*, were evaluated in experiments containing xylose as sole carbon source.

The performance of the yeasts was greatly influenced by oxygen availability. All the strains showed higher biomass yields under oxygen limitation whereas the production of ethanol was variable according the considered. While *Scheffersomyces stipitis* and *Spathaspora passalidarum* exhibited the highest xylose consumption specific rate under oxygen limitation, *Ogataea siamensis, Pachysolen tannophilus, Kluyveromyces marxianus, Meyerozyma guilliermondii, Yamadazyma sp*. and *Candida sp*. showed an intermediate performance among the assessed yeasts.

A12

Use of oleaginous yeasts for conversion of agro-industrial biomasses in high added-value bio-products

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In recent years, oleaginous yeasts have attracted scientific attention as a sustainable route of implementing circular economy. These microorganisms are extremely versatile since they are able to grow on several low-cost carbon sources and accumulate high titer lipids, up to 70% of their dry cell weight. The main metabolic products are triglycerides, accumulated as neutral intracellular lipids in conditions of nitrogen starvation and excess carbon source. These microbial lipids, better known as Single Cell Oils, show a fatty acids profile very similar to some vegetable oils, representing a sustainable resource of oils for producing biofuels, bioplastic, biosurfactant, etc...

The present work optimized the use of an oleaginous yeast, *Lypomices tetrasporus* (DSM 70314), for the conversion of wheat straw after the steam-explosion pretreatment, in triglycerides. A detailed study on the microorganism metabolism was carried out to overcome the inhibiting effect, typical of lignocellulosic biomass, and improve the performance of microbial growth and lipid production. Data showed that *L. tetrasporus* was able to metabolize lignocellulosic sugars, almost all the sugars in the biomass hydrolyzates, namely glucose, galactose, and xylose. Arabinose was not converted even in prolonged fermentations. Furthermore, the yeast produced mainly polyols or triglycerides depending on the initial sugar concentration. The results obtained showed that the metabolic phase of inoculum plays an important role in reducing the hydrolyzate toxicity and the overall productivity. Under optimized conditions, such as pH, sugar concentration, C / N ratio, and nitrogen source, a final lipid yield (g lipid/g sugars consumed) of 23% was obtained, corresponding to 62% of the lipid content (g lipid/ g cellular biomass produced).

These preliminary results indicate that *L. tetrasporus* might represent a promising microbial cell factory for the sustainable conversion of agri-food wastes in added value-products for several applications in the food and chemical fields.

A13

Metabolic regulation adapting to high methanol environment in the methylotrophic yeast *Ogataea methanolica*

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Efficient conversion of high methanol into value-added products contributes to the construction of a revolutionary carbon-neutral material circulation system. However, the methylotrophic yeast, capable of using methanol as sole carbon and energy source for proliferation, shows a severe growth retardation under high methanol (5% or more) condition. In this study, metabolite profiling of methylotrophic yeast *Ogataea methanolica* was conducted under glucose and low and high methanol (L- and H-MeOH) conditions to show the adaptation mechanism to a H-MeOH environment. The yeast strain responded not only to the presence of methanol but also to its concentration based on the growth condition. Under H-MeOH conditions, O. methanolica downregulated the methanol utilization, glycolytic pathway and alcohol oxidase (AOD) isozymes and dihydroxyacetone synthase (DAS) expression compared with L-MeOH-grown cells. However, levels of energy carriers, such as ATP, were maintained to support cell survival. In H-MeOH grown cells, reactive oxygen species (ROS) levels were significantly elevated. Along with increasing ROS levels, ROS scavenging system expression was significantly increased in H-MeOH-grown cells. Thus, we concluded that formaldehyde and H_2O_2, which are products of methanol oxidation by AOD isozymes in the peroxisome, are overproduced in H-MeOH grown cells, and excessive ROS derived from these cells is generated in the cytosol, resulting in upregulation of the antioxidant system and downregulation of the methanol utilizing pathway to suppress overproduction of toxic intermediates. Our results might provide insights into improving high methanol utilization by methylotrophic yeasts from the perspective of synthetic biology and metabolic engineering.

A14

A novel in-vivo model for BCAA self-assembly and therapeutic targeting in Maple Syrup Urine Disease

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It was recently shown in our lab that metabolites, as simple as single amino acids and nucleobases, can form amyloid-like structures, thus providing a novel paradigm for inborn error of metabolism (IEM) disorders. Here, we wish to explore a never tested hypothesis suggesting that the systemic pathology following BCAAs accumulation in the metabolic disorder Maple Syrup Urine Disease (MSUD) may be related to the formation of amyloid-like structures. Our preliminary data provides a proof-of-concept for this hypothesis, indicating that BCAAs can forms unique assemblies with amyloid-like characteristics. Therefore, we postulate that high levels of BCAAs can lead to the formation of toxic structures that in turn can be involved in the cytotoxicity observed in the disorder. This discovery can offer new prospects for understanding the complex etiologies of the disease in order to find proper treatment for MSUD patients. Recently, the first in vivo yeast model for IEM disorder was established in our lab by genetically modifying the adenine salvage pathway to reflect the situation in patients coping with IEMs that are associated with the accumulation of adenine and its derivatives. Here, we utilize our unique expertise and knowledge in metabolite self-assembly and yeast models for IEMs to address fundamental issues concerning MSUD and for identification of therapeutic leads.

A15

Understanding erythritol biosynthesis pathway in *Saccharomyces* involved in osmotic stress response

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Recently, clear metabolic differences among the yeast species *S. cerevisiae*, *S. uvarum*, and *S. kudriavzevii* have been described. *S. cerevisiae* directs the carbon flux through ethanol production meanwhile *S. uvarum* and *S. kudriavzevii* produce higher amounts of glycerol, succinic acid, 2,3-butanediol, erythritol and 2-phenyl ethanol than *S. cerevisiae*. This indicates that the NADH/NADH+ cofactor regeneration is different among these species. The genes involved in erythritol production have never been described in *Saccharomyces*, thus the study of the origin of the erythritol synthesis pathway is interesting. Erythritol is also produced by yeasts such as *Candida magn*oliae and *Yarrowia lipolytica* in which its synthesis pathway has recently been elucidated. This pathway starts with the pentose phosphate pathway, where erythrose-4-P is produced and is subsequently dephosphorylated to erythrose, which is reduced by an erythrose reductase into erythritol.

To determine the putative genes responsible for the reduction of erythrose to erythritol in *Saccharomyces*, we used phylogenetic and genetic comparative approaches with *Y. lipolytica* erythrose reductases. Consequently, the genes YOR120W, YDR368W, YHR104W, YBR149W and YJR096W were selected in *Saccharomyces*. To test if these putative genes are responsible for the reduction of erythrose to erythritol, they were knocked out by CRISPR/Cas 9 in *S. uvarum*, which was the species with a higher erythritol production. Afterwards, fermentations were performed in different media with the knockouts and the production of erythritol and other metabolites was measured by High Performance Liquid Chromatography (HPLC).

Our results showed a decrease in the production of erythritol by the single, double, and triple mutants compared to the wild type, indicating that several genes could be involved in the synthesis of erythritol. Moreover, the production of erythritol was higher under osmotic stress, which is in accordance with the presence of regulatory elements, involved in osmotic stress response, in the promoters of these putative genes.

A16

Metabolic Flux towards trehalose de novo synthesis in MAF1 mutant can be reversed by Bcy1

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Comprehensive understanding of microorganism's cellular metabolism is required for efficient engineering of "cell factories".
We reported (Adamczyk and Szatkowska, 2017, Szatkowska et. al. 2019), that RNA Polymerase III (RNAP III), as well its negative regulator Maf1, can act as intracellular factors that indirectly regulate the glycolytic flux in yeast cells.
RNA Polymerase III (RNAP III) synthesizes transfer RNA (tRNAs), the essential carrier for amino acid delivery to protein synthesis, and therefore promotes efficient growth. RNAP III activity is regulated by glucose availability through Maf1, a mediator of a range of stress signals including glucose starvation, oxidative stress, DNA damage that is highly conserved from yeast to human (Boguta 2013).
Cells lacking Maf1 demonstrate proficient sugar metabolism and storage carbohydrates accumulation under glucose rich conditions without heating the culture (Patent PL426329). This is not due to Snf1 kinase or Pho85 phosphatase, the modulators of transcriptional and posttranslational activity of metabolism, instead it is dependent on PKA signaling pathway activity.
The observation opens entirely new perspectives for regulation of carbon metabolism in yeast.
This report has direct consequences for biotechnologists, seeking to engineering metabolic pathways in yeast affecting glycolysis, where inadvertent growth issues or protein imbalance can be introduced. It is also relevant to cancer biologists, since hMaf1, consistent with its role as a tumor suppressor, suppresses cellular transformation and tumorgenesis by inhibiting RNAP III from unrestricted tRNA synthesis.

We acknowledge financial support from Faculty of Chemistry WUT Project RND NCHEM2 no. 504/04632/1020/43.120010

1. Adamczyk and Szatkowska 2017 PlosOne
2. Szatkowska et.al. 2019 Biochemical Journal
3. Boguta 2013 BBA Gene Regulatory Mechanisms
4. PL426329 registered at the Patent Office of the Republic of Poland

A17

The toxic effects of Ppz1 overexpression involve Nha1-mediated deregulation of K+ and H+ homeostasis

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Modulation of protein function by phospho-dephosphorylation is a major mechanism for controlling cellular processes, and alteration of the fine-tuned balance between protein kinases and phosphatases often results in functional disturbance. In the yeast *Saccharomyces cerevisiae*, overexpression of Ser/Thr phosphatase Ppz1 drastically blocks cell proliferation, causing profound changes in the transcriptomic profile and in the phosphorylation status of hundreds of proteins. While this effect seems to derive from the alteration of multiple targets, the precise mechanisms are still obscure. Ppz1 is a negative effector of potassium influx. However, we have found that the toxic effect of Ppz1 overexpression is unrelated to the Trk1/2 potassium importers. We show that cells overexpressing Ppz1 display decreased K+ content and increased intracellular acidification and fail to properly acidify the medium. These effects are counteracted by deletion of NHA1, encoding a plasma membrane H+/Na+,K+ antiporter. Lack of Nha1 also attenuates the growth defect of cells overexpressing Ppz1. We postulate that high levels of Ppz1 hyperactivate Nha1, leading to an exacerbated entry of H+ and efflux of K+ that would be detrimental for growth. Consistently with this model, the beneficial effect of lack of Nha1 vanishes as pH of the medium approaches neutrality.

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A18

Phosphoproteomic responses of AMPK/Snf1 pinpoint multilayered interactions with TORC1 signalling

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The eukaryotic AMP-activated protein kinase (AMPK/Snf1) and the target of rapamycin complex 1 (TORC1) signalling pathways sense the availability of energy and nutrients to control cell growth in opposing manners. Accordingly, AMPK/Snf1 promotes catabolic and stress response pathways when cellular energy or glucose levels are low, while TORC1 inhibits these processes and stimulates anabolic metabolism when nutrients are abundantly available. In mammals, coordination of AMPK and TORC1 involves crosstalk between these signalling pathways at different levels (e.g., AMPK phosphorylates TSC2, a GAP for the TORC1 activator RHEB, and controls TORC1 by phosphorylating its subunit RAPTOR, while TORC1 regulates AMPK by phosphorylating its α1/α2 subunits), but these mechanisms are only in part conserved across eukaryotes. Here, we studied the potential crosstalk between AMPK/Snf1 and TORC1 in *Saccharomyces cerevisiae* using a CRISPR/Cas9-generated, 2NM-PP1 ATP-analog-sensitive Snf1-I132G mutant. Interestingly, we found that inhibition of Snf1-I132G with 2NM-PP1 prevented the proper dephosphorylation of the bona fide TORC1 target residue Thr737 in Sch9 in glucose-limited cells. This observation highlights a role of Snf1 in downregulation of TORC1 and/or in mediating the dephosphorylation of the TORC1 target Sch9. To discriminate between these possibilities and to potentially identify additional points of convergence between the AMPK/Snf1 and TORC1 pathways, we performed a set of SILAC-based quantitative phosphoproteomic experiments using 2NM-PP1 and the Snf1-I132G strain. Our data indicate that Snf1 may act upstream of TORC1 by impinging either on Lst4, a subunit of the TORC1-regulatory Rag GTPase GAP Lst4-Lst7, or on Pib2, a presumed glutamine sensor that acts upstream of TORC1. In parallel, Snf1 also phosphorylates Sch9 and thereby modulates the impact of TORC1 on this key effector. Hence, our data suggest that the AMPK/Snf1 and TORC1 signalling pathways are intricately wired to each other, which likely serves to ensure coordinated and homeostatic responses to cellular energy and nutrient levels.

A19

Indole-3-acetic acid is a physiological inhibitor of TORC1 in budding yeast

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In eukaryotes, the Target of Rapamycin Complex 1 (TORC1) is a conserved kinase complex responsible for the regulation of crucial cell functions, such as growth and metabolism, acting on processes like protein synthesis, ribogenesis and autophagy. The signals impinging on TORC1 vary from basic building blocks like amino acids, which are sufficient to activate TORC1 in organisms like the budding yeast *S. cerevisiae*, to hormones and growth factors, which, combined with amino acids, are primarily important to activate TORC1 in higher eukaryotes. Whether TORC1 can also be negatively regulated by physiologically relevant metabolic inputs is currently not known. In this context, we have discovered that indole-3-acetic acid (IAA), best known for its role as a hormone that regulates cell division and growth in plants, potently inhibits growth of yeast cells. Furthermore, through a chemical-genetic screening and a SAturated Transposon Analysis in Yeast (SATAY), we pinpointed the TORC1 pathway as a target of IAA in vivo.
We thus measured the effects of IAA on TORC1 activity in vivo (by measuring the phosphorylation of its target Sch9) and in vitro (by assaying the kinase activity of highly purified TORC1 complexes). Surprisingly, IAA caused a rapid, dose-dependent inactivation of TORC1 both in vivo and in vitro. In addition, we observed that a yeast strain which bypasses the essential function of TORC1 was highly resistant to IAA, indicating that TORC1 is the major target of IAA in yeast. Notably, we demonstrated that yeast cells appear to synthesize IAA at levels that are relevant for TORC1 inhibition, supporting the intriguing possibility that the physiological levels of IAA may in fact represent an endogenous signal to modulate TORC1 activity in vivo.

A20

Regulatory role of kinases on K+ homeostasis in *Saccharomyces cerevisiae*

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S. cerevisiae is widely used as a model organism to study ion homeostasis. It can tightly regulate its intracellular ion concentration. K+, as the major cation, plays important role in physiological processes such as osmotic regulation, protein synthesis, and enzyme activities. It is also essential to maintain cytosolic pH and membrane potential. In *S. cerevisiae*, five different plasma membrane transport proteins (Trk1, Trk2, Nha1, Ena1-5, and Tok1) regulate the intracellular concentration of this cation. Tok1, Trk1, and Trk2 are potassium specific, whereas Nha1 and Ena1-5 are also required for the efflux of other monovalent cations. We have identified five kinases (Ptk2, Sky1, Snf1, Hal5, and Hog1), which might play a regulatory role for these transport proteins. To elucidate the role of kinases in cation homeostasis and potassium transport in detail, corresponding genes were deleted in strains lacking various combinations of genes encoding cation transporters. The resulting phenotypes of strains were compared and analyzed by growth assays, intracellular pH, and membrane potential measurements. Deletion of kinase genes in the BY4741 wild type, in the BYT12 strain lacking potassium importers (*trk1*Δ *trk2*Δ) and in the BYT45, strain without the two main potassium exporters (*nha1*Δ *ena1-5*Δ) revealed the role of most of the tested kinases in potassium homeostasis. The observed phenotypes also depended on the source of nitrogen in the growth medium and on its pH.

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A21

Towards the characterization of a protein-protein interaction that is essential for overcoming stress

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The eIF2α protein kinase Gcn2 is present in virtually all eukaryotic cells, from yeast to human. It helps cells to cope with nutrient starvation. Under amino acid depletion, Gcn2 phosphorylates eIF2α. Increased eIF2α phosphorylation leads to reduced global protein synthesis and at the same time to increased translation of the transcription factor Gcn4. Gcn4 then changes the cell's transcription profile to help adapt and overcome starvation. Activation of Gcn2 in response to amino acid starvation requires the physical interaction between Gcn2 and Gcn1. Gcn1-Gcn2 complex formation is mediated by the N-terminal RWD domain in Gcn2 and the RWD-binding domain (RWDBD) in Gcn1. Gcn2 has been implicated in various diseases such as cancer and Alzheimer's. Cancer cells take advantage of Gcn2 and are dependent on Gcn2 to satisfy their high nutritional demand. Our aim is to investigate in detail the Gcn1-Gcn2 interaction using yeast as a model organism, site-directed mutagenesis and in vivo Gcn2 activity screening.

A22

K+-specific importers Trk1 and Trk2 play different roles in Ca2+ homeostasis and signalling in *Saccharomyces cerevisiae* cells

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The maintenance of K+ and Ca2+ homeostasis is crucial for many cellular functions. Potassium is accumulated in cells at high concentrations. To ensure its signalling function, the cytosolic level of calcium is kept at low levels and transiently increases in response to stresses via a transport of Ca2+ both from the extracellular environment and from intracellular stocks. To find out a possible interconnection between K+ and Ca2+ homeostasis in *S. cerevisiae* cells, we examined in detail Ca2+ tolerance, total Ca2+ content, and Ca2+ cell response to stress in a set of strains with disturbed K+ homeostasis, i.e., lacking plasma-membrane K+ influx (Trk1, Trk2) and/or efflux (Tok1, Nha1, Ena1-5) systems.

The absence of K+-importer Trk1 highly increased cell sensitivity to elevated extracellular Ca2+, though these cells contained a similar amount of total Ca2+ to wild-type strain. In contrast, the lack of Trk1 homolog, Trk2, decreased cell Ca2+ tolerance marginally, but it doubled steady-state intracellular Ca2+ levels. Monitoring of cytoplasmic changes of the concentration of Ca2+ revealed that Ca2+ response to osmotic or CaCl2 stresses was highly enhanced in cells without K+ importers. We experimentally proved that mainly the part of the Ca2+ flux through the plasma-membrane was diminished in cells lacking both Trk transporters. It shows that the absence of Trk1 (and Trk2) altered the balance between Ca2+ flux from external media and intracellular compartments. The deletion of genes encoding K+ exporters only slightly increased the cytosolic Ca2+, but it did not change other tested Ca2+ related phenotypes.

All our data show that K+-importers Trk1 and Trk2 play important and distinct roles in the maintenance of Ca2+ homeostasis and we describe new interconnections between potassium and calcium homeostasis in *S. cerevisiae*.

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A23

Ribosomal protein defects unravel a function of the Arf1 GTPase in controlling TORC1 activity

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Living organisms require nutrients and energy to grow and survive. To sense and respond these cues, eukaryotes employ, among others, the TORC1 signalling pathway, which balances cellular anabolism and catabolism preferentially in response to amino acids. While the intracellular levels of these amino acids strongly depend on their extracellular availability, genetic mutations can alter the intracellular amino acid profile, as demonstrated by a study of the amino acid metabolome and its response to systematic gene deletions in yeast (Mülleder et al., 2016). Notably, TORC1 pathway mutants exhibited elevated levels of asparagine and glutamine, which may result from the combined inhibition of protein synthesis and stimulation of cataplerotic reactions when TORC1 activity is low. Cluster analyses further indicated that some ribosomal protein gene mutants (i.e., *rps10A*Δ, r*pl20B*Δ, and *rps12*Δ), as well as *ygl188C-a*Δ that carries a deletion of a non-coding ORF in the 5'UTR of RPS26A, exhibited a similar amino acid profile signature like TORC1 pathway mutants, suggesting a functional link between ribosomal proteins and TORC1. Here, we describe that loss of Rps26a transiently protects TORC1 from inactivation during nitrogen starvation. This effect is independent of the known amino acid-driven TORC1 regulators Gtr1/2 Rag GTPases or Pib2 and is not modulated by the Gcn2 kinase that has been suggested to inhibit TORC1 in amino-acid-starved cells (Yuan et al., 2017). Interestingly, loss of the small GTPase Arf1, which has been implicated in mediating asparagine and glutamine signals to TORC1 in mammals (Meng et al., 2020), rendered TORC1 in *rps26*Δ cells again sensitive to nitrogen starvation. Therefore, we entertain a working hypothesis according to which loss of Rps26a causes, likely via a reduction in protein synthesis rates, an increase in intracellular amino acids that transiently buffer the amino acid pool during amino acid starvation and thereby contribute to Arf1-dependent stimulation of TORC1.

A24

Functional characterization of the SUR2 gene encoding sphinganine hydroxylase in oleaginous yeast *Yarrowia lipolytica*

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Sphingolipids are essential membrane components found in mammalian cells as well as in plants and microbes. The biosynthesis of sphingolipid begins with the condensation of L-serine and palmitoyl-CoA to yield the C18 carbon unit 3-ketosphinganine, which is reduced to yield sphinganine (dihydrosphingosine). In yeast, significant portion of dihydrosphingosine is converted to phytosphingosine via hydroxylation at the C4 position by sphinganine C4-hydroxylase. Phytosphingosine is used exclusively for the synthesis of inositol-containing ceramides, whereas dihydrosphingosine is mainly used for glucosylceramide synthesis in non-Saccharomyces yeasts. In this study, to block the phytosphingosine-based sphingolipid biosynthetic pathways in the oleaginous yeast *Yarrowia lipolytica*, a mutant strain with the deletion of the SUR2 gene, encoding sphinganine C4-hydroxylase, was constructed and functionally characterized. The resultant *Y. lipolytica* sur2 null mutants (*Ylsur2*Δ) exhibited a retarded growth with increased pseudohyphal formation and displayed the increased sensitivity to high temperature, osmotic, and cell wall stresses compared to the wild-type strain. The treatment with myriocin or exogenous addition of phytosphingosine did not rescue the growth defect of the *Ylsur2*Δ mutant, suggesting that neither the accumulation of dihydrosphingosine or the deficiency of phytosphingosine is associated with the growth defect. Notably, the *Ylsur2*Δ mutant showed increased production of dihydrosphingosine, which was mostly secreted to the cell surface or extracellular medium even without acetylation. Also, a higher amount of dihydrosphingosine was observed in *Ylsur2*Δ cultured in GB medium compared to YPD medium. Furthermore, the *Ylsur2*Δ mutant showed the remarkably increased synthesis of glucosylceramides in the form of sphingosine-based ceramides, which are also mostly detected at the cell surface. Taken together, our results suggest the potential of *Ylsur2*Δ strain as a production host for cell surface-associated sphingosine-based ceramides, as well as a sphingosine precursor, dihydrosphingosine, which are useful ingredient for cosmeceutical or nutraceutical formulations.

A25

Yeasts associated with juice extraction areas for sucrose production in a cane mill from Colombia

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Reducing sucrose losses in sugar production, mainly affected by physical, thermal, microbiological, enzymatic, and operational activity, is one of the main problems of the sugar industry. This work aimed to document the cleaning routines used in the tandem mills of a cane mill, demonstrating how the changes made in the structure of these routines affect indicators such as the estimated inversion and the difference in the purity of cane juice. The assessment of some of the metabolites produced by lactic acid and acetic acid bacteria allowed the identification of two possible points of contamination, which could be related to cleaning issues. The microbiological analysis of these critical points permitted us to get 41 yeast strains, that were grouped in 12 morphotypes. One representative yeast for each morphotype was our chosen for molecular identification, using the ITS region, showing genera such as *Kluyveromyces, Candida, Wickerhamiella, Rhodotorula*, and *Saccharomyces*. Only two yeasts were identified to the taxonomic level of species, belonging to *Kluyveromyces marxianus* and *Candida tropicalis*. Other yeasts were not identified when were compared to type sequences documented in the NCBI databases, suggesting that the juice extraction area from this cane mill could be a reservoir for new yeast species

A26

Investigating the interactome of ubiquitin ligases using a sensitive protein-fragment complementation assay

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The process of protein ubiquitination is essential for the proper functioning of eukaryotic cells. Regulation of many cellular processes, e.g., cell growth and proliferation, depends on this modification. Three types of enzymes work in concert to conjugate ubiquitin to target substrate proteins. Among them, the ubiquitin ligases recognize substrates to be modified and ensure ubiquitination specificity. Thus, they have become attractive targets for drugs against diseases caused by perturbed ubiquitination pathway. For instance, overexpression of some of the ubiquitin ligases has been linked to different types of cancers. Therefore, understanding of the molecular functions of ubiquitin ligases is essential for the development of processes-specific therapies.

Here, we focus on two ubiquitin ligases, which have been shown to be involved in the cellular response to DNA damage in the yeast *Saccharomyces cerevisiae*. We investigate the mechanism of action, and substrates' recognition mechanisms to explain their cellular consequences. We use a modern protein-fragment complementation assay (the NanoBiT® technique) to dissect the interactome of these ligases and decipher their substrates. This assay is performed in live cells with minimal perturbation.

Our preliminary results allowed to uncover the interactome of ligases of interest. We confirmed previously described interactions and identified new potential interactions. We were also able to confirm that the variants of the complementation system we use, enable the study of proteins involved in ubiquitination. This technique will enable us to learn about the effects of their ubiquitination in a broader range of cellular processes. In addition, the optimized system used in a high-throughput format allows various types of research investigating both ubiquitination and other pathways, and may contribute to the development of novel therapies.

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A27

Transcriptome analysis of nitrogen-starved *Pseudozyma hubeiensis* BOT-O, an oleaginous yeast that utilizes glucose and xylose at equal rates

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A majority of the yeasts known to be able to utilize xylose does so at lower rates than that of glucose. One species that has been shown to grow equally well on both sugars, and also to have the capacity to co-ferment glucose and xylose, is the basidiomycete *Pseudozyma hubeiensis* (Tanimura et al, 2016). While most of the research on this species has been focused on its ability to produce and secrete biosurfactants (glycolipids; Morita et al, 2013), it is also capable of accumulating high amounts of storage lipids intracellularly. Previously, *P. hubeiensis* BOT-O was isolated in the Gothenburg botanical garden and found to produce storage lipids up to 40% of biomass during nitrogen-starvation with xylose as a carbon source. In the current study, we have sequenced and annotated the BOT-O genome using a combination of long-read sequencing (MinION) and Illumina paired-end sequencing.

We have subsequently compared the growth on glucose and xylose, in log phase and during early nitrogen starvation. Similar to previous reports on *P. hubeiensis*, BOT-O grew equally well on glucose and on xylose with comparable growth and consumption rates (0.23 h-1, 0.20 g/h and 0.21 h-1, 0.19 g/h for glucose and xylose respectively), but produced more total lipids on glucose. Samples were also analyzed by RNAseq. A high number (~900) of significantly up- or down-regulated genes were detected after N-starvation on either of the sugars, but when exponential growth on glucose was compared to that on xylose, the difference was much less pronounced (~70 genes). To our knowledge, this is the first time the transcriptome of *P. hubeiensis* has been studied, which will, among other things, give an insight into the fatty acid metabolism of this less characterized oleaginous yeast.

Morita et al. FEMS yeast research (2013), 13(1), 44-49.
Tanimura et al. AMB Expr (2016) 6:58

A28

Uncovering the potential of *Candida intermedia* as a cell factory by understanding lactose metabolism

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Cheese whey run off from the Dairy industry contains large amounts of lactose. Using microbial cell factories, this inexpensive carbon source can be used for the production of biochemicals (Liu et al., 2016). *Candida intermedia* is a haploid yeast belonging to the CTG clade of fungal species and is one of the few yeasts with the ability to utilize lactose (Riley et al., 2016). In this project we aim to elucidate the gene clusters and pathways involved in lactose utilization in C. intermedia, and to use this knowledge to engineer the yeast's metabolism to produce value-added chemicals.

We have adopted a systems biology approach to map out the genes and pathways involved in lactose metabolism in this yeast by combining experimental and computational data. Preliminary data suggest that the genes involved in lactose utilization in *C. intermedia* are co-localized in the genome, and deletion mutant analysis shows that a novel gene cluster plays a crucial role in *C. intermedia's* growth on lactose. Reconstruction of a Genome Scale Metabolic Model (GEM) followed by Reporter Metabolite Analysis (Patil & Nielsen, 2005) indicates that *C. intermedia* uses an oxidoreductive pathway in concert with the conventional Leloir pathway for lactose metabolism. We also show that *C. intermedia* can be metabolically engineered to produce Galactitol, an industrially important polymer (Natarajan et al., 2017) and precursor for chemotherapeutic agent (Jiang et al., 2017), by growth on lactose, thus, exploring *C. intermedia's* potential as a future cell factory.

A29

Predicting sluggish fermentation dynamics requires knowledge on yeast biomass composition.

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The yeast *Saccharomyces cerevisiae* is an essential microorganism in food biotechnology; particularly, in wine and beer making. During wine fermentation, yeasts transform sugars present in the grape juice into ethanol and carbon dioxide. The process occurs in batch conditions and is, for the most part, an anaerobic process.

Problematic fermentations still occur in the winemaking industrial practice. Problems include sluggish rates of fermentation, which have been linked to insufficient levels of assimilable nitrogen. Data and relevant models can help anticipate poor fermentation performance. Previous studies linked limited-nitrogen conditions with problematic fermentations, with negative consequences for the performance of the process and the quality of the final product. It is, therefore, of the highest interest to anticipate such problems through mathematical models.

In this work, we proposed a model to predict biomass growth and fermentation rate under nitrogen-limited conditions and tested its performance with previously published experimental data. We separated the biomass formation into two phases: growth and carbohydrate accumulation. Growth was modelled using the well-known Monod law while carbohydrate accumulation was modelled by an empirical function, analogous to a proportional controller activated by the limitation of available nitrogen. We also proposed to formulate the fermentation rate as a function of the total protein content when relevant data are available. The final model was used to successfully explain experiments taken from the literature, performed under normal and nitrogen-limited conditions.

Our results revealed that Monod law is insufficient to explain biomass formation kinetics in nitrogen-limited fermentations of *S. cerevisiae*. The goodness-of-fit of the herewith proposed model is superior to that of previously published models, offering the means to predict and control fermentations.

A30

NADH kinase-NADP+ phosphatase cycling as proposed mechanism for redox balance alleviation during xylose fermentation in *Scheffersomyces stipitis* and *Saccharomyces cerevisiae*

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To become economically feasible, industrial implementations of sustainable biological pathways such as the conversion of lignocellulosic xylose into ethanol and other value-added products, require careful optimization of process conditions. For example, having to maintain a well-defined oxygen uptake rate is significantly more expensive than being able to run a process anaerobically. The yeast *Scheffersomyces stipitis* is able to ferment xylose to ethanol under oxygen-limited conditions using NADPH-preferring xylose reductase (XR) and NAD+-dependent xylitol dehydrogenase (XDH), while the industrial workhorse *Saccharomyces cerevisiae* expressing the same genes requires full aerobicity for xylose catabolism. The differences in phenotype between these species could be partly attributed to a superior NADPH regeneration capacity in *Sc. stipitis*, but the exact mechanisms have yet to be elucidated. In the present work, we used a genome-scale network reconstruction of *Sc. stipitis* to identify an NADH kinase-NADP+ phosphatase cycle (KPC), putatively encoded by SsUTR1 and SsPHO3.2, respectively. Indeed, purified SsUtr1p and SsPho3.2p were found to exhibit KPC activities in vitro. In contrast, while the *Sa. cerevisiae* genome was found to contain several potential SsUTR1 homologs, no SsPHO3.2 counterpart could be identified, indicating an incomplete KPC. Furthermore, in silico flux balance analysis of *Sa. cerevisiae* predicted that implementation of a KPC would allow for oxygen-limited xylose fermentation into ethanol at rates comparable to fully aerobic conditions. The modelling results were tested by simultaneous overexpression of SsPHO3.2 and native SsUTR1 homologs in a *Sa. cerevisiae* background strain utilizing xylose using *Sc. stipitis* XR/XDH. The resulting strains exhibited KPC activities in vitro, but no improvement in xylose fermentation capacity was observed. In fact, overexpression of certain NADH kinases (e.g., cytosolically targeted ScPOS5) resulted in growth defects, possibly due to depletion of NADH and/or ATP. These results indicate that for the KPC to be effective, careful regulation of its expression is required.

A31

Study the effect of overexpression of methanol dissimilation pathway genes on the recombinant eGFP production in *Pichia pastoris*

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Expression and secretion of heterologous proteins is a highly energy-demanding process and usually led to a limitation in co-factor availability and altered metabolic flux distribution. *Pichia pastoris* is a well-known expression platform widely used for the production of heterologous proteins, but there is still a considerable gap of improvement for this host system. Engineering of metabolic pathways in *Pichia pastoris* can efficiently improve the energy supply and reduce the metabolic burden for recombinant protein production. Herein, we evaluate the overexpression of three genes (FLD, FGH, and FDH) involved in the methanol dissimilation pathway, on the production of eGFP as the model recombinant protein. The recombinant clone of *Pichia pastoris* expressing secretary eGFP under the control of AOX1 promoter was constructed using PichiaPink™ Expression system. The nucleotide sequences of FLD, FGH, and FDH were obtained from *Pichia pastoris*' genomic DNA, cloned in the pPICZA, and transferred to the previously obtained clones expressing eGFP. The clones with zeocin resistance (100 µg/ml) were examined for the presence of both model protein and each of the FLD, FGH, and FDH by PCR with specific primers and sequencing. To discriminate the intrinsic *Pichia pastoris* FLD, FGH, and FDH from the newly introduced one, the PCR with the forward primer of pAOX1 and reverse specific primer (and vice versa) have been applied. The results indicated no significant difference in eGFP fluorescent intensity when model protein was co-expressed with each of the candidate genes and fed with 1% methanol. However, the increased concentration of methanol (2.5%) caused a 2.6 fold increase in the detected fluorescence when eGFP was co-expressed with FGH. These results may be due to the dual functions of FGH in the methanol dissimilation pathway and as a reaction that supplies the intracellular glutathione pool.

A32

Establishing *Pichia pastoris* peroxisomes for compartmentalized 3-Hydroxypropionic acid synthesis using oleic acid as a carbon source

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The yeast *Pichia pastoris* (*Komagataella phaffi*i) is well-established as a model organism for the study of peroxisome biogenesis and proliferation. Compartmentalization of metabolic pathways within this organelle has also been suggested due to some advantages: i) Easiness of protein translocation using the PTS signal. ii) Peroxisomes may represent up to 80% of the total cell volume in methanol or oleic acid-grown cells. iii) The fatty acid □-oxidation cycle is located in the peroxisomes, generating acetyl-CoA and NADH.

Recently, we have successfully expressed the malonyl-CoA pathway in *P. pastoris* to produce 3-Hydroxypropionic acid (3-HP) from glycerol, achieving promising results. In the present study, we have targeted this metabolic pathway into the peroxisomes to produce 3-HP from oleic acid, whose oxidation supplies the building blocks required for the synthesis of this value-added chemical. To this end, a *P. pastoris* strain expressing separately the genes encoding for the two domains of the Malonyl-CoA Reductase (MCR) from *Chloroflexus aurantiacus* was grown on a minimal medium containing 1% (v/v) of oleic acid and Tween 80, yielding an amount of 0.025 g/l of 3-HP in shake flask cultures, which is far from the 0.19 g/l obtained when the pathway was located at the cytosol. To further increase this titer, an acetyl-CoA carboxylase (ACC) from *Yarrowia lipolytica* and a mitochondrial NADH kinase (POS5) from *Saccharomyces cerevisiae* were also targeted to this organelle, aiming at improving the malonyl-CoA supply and NADPH availability, respectively. Nonetheless, 3-HP production was only slightly higher in the final strain.

Although this study demonstrates the workability of the malonyl-CoA pathway compartmentalization into the peroxisomes, further metabolic engineering of *P. pastoris* would be required to develop this yeast as a platform for the efficient conversion of low-value long chain fatty acids from sustainable resources into value-added chemicals.

A33

Biodegradation of aliphatic polyester by genetically engineered strains of the yeast *Yarrowia lipolytica*.

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The increased production and consumption of plastic resulted in the global waste problem caused by its limited biodegradability. Up to date, some enzymes, such as cutinase, have been found as capable for hydrolysis of plastic. The problem with the use of organisms that naturally produce these enzymes is a very low efficiency of plastic degradation.

The use of genetic engineering methods along with the knowledge of enzymes that have the ability to degrade plastics may be the solution to the difficulties associated with plastic waste. *Yarrowia lipolytica* is a suitable host for heterologous expression of proteins due to wide range of genetic engineering methods designed for this yeast. An additional advantage of *Y. lipolytica* is its ability to assimilate various hydrophobic carbon sources such as n-alkanes or fatty acids. This yeast produces large amounts of lipases that can assist in the hydrolysis of ester bonds present in plastic polymer molecules.

In this study we overexpressed cutinases from *Fusarium solani f. sp*. *pisi* and *Trichoderma reseei* and the native *Y. lipolytica* lipase 2. The capability of the obtained strains was checked using ε-polycaprolactone (PCL) as a model compound. PCL degradation was carried out directly in the culture with modified strains. The supernatants were investigated for the presence of ε-caprolactone, which is a degradation product of PCL. Enzymatic activity of the enzymes produced by engineered *Y. lipolytica* strains and weight loss of plastic film were also examined.

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A34

Metabolic engineering of *Yarrowia lipolytica* for polyethylene terephthalate degradation

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Yarrowia lipolytica is well known unconventional yeast with capability to assimilate atypical carbon sources such as alkanes or polyols. *Y. lipolytica* produces large amounts of bio surfactants, lipases and organic acids, it is resistant for high osmotic pressure and low or high pH. In addition, for this yeast a wide range of the molecular tools have been developed.
Polyethylene terephthalate (PET) is the most common plastic material which scale of world's production causes huge problems due to its strong anti-biodegradability. Up to now the enzymes from hydrolases class had been found capable to degrade PET.
In this study we performed heterologous overexpression of two genes, cutinase from *Fusarium solani* and PETase from *Ideonella sakaiensis*. PET degradation experiments were performed directly in cultures with modified yeast strains. The ability of PET hydrolysis by enzymes produced by the engineered strains was verified by the amount of released degradation products in supernatants, such as terephthalic acid (TPA) and mono-(2-hydroxyethyl)-terephthalic acid (MHET). In this study, the influence of oil supplementation on PET biodegradation was investigated.

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A35

Analysis of erythrose reductase homologue in yeast *Yarrowia lipolytica*

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Erythritol is a four carbon polyol commonly used as a sweetener. The constant increase in demand of this compound influence the development of new methods and novel strains of microorganisms to increase the production. On the list of the microorganisms used to produce erythritol is an oleaginous yeast *Yarrowia lipolytica*. One of its most valuable features is an ability to produce erythritol from glycerol as a sole carbon source. Erythrose reductase (ER) is an enzyme responsible for conversion of erythrose to erythritol, which is the last step of erythritol synthesis in yeast *Y. lipolytica*. The metabolic pathway resulting in erythritol production is already well described, yet the final enzyme of this process is in debate. In silico analysis of known ER genes in different organisms producing erythritol suggest many homologues of ER in *Y. lipolytica*. The work focused on newly identified ER homologue due to the discrepancy between the genes N-terminal end in otherwise highly similar sequences of potential ER homologues. A series of experiments indicated the role of ER homologue as an erythrose reductase as well of its influence on overall polyol production. The results of this study may help to finally decipher the erythritol synthesis pathway as well as to further increase the erythritol production on an industrial scale by overexpression of the native genes.

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A36

Engineering advanced biofuels production in the yeast *Pichia pastoris*

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As the effects of climate change become increasingly severe, metabolic engineers and synthetic biologists are looking towards greener sources for transportation fuels. The design and optimization of microorganisms to produce gasoline, diesel, and jet fuel compounds from renewable feedstocks can significantly reduce dependence on fossil fuels and thereby produce fewer emissions. Over the past two decades, a tremendous amount of research has contributed to the development of microbial strains to produce advanced fuel compounds, including branched-chain higher alcohols (BCHAs) such as isopentanol (3-methyl-1-butanol; 3M1B) and isobutanol (2-methyl-1-propanol). Here, we engineered *Pichia pastoris*, an industrial workhorse in heterologous enzyme production, to produce the biofuels isobutanol and isopentanol from two renewable carbon sources from renewable carbon sources. Our strategy exploited the yeast's amino acid biosynthetic pathway and diverted the amino acid intermediates to the 2-keto acid degradation pathway for higher alcohol production. Rewiring of the cells' primary metabolism using CRISPR/Cas9 further improved product yields. Efforts are underway to create a consolidated bioprocessing platform based on a single microorganism that directly converts sugarcane bagasse, one of Thailand's most abundant agricultural wastes into advanced biofuels. We envision that our CBP platform will enable the efficient utilization of agricultural wastes and provide an economic route to the production of advanced biofuels and chemicals.

A37

Expression of a heterologous acetyl-CoA carboxylase devoid of Snf1 kinase target sites and its effect on Malonyl-CoA concentration in yeast

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Saccharomyces cerevisiae is one of the preferred cell factories for the production of added value compounds. However, it is not capable of generating economically viable amounts of malonyl-CoA derived products. Malonyl-CoA is generated via the ATP-dependent carboxylation of acetyl coenzyme A catalyzed by acetyl-CoA carboxylase (ACC). ACC activity is negatively regulated via phosphorylation mediated by AMP-activated protein kinase Snf1p. In this work, a biopython based bioinformatics approach was used to search the GenBank database for putative ACCs that do not contain the Snf1p phosphorylation recognition motif. A fungal ACC matching the query was found and used for further testing. Expression of this ACC from a plasmid complemented the phenotype of a *S. cerevisiae* strain with a tetracycline repressible ACC1. Results of in vivo measurements of malonyl-CoA levels in the recombinant strain will be discussed.

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A38

Saccharomyces cerevisiae as host for the production of capsaicinoids

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Capsaicinoids are molecules found in Capsicum sp., giving rise to the chilli peppers' spiciness. The pungent sensation is caused by the agonistic activity of capsaicinoids on the transient receptor potential vanilloid type 1 (TRPV1) cation channel, which is an important drug target for the treatment of pain conditions. In the chilli plant, capsaicinoids are synthesised from the precursors vanillylamine and a medium-chain acyl-CoA with variation in saturation, branching and carbon chain length. Transfer of the biosynthetic machinery into a microbial host cell opens up the possibility to achieve synthesis of novel and specific capsaicinoids with potentially improved drug properties. In this project, we are evaluating yeast as a host for a recombinant N-acyltransferase-CoA ligase (NAT-CL) cascade carrying out the key amidation step. Results demonstrating successful whole-cell synthesis of capsaicinoids from supplemented amine and acid precursors, as well as challenges associated with the biocatalytic process will be presented.

A39

Novel carotenogenic gene combinations from red yeasts enhanced lycopene and beta-carotene production in *Saccharomyces cerevisiae* from the low-cost substrate sucrose

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Tetraterpenoids (C40H56) including lycopene and beta-carotene are well-known antioxidants that provide beneficial effects to cellular functions in human body. At present, market demand of these carotenoids for application in food supplement, functional food, and cosmetics has been significantly increasing. Thus, improving their production efficiency is definitely required. This study was aimed to develop bench-scale bioprocess for lycopene and beta-carotene production in *Saccharomyces cerevisiae*. The recombinant yeast capable of producing lycopene or beta-carotene was constructed by employing markerless chromosomal integration of carotenogenic (Crt) genes from red yeasts under the control of galactose-inducible GAL promoters, mevalonate pathway-wide upregulation, and downregulation of the galactose metabolism regulatory protein GAL80. By screening of potential red yeast candidates, we found *Rhodosporidium paludigenum* A1, *Sporidiobolus pararoseus* B2, and *Rhodotorula taiwanensis* C3 accumulated relatively high estimated carotenoid contents with well-annotated genomic sequence data, representing appropriate source for Crt genes. Plasmid-based overexpression of different combinations of cloned Crt genes identified that SpCrtE/YB/I genes from *Sp. pararoseus* B2 were promising for strain engineering to overproduce lycopene and beta-carotene. Media optimization demonstrated that sucrose, an alternative carbon source cheaper than glucose, could enhance carotenoid production, possibly by relieving catabolite repression on GAL promoters. Batch fermentation showed that the recombinant lycopene-producing strain co-overexpressing SpCrtI gene, with the benchmark TmCrtE gene from Taxus X media and PaCrtB gene from *Pantoea agglomerans*, efficiently produced approximately 70% higher lycopene from sucrose than did the benchmark strain that overexpressed TmCrtE, PaCrtB, and BtCrtI gene from *Blakeslea trispora*, suggesting higher in vivo phytoene desaturase activity of SpCrtI. Moreover, the recombinant beta-carotene-producing strain overexpressing SpCrtE/YB/I genes effectively produced high beta-carotene from sucrose. With this simple process, it is plausible to reduce the production costs, and enhance production efficiency of lycopene and beta-carotene. This could be applied for the production of other high-value carotenoid compounds.

A40

Expression of a branched-chain α-ketoacid dehydrogenase (BCKDH) from a non-conventional yeast in *Saccharomyces cerevisiae*

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The rapid developments in the fields of metabolic engineering and synthetic biology combine to make sustainable production of chemicals using microbial cell factories an ever more promising alternative to chemical synthesis. Branched-chain fatty acids (BCFAs) are important building blocks for a range of economically and biotechnologically important molecules. These fatty acids are often sourced from polluting or non-sustainable sources. BCFAs occur in many gram-positive bacteria as major constituents of the cell membrane. Biosynthesis of BCFAs requires a branched-chain α-ketoacid precursor deriving from the branched-chain amino acid catabolism that undergoes an oxidative decarboxylation to form a branched-chain α-ketoacyl-CoA. This reaction is catalyzed by the branched-chain α-ketoacid dehydrogenase complex (BCKDH). The branched-chain α-ketoacyl-CoA is then incorporated in the first condensation step of fatty acid biosynthesis. In this work we have expressed the BCKDH from *Yarrowia lipolytica* in *Saccharomyces cerevisiae* concomitantly with enzymes involved in the lipoylation of lipoate-dependent enzymes. Mitochondria targeting sequences were excised from the enzymes for cytosolic expression. The enzyme activity experiments were performed using different substrates and lipoylation conditions. The results of this work will be further discussed.

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A41

Towards the development of a *Saccharomyces cerevisiae* cell factory to produce the polyphenol curcumin

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Curcumin, a polyphenol produced by turmeric (Curcuma longa), has attracted increased attention due to its potential as a novel cancer-fighting drug. However, to satisfy the required curcumin demand for health-related studies, high purity curcumin preparations are required, which are difficult to obtain and extremely expensive. Curcumin accumulates in low amounts over long periods in the plant and its extraction process is costly and not environmentally friendly. In addition, its chemical synthesis is complex. All these reasons block the advances in studies related to the in vitro and in vivo curcumin biological activities. The curcumin biosynthetic pathway in plants starts with the phenylpropanoid pathway, whose reactions convert the aromatic amino acids (phenylalanine/tyrosine) to the curcumin precursor ferulic acid. Afterwards, curcumin is produced under the catalysis of 4-coumarate-CoA ligase (4CL) and type III polyketide synthases (PKSs) with the involvement of one malonyl-CoA molecule. Herein, we intend to understand if the GRAS yeast *Saccharomyces cerevisiae* is a suitable platform to genetically engineer towards curcumin biosynthesis. For that purpose, a combination of different curcumin biosynthetic pathways was designed and expressed in *S. cerevisiae*, and the curcumin production from supplemented ferulic acid was evaluated. The enzymes tested included Arabidopsis thaliana 4CL or feruloyl-CoA synthetase (FerA) from Pseudomonas paucimobilis and type III PKSs from C. longa (diketide-CoA synthase (DCS) and curcuminoid synthase (CURS)) or curcumin synthase (CUS) from Oryza sativa. The recombinant yeast strain that resulted in higher curcumin productivity was constituted by ferA, DCS and CURS reaching 1.8 mg/L of curcumin produced. In the future, additional genetic modifications will be constructed in the yeast chassis to increase the natural supply of precursors, such as malonyl-CoA, and consequently the curcumin productivity. Additionally, the enzymes from phenylpropanoid pathway will be included in the heterologous pathway to allow the use of simpler substrates.

A42

Development of a second-generation lactic acid producing industrial *S. cerevisiae* strain

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Currently, lactic acid is mainly produced by fermentation with lactic acid bacteria. This method has some drawbacks of which the most prominent one is the accumulation of gypsum, which presents a huge economic and environmental burden. The cause for gypsum accumulation is the addition of a large amount of the base calciumhydroxide/ calciumcarbonate to the fermentation broth to maintain a neutral pH, after which addition of sulfuric acid is used to convert lactate into lactic acid, creating calcium sulfate. An alternative microorganism to ferment sugar into lactic acid is metabolically engineered yeast, which because of its high acid tolerance is able to directly produce lactic acid at low pH. Currently, we are developing a second-generation lactic acid producing industrial *S. cerevisiae* strain in which all the genes encoding pyruvate dehydrogenase (PDC) have been deleted. This creates some metabolic issues, mainly the lack of cytosolic acetyl-CoA due to deletion of the PDC genes, ATP depletion due to active export of lactic acid from the cells and intracellular acidification due to lactic acid accumulation. These issues are addressed with different approaches. In addition, we are performing polygenic analysis of high lactic acid tolerance to identify causative genetic elements enabling improvement of lactic acid tolerance in acidic conditions in the second-generation industrial strain. The final aim is to develop a highly efficient industrial *S. cerevisiae* strain able to produce lactic acid with high efficiency from lignocellulosic substrates under anaerobic conditions.

A43

Co-expression of formate dehydrogenase from *Hansenula polymorpha*, improved production of recombinant model protein in *Pichia pastoris*

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Pichia pastoris is a popular host that has been used for the production of different recombinant proteins. This eukaryotic system can produce high yields of recombinant proteins by using methanol as the sole carbon source. The alcohol oxidase 1 (AOX1) converts methanol to formaldehyde which is subsequently either assimilated by dihydroxyacetone synthase (DAS) or dissimulated to CO_2 through glutathione-dependent formaldehyde dehydrogenase (FLD), S-formyl glutathione hydrolase (FGH), and formate dehydrogenase (FDH). Metabolic flux analysis has revealed significant redistribution of carbon fluxes in the central carbon metabolism, including higher dissimilation rates, in recombinant strains of *Pichia pastoris*. The metabolic networks are valuable targets for the optimization of protein production in hosts. Here, we engineered the methanol utilization pathway of *Pichia pastoris* by overexpression of formate dehydrogenase (FMDH) gene from another methylotrophic yeast (*Hansenula polymorpha*) which includes associated FGH and FDH activity and functions as the main enzyme in the methanol dissimilation pathway. The recombinant clone of *Pichia pastoris* expressing secretary enhanced green fluorescent protein (eGFP) under the control of AOX1 promoter was constructed using PichiaPink™ Expression system. The nucleotide sequence of FMDH was obtained from Hansenula polymorpha's genome, cloned in the pPICZA, and transferred to the previously obtained clones expressing eGFP. The clones with zeocin resistance (100 µg/ml) were examined for the presence of both eGFP and FMDH by PCR with specific primers and sequencing. The results indicated a two-fold increase in the supernatant fluorescent intensity when eGFP was co-expressed with the FMDH. This result may be in part due to the beneficial effect of intracellular FMDH in NADH regeneration, which is an important cofactor in enzyme-catalyzed synthetic reactions.

A44

Establishment of Weimberg pathway for D-Xylose conversion by *Saccharomyces cerevisiae*

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Among different heterologous pathways that were established for utilization of D-xylose in *Saccharomyces cerevisiae*, the Weimberg pathway is especially attractive for production of α-ketoglutarate (αKG) and derived compounds.

In present work, we expressed a Weimberg pathway comprising the genes xylB, xylD and xylX from *Caulobacter cresentus* as well as ksaD from *Corynebacterium glutamicum*. However, no growth of the engineered strain was observed with D-xylose as a sole carbon source. Therefore, the strain was subjected to adaptive laboratory evolution, where the cells were grown in media with D-xylose and a low concentration of D-galactose to support a basal growth. The evolutionary experiment has resulted in a significant growth of cells on pure D-xylose. A more detailed analysis of the evolved strain is being performed.

Furthermore, we present a strategy for screening of the αKG production from D-xylose, where even a conversion of trace amounts of D-xylose could be sufficient to prove the functionality of the Weimberg pathway. For that, a 5-aminolevulinate (ALA) auxotrophic strain was created by deleting the HEM1 gene, encoding 5-aminolevulinate synthase that converts succinyl-CoA to ALA in the mitochondrial matrix. In the cytosol of the ALA auxotrophic strain, the Weimberg pathway genes are expressed together with genes required for a functional αKG dehydrogenase (KGDH) from E. coli and the ALA synthase hemA from *Rhodobacter sphareoides* (Baldi et al., 2019). In this scheme, KGDH converts αKG produced by the Weimberg pathway in the cytosol to succinyl-CoA, which is subsequently converted to ALA by hemA. Since the mitochondrial membrane is permeable for ALA, the resulting strain is supposed to grow only when ALA is cytosolically produced via αKG derived from the Weimberg pathway. This strategy may be used not only for screening αKG production, but also could be beneficial for increasing D-xylose flux through the Weimberg pathway.

A45

Production of medium chain fatty acids and derivatives by an improved reverse β-oxidation pathway

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Medium chain fatty acids are interesting precursors for biofuels and for more complex molecules like cannabinoids (Luo et al. (2019)). The reverse β-oxidation pathway has been broadly studied to produce n-butanol and medium chain fatty acids (Schadeweg and Boles (2016), Lian et al. (2014)). This pathway utilizes acetyl-CoA as starting and carbon elongation unit, which is less energy and carbon consuming than using malonyl-CoA for elongation, as it is done in the fatty acid biosynthesis. Until now, the production of medium chain fatty acids using the reverse β-oxidation has been mostly limited to hexanoic acid, and the inability of elongating the pathway further than six carbon has remained a limitation. Here, we used a schematic approach and screened different enzymes at each reaction of the pathway. Our strategy led to a pathway variant that produced octanoic acid as the main product in the yeast *Saccharomyces cerevisiae*. For this, we assessed the role in medium chain fatty acid formation of PaaH1, Crt2 and BktB from Cupriavidus necator, Hbd and Crt from *Clostridium acetobutylicum*, Ter from *Euglena* gracilis or *Treponema denticola* and Had and Ech from *Yarrowia lipolytica*. In the case of the enoyl-CoA hydratases Crt2 and Ech, their ability to produce octanoic acid at relatively high titers was observed for the first time. When we combined the best pathway variants for hexanoic and octanoic acid with the metabolic engineering of this organism, we produced 60 mg/L of octanoic acid and 80 mg/L of hexanoic acid, so far, the highest titers of these medium chain fatty acids produced in this yeast using the reverse β-oxidation pathway. Finally, these engineered strains served as a platform for producing cannabinoid precursors and medium chain fatty alcohols.

A46

A synthetic Calvin cycle enables autotrophic growth in *Pichia pastoris*

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Synthetic biology offers several routes for CO_2 conversion into biomass or bio-chemicals, helping to avoid unstainable use of organic feedstocks, which negatively contribute to climate change. Engineering well-known production organisms, such as the methylotrophic yeast *Pichia pastoris* (*Komagataella phaffi*i), with the scope to create novel CO_2-based platforms could wean biotechnology from feedstocks with alternative use in food production. Starting with a rational engineering approach, we re-wired the natural peroxisomal methanol-assimilation pathway of P. pastoris into a synthetic version of the Calvin-Benson-Bassham (CBB) cycle. This strategy involved knocking out of three endogenous genes combined with overexpression of 8 heterologous genes including the key genes from the CBB cycle. A modular design separates carbon assimilation from energy production and allows use of any NADH yielding energy source. At present methanol oxidation is employed to fuel CO_2 fixation. The resulting autotrophic strains grew continuously with a µmax of 0.008 h-1, which was further improved to 0.018 h-1 by adaptive laboratory evolution. Using reverse genetic engineering of single-nucleotide polymorphisms (SNPs) occurring in coding regions after evolution, we verified their influence on the improved autotrophic phenotypes. The reverse engineered SNPs lead to lower enzyme activities in branching point reactions and in reactions involved in energy balancing. Beyond this, we show how further evolution facilitates peroxisomal import and increases growth under autotrophic conditions. The engineered P. pastoris strains are basis for the development of a platform technology, which uses CO_2 for production of value-added products, such as cellular biomass, technical enzymes and chemicals and which further avoids consumption of organic feedstocks with alternative use in food production.

A47

Heterologous expression and biochemical characterization of lignocellulolytic enzymes and auxiliary proteins in *Komagataella phaffii*

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Fossil fuels represent a significant portion of the energy matrix and have been extensively utilized over time. These resources are finite and due to the increase in energy demand, it is becoming more expensive, generating higher prices in essential sectors around the world. Considering economic and environmental factors, biotechnological processes for biofuels and renewable chemicals production from biomass are under development as an alternative energy source. Lignocellulolytic enzymes act in the deconstruction of biomass, and the identification and characterization of new enzymes are of great interest. Cellulases, hemicellulases, and proteins with auxiliary activity help in the process of converting biomass into value-added products. A combination of these enzymes with esterases, lyases and auxiliary proteins (expansins, expansins-like and lytic polysaccharides monooxygenase - LPMOs) can be employed for the synergistic deconstruction of biomass components. Moreover, these enzymes have important applications in various sectors of the industry: such as pharmaceuticals, food, detergents, and textiles. For the high-yield production of these proteins and cost savings, the optimization of recombinant expression systems in microorganisms is highly desired. In this work, gene sequences from the fungus Thermomyces lanuginosus that possibly code for the proteins polygalacturonase (TLPG), lytic polysaccharide monooxygenases (TLPMO), expansins-like (TLEX2), and feruloyl esterases (TLFE1) were obtained for heterologous expression in *Komagataella phaffii* X33. These gene sequences were optimized and cloned into pGAPZB or pPICZ□A expression vectors. After the genetic transformation of K. phaffii, positive clones were selected and cultivated in Erlenmeyer flasks. The production of TLPG, TLPMO, TLEX2, and TLFE1 proteins was evaluated by SDS-PAGE and Western-blot techniques to detect the heterologous protein. The enzymatic activity was verified, and initial biochemical characterization tests were carried out.

A48

Assessing flux rearrangements of an alternate methanol metabolism that enables efficient recombinant protein production in *Pichia pastoris*

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Until recently, it was commonly believed that the initial reaction of methanol utilisation in *Pichia pastoris* (syn. *Komagataella spp.*) was only catalysed by alcohol oxidase, encoded by *AOX1* and *AOX2*. The Mut^- strain (lacking both *AOX1* and *AOX2*) was commonly regarded as unable to utilise methanol. Yet, despite being unable to generate biomass, we demonstrated that the Mut^- strain can consume methanol via a native, promiscuous, alcohol dehydrogenase (Adh2) [1].

Using the most recent metabolic model for *P. pastoris* [2], constraint-based approaches, and fermentation data of protein producing *P. pastoris*, we explored the effects on the metabolic flux distribution of changing this initial oxidation step in a recombinant protein producing strain. Results suggest that the NADH yield of the Mut^- strain is higher than the industry standard Mut^S (lacking AOX1), through the combination of an alternative cofactor for the initial methanol oxidation step and the increased dissimilation of formaldehyde to CO_2. The outcome of which, is that the Mut^S and Mut^- strains share a similar ATP yield, despite reduced assimilation of carbon into central carbon metabolism. Compared to the Mut^S strain, metabolic flux is reduced in metabolite shuttling pathways for oxidative phosphorylation, suggesting an abundance of reducing equivalents. In addition, flux is increased to redox balancing pathways associated with protein folding and processing, highlighting possible mechanisms to maintain the redox state of the strain.

The combined effect of which, is that the Mut^- phenotype and *ADH2* overexpression leads to recombinant protein production at similar levels as the industry-standard Mut^S strain albeit with significantly less oxygen demand and heat generation.

[1] Zavec, D. *et al. FEMS Yeast Research, accepted with minor revision.* (2021)

[2] Gamisans, M. *et al. Microbial Biotechnology.* **11.1**, 224-237 (2018)

A49

Metabolic engineering of *Komagataella phaffii* for the production of xylonic acid from xylose

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Building block chemicals are versatile molecules of industrial interest that can be used to produce a wide range of compounds in the pharmaceutical, food, chemical, and construction industries. Currently, the industrial production of most of these compounds is not sustainable or renewable, therefore, the search for biotechnological solutions to produce them in an environmentally friendly manner has been growing. Thus, the concept of biorefineries, based on the integration of biomass conversion processes to produce fuels, energy, and chemicals have been pointed out as the most viable way to increase the use of waste for the formation of valuable co-products. In this context, this work has aimed to obtain recombinant *Komagataella phaffii* (previously known as Pichia pastoris) yeast strains capable of producing xylonic acid by the overexpression of newly identified xylose dehydrogenases (XDH) encoding genes. For this, 11 putative xylose dehydrogenases (XDH) from bacterial and fungi were identified through in silico analysis using the sequences of the two XDH previously described, encoded by xylB from Caulobacter crescentus or XYD1 from *Trichoderma reesei*. Optimized sequences of 6 genes were successfully cloned and expressed in *K. phaffii* as demonstrated by the ability of the strains to produce xylonic acid. The best strain could produce up to 37.1 ± 1.9 and 11.7 ± 1.6 g/L of xylonic acid with yields of 0.96 ± 0.02 and 0.40 ± 0.06 g/g in mineral medium and sugarcane bagasse hydrolysate, respectively. The results presented here demonstrated the functionality of 6 newly identified xylose dehydrogenases, increasing the number of XDH described. In addition, the efficiency of *K. phaffii* as a xylonic acid producer both in synthetic media and lignocellulosic hydrolysate is showed for the first time.

A50

Methanol induced changes on the transcriptome, proteome, metabolome and fluxome of *Pichia pastoris*

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Pichia pastoris is an established host for the production of heterologous proteins. Many processes focus on the methanol inducible alcohol oxidase (AOX) promoter system, requiring methanol as carbon and energy source (as single substrate or in mixed substrate feeds). Alternatively, the constitutive glycolytic GAP promoter is commonly used with glucose as substrate.

In recent years, post-genomic research has driven the investigation of (sub)cellular regulatory mechanisms and interrelations. Apart from the methanol utilization pathway, regulations on a gene level when using methanol are largely unknown. This study presents genome scale transcriptomic, proteomic, metabolomics and flux analysis of *P. pastoris* (mut^S) cultures grown on glucose or glycerol/methanol (mixed substrate feed), respectively. A carbon limiting feed strategy was chosen with regard to its use in yeast bioprocesses.

The carbon source affects formation and use of intermediate metabolites. ^{13}C-metabolic flux analysis in combination with the quantitative analysis of metabolites using LC-MS and GC-MS was used to determine the influence of methanol on intracellular fluxes through the metabolism and accomplished a comprehensive analysis of as many metabolites as possible.

Along with sugar transporters, we observed differences in energy metabolism, biosynthetic pathways and cell wall and membrane organisation on the transcriptome and proteome level. In total, 403 genes and approximately 250 proteins were significantly up- or down-regulated by at least 1.5 fold in the chemostat experiments

To the best of our knowledge, we report the first whole systems level analysis of methanol metabolism, which determines the influence of carbon source on all cellular levels of *P. pastoris*.

A51

BFAIR: Automated metabolic flux analysis

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Laboratory automation allows researchers to escalate the number of samples in their studies; robots can process tens if not thousands more samples than human operators. With an increase in sample number, a need for faster analysis methods and subsequent data processing arises. BFAIR was created as a python package for standardized high throughput metabolomics data analysis. A strong focus lies on different approaches to making as much use as possible of metabolomics data through metabolic modeling. It is part of a bigger effort to set up a big-omics data processing platform following the FAIR principle. Here, the tools for performing Metabolic Flux Analysis (MFA) are presented. Currently, MFA is the gold standard for intracellular flux determination but is very laborious to perform. Together with BFAIR, we can perform MFA on practically any strain that can be cultivated in our automated cultivation platform; only a strain and a corresponding constraint-based model are required.

Room B – Belvedere

B01

The unfolded protein response in methylotrophic yeast *Pichia pastoris*

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The dysfunction or overload of the endoplasmic reticulum (ER) caused by an accumulation of misfolded proteins is designated ER stress. In response to such stress, eukaryotic cells accordingly reprogram their gene-expression profiles to restore ER homeostasis. The Unfolded Protein Response (UPR) profoundly mitigates ER stress, which serves as a protective mechanism initially revealed through pioneering studies of *Saccharomyces cerevisiae*. In *S. cerevisiae*, the transmembrane endoribonuclease Ire1, which resides in the ER, is specifically activated in an ER-stress dependent manner. This event promotes splicing of HAC1 mRNA, which is subsequently translated to produce a transcription-factor that activates the genes required to mount the UPR.

Here we investigated the UPR of the methylotrophic yeast *Pichia pastoris* (*Komagataella phaffi*i). Our findings partially contradict previously published studies of others. Unlike *S. cerevisiae*, rapidly proliferating P. pastoris, cultured in nutrient-rich medium in the absence of stressors, partially spliced HAC1 mRNA. This may be explained by the intrinsic overload of the ER in P. pastoris because of its robust protein secretion. Consistent with this possibility, the *ire1*Δ or *hac1*Δ mutation slowed the growth of P. pastoris. RNAseq analysis of P. pastoris showed that the *ire1*Δ mutation decrease the expression of certain genes encoding ER proteins, which serve as targets of the UPR in *S. cerevisiae*.

We were interested in observations that the *ire1*Δ mutation induced the transcription of genes encoding cytosolic molecular chaperones and factors involved in the degradation of misfolded-protein in the cytosol. Most chaperones belong to the family of heat shock proteins, leading us to conclude that the heat shock response was activated in cells harboring the *ire1*Δ mutation to eliminate excess misfolded proteins in the cytosol. Thus, these findings strongly support the conclusion that the control and surveillance of ER proteins are tightly linked to those of cytosolic proteins.

B02

The ER protein quality control sensor UDP-Glc: glycoprotein glucosyltransferase plays critical roles in cellular growth and pathogenicity of *Cryptococcus neoformans*

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Eukaryotic cells have evolved a N-glycan-dependent endoplasmic reticulum-mediated glycoprotein quality control system, in which UDP-glucose: glycoprotein glucosyltransferase (UGGT) selectively reglucosylates misfolded glycoproteins for binding to lectin/chaperones. The human pathogenic yeast *Cryptococcus neoformans* has evolutionary unique unfolded protein response (UPR), mediated by a novel bZIP transcription factor Hxl1, and N-linked glycosylation pathway lacking the glucose addition steps before assembling of the mature precursor N-oligosaccharides on proteins. To investigate the physiological roles of UGGT in *C. neoformans*, we constructed and characterized a mutant strain lacking UGGT (*uggt*Δ). Notably, the *C. neoformans uggt*Δ strain showed a delayed growth rate with aggregated forms even at normal growth condition and severe growth defects under several stress conditions, including high temperature, ER stress, and cell wall stress. In addition, the *Cnuggt*Δ mutants exhibited increased sensitivity to the leucine analog 5',5',5'-trifluoroleucine (TFL) and triggered UPR in the absence of an exogenous ER stress, implying a defect in eliminating misfolded glycoproteins generated during cellular growth. Furthermore, the *C. neoformans uggt*Δ strain displayed defect in the formation of virulence-associated factors, such as capsule and melanin, and in vivo virulence using a mouse intranasal infection model. Our results demonstrated that UGGT plays critical roles in maintaining various cellular processes of *C. neoformans*, including normal cell growth, stress resistance, and pathogenicity.

B03

Redox balancing in recombinant protein-producing *Pichia pastoris* with altered protein folding

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Disulphide bond formation is one of the key rate-limiting steps in protein folding. In most eukaryotic cells, oxidative protein folding is carried out in the endoplasmic reticulum (ER). The ER, in fact, provides a chaperone-rich oxidizing environment, which is highly beneficial. The sole formation of S-S bonds is a relatively simple electron-transfer process, which starts from the oxidation of two partnering cysteines, continuing onto the ER oxidoreductases PDI and Ero1, and culminating into the formation of hydrogen peroxide (Tu et al. (2004) J. Cell Biol. 164(3), 341). The notorious toxicity of this by-product contributed to the evolution of a tight regulation of Ero1 and consequent stress responses. Heterologous overexpression of disulphide-bonded proteins remains, thus, challenging (Delic et al. (2014) Antioxid Redox Sign. 21(3), 414). One of the current aims of this project is to hack the kinetics of PDI and Ero1 through enzyme engineering, and expression fine-tuning in the yeast *Pichia pastoris* (syn *Komagataella spp*). Altogether, these approaches will allow a better mechanistic comprehension of disulphide bond formation in *P. pastoris* and how to use it for enhanced heterologous protein production.

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B04

Investigating the role of oxidative stress response genes in protein secretion using a novel Golden PiCS-based approach

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One of main challenges for the efficient production of recombinant protein by *Pichia pastoris* is the redox stress generated in the endoplasmic reticulum during protein folding. However, apart from the ER and its players, many other cellular compartments and components participate in this context. Among them, genes involved in the defence against oxidative stress are believed to be essential for maintaining an optimal redox state in *P. pastoris* during protein production and secretion. Moreover, rather than the effect of a single gene, it is likely that the combination of multiple genes is responsible for providing better redox conditions in P. pastoris. Therefore, the Golden Gate-based Golden PiCS cloning system (Prielhofer et al. 2017. BMC Syst Biol 11(1):123) presents itself as an amazing ally, as it is a very powerful tool for making fast, versatile and efficient cloning of different genes.

As a starting point, candidate genes were selected for the creation of a combinatorial plasmid library. The library was created by using completmentary fusion sites, allowing genes to be cloned in different positions of the final expression vector. Thus, as result of a Golden Gate Assembly using all combinatorial plasmids, random combinatons of the genes were generated. The library was evaluated in a trypsinogen secreting *P. pastoris*. The reporter fluorescent protein mCherry was tagged to trypsinogen to allow fluorescence output analysis.

Here, a novel application of the Golden PiCS system is presented. This strategy can be applied in future studies that desire quick, versatile and efficient combination of multiple genes.

B05

Enhanced production of recombinant Lignin peroxidase in *Pichia pastoris* via medium optimization

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Lignin peroxidase (LiP) is one of the major enzymes secreted by white-rot basidiomycetes to degrade lignin in response to nutrient depletion. Furthermore, LiP presents significant potential for application in various industrial sectors such as second-generation biofuels, cosmetics, food, bio-pulping and biobleaching. However, the lack of commercial LiP preparations has hindered its industrial application. In addition, they are unstable at high temperatures, deactivated by solvents, susceptible to inactivation by hydrogen peroxide and challenging to produce in ample quantities. Thus, there is a need for the development of new strategies that would enable high yields of recombinant LiP (rLiP) production.
The lipH8 gene from Phanerochaete chrysosporium was successfully expressed in *Pichia pastoris* and secreted in the induction medium. Subsequently, the optimisation of cultivation conditions was evaluated in 96 deep-well plates. Our results indicate that the individual synergistic effects of co-feeding glycerol or sorbitol with methanol, urea and adding FeSO¬4 in the growth and inducing media increase rLiP production by 2.4, 5.9, 3.3 and 6.0-fold, respectively, compared to the control conditions. The results show that medium optimization plays a significant role in improving recombinant protein production in *P. pastoris.*

B06

Optimizing protein production by looking outside the secretory pathway

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Many different yeast species are currently being used for the production of (pharmaceutical) proteins in industry. For a production process to be economically feasible a major factor is the titer of product in the media. Therefore, an interesting research topic within yeast biology and engineering is to increase the efficiency of recombinant protein production and secretion. When improving recombinant protein production most research is dedicated towards the secretory pathway. Often the studies focus on folding chaperones in the ER or the translocation of target proteins to the cell exterior. Using these approaches have shown very successful and enabled us to learn about the complexity of the secretory pathway and the bottlenecks in recombinant protein production and secretion.

In our lab our strategies have been similar to improve recombinant protein production in *Saccharomyces cerevisiae*, our model organism. With this model organism many researchers in our lab have worked on increasing the production of amylase by using both targeted engineering but also UV-mutagenesis.

For this work however, we decided to look at another part of the cell to even further increase the flux of proteins into the media in case of a high production strain. Since the high protein production can be a burden to the cell and potentially inhibit the protein production mechanism itself. To test our theory, we removed one gene in the center of this potential negative feedback loop and nearly doubled the amylase titer in the media. We are momentarily working on the underlying mechanisms of this gene and negative feedback loop in relation to protein production.

B07

Heterologous production of a novel *Coriolopsis polyzona* laccase in *Saccharomyces cerevisiae*

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In recent years, thanks to an increased awareness of the potential of biodiversity and to the versatility of biomolecular tools, the use of eco-friendly biocatalysts has increased further its biotechnological interest.
Laccases are considered very versatile biocatalysts since they catalyze the oxidation of various aromatic and related compounds with the concomitant reduction of oxygen to water as the only by-product, without the need of additional cofactors.
Laccases can be exploited both for synthetic and degradative reactions and have gained a prominent role in different industrial fields for very diverse purposes, ranging from food additive and beverage processing to biomedical diagnosis, from pulp delignification to bleaching and textile dye transformations. Furthermore, their ability to transform complex xenobiotics makes them useful in enzymatic bioremediation and detoxification. For these reasons, new laccases are continuously sought as their different characteristic can match specific industrial requirements.
It is known that laccases are widely distributed in higher plants and bacteria, but mostly in white-rot fungi.
To identify novel enzymatic laccase activities of potential industrial interest, we tested several poorly characterized white-rot fungi that were screened on substrates known to be oxidized by the laccases. We focused on the best candidate, *Coriolopsis polyzona*, which was further studied for laccase production. Moreover, to describe potential traits of interest of single enzymes, we overexpressed a putative *C. polyzona* laccase encoding gene in the yeast *Saccharomyces cerevisiae*. Here we will illustrate the cloning, expression and production strategies together with first results and perspectives on the potential interest of this novel laccase.

B08

Insight into heterologous secretory proteins synthesis and physiology of *Yarrowia lipolytica* under exposure to combination of selected stress factors

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While overproduction of recombinant secretory proteins (rs-Prots) itself triggers multiple changes in physiology of the producer cell, exposure of the yeast host to suboptimal / stressful environmental conditions further modulates that biological response. When challenged with environmental perturbations, the producer cell may respond with impaired cell growth and substrate consumption, increased mortality, induction of morphological transition (in case of dimorphic yeasts) or altered metabolic profile, including the target rs-Prot production level. The environmental conditions may modulate the rs-Prot genes transcription intensity, translation efficiency and the nascent polypeptides folding, by increased provision of chaperones. Insight into responses awaken by different environmental factors and their combinations on the rs-Prots synthesis and the yeast host physiology contributes to better understanding of fundamental biology processes and potentially leads to development of new strategies of bioprocess engineering.

In this study we conducted a series of batch cultivations of *Yarrowia lipolytica* strains overproducing three model rs-Prots, differing in molecular weight and posttranslational modifications. Combination of different settings of pH (3.0/7.0) and oxygen availability (kLa 30/110) was studied in terms of their impact on the strains' growth rate, substrate consumption, morphology and viability of the cells, the genes expression level and secretion of the rs-Prots.

Our results suggest significant impact of both the produced rs-Prot characteristics and the stress conditions on physiological response of the cells in terms of the abovementioned parameters. OA emerged as the dominant factor over pH. Induction of synthetic promoter 4UASpTEF was found to be environmental conditions-independent and affected solely by the overall physiological condition of the cells. Secretion of active rs-Prots strongly depended on the rs-Prot's biochemical characteristics and OA. Combination of acidic pH and limited OA was particularly adverse for the *Y. lipolytica* in terms of all the selected parameters.

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B09

Pichia pastoris: QTL mapping for strain development

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The majority of research on recombinant protein production in *Pichia pastoris* (*Komagataella phaffi*i) is based on the strain background CBS7435, with other *Komagataella* sp. strains and species being largely neglected. Yet, we see considerable inter- and intraspecies variation in strain performance among natural isolates within this genus. Protein secretion is regulated in a quantitative and complex manner in the cell; therefore, making it difficult to pinpoint the exact genetic determinants contributing to observed production phenotypes.

To identify the genetic loci underlying a high protein production phenotype, we investigated the secretion potential among natural isolates and designed a Quantitative Trait Loci (QTL) mapping experiment, ultimately aiming at the identification of the best allelic variants of all identified candidate genes.

We determined the protein production potential of various natural isolates belonging to all seven currently known *Komagataella* species. Following identification of parent strains that show a large phenotypic and genotypic variation, we established an efficient mating protocol for bulk segregant analysis of *K. phaffi*i hybrids. Consecutive rounds of mating resulted in a F12 hybrid generation showing highly variable protein secretion, with a number of clones exceeding the initial parent strains.

Ongoing production screenings and bioinformatical analysis will be used to identify the beneficial genetic determinants and to answer the question why some isolates are naturally better producers than others. The results will yield novel engineering targets for the improvement of protein secretion in industrial *Komagataella* strains.

B10

Scale-dependent effect of helper factor co-expression in *Pichia pastoris*

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Pichia pastoris (*Komagataella phaffi*i) is widely used as expression host for heterologous proteins. Expression and secretion of heterologous proteins in *P. pastoris* can be influenced by many factors such as gene copy number, promoter usage or co-expression of helper factors. In this study, we tested the effect of co-expression of a helper factor on secretion of a carboxylesterase. Four strains were cultivated which were based on *P. pastoris* CBS7435, expressing the model protein under control of a methanol-inducible promoter. Cultivations were performed in deep well plates in repeated-batch mode, a common strategy for clone screening, as well as in small-scale (1 L working volume) fed-batch bioreactor cultivations. Enzyme activity in the supernatant was compared for both scales. While there was no difference in volumetric enzyme activity between the four strains in deep well plates, enzyme activities differed in the bioreactor cultivations. Our results indicate that cultivation conditions can have a strong impact on the effect of helper factors on expression of heterologous proteins, which needs to be considered when setting up a screening procedure supposed to represent a downscaled bioreactor process.

B11

Effects of mutations in endocytosis and glycosylation on efficiency of surface display of recombinant proteins in yeast

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Surface display in yeast provides an alternative to standard protein immobilization on solid surfaces as it reduces the costs of protein purification and possibility of protein denaturation during the immobilization. Also, it provides continuous synthesis of recombinant protein composed of enzyme of interest fused with native yeast cell wall proteins. However, its major disadvantage is low surface display efficiency. Lately, it has been reported that mutations in genes encoding proteins involved in endocytosis may increase the amount of secreted heterologous proteins from the yeast cells. Glycosylation can also affect the efficiency of surface display as it could possibly have negative effect on conformation of displayed proteins and cause high density of wall outer mannan layer, limiting accessibility of the substrate. In order to test whether inactivation of genes involved in these processes would enhance surface display efficiency, two different systems using β-lactamase as a reporter were developed. In one, bla gene was fused with a fragment of CCW12 gene coding for its C-terminal GPI anchoring signal sequence. Other system consisted of bla gene coding for β-lactamase fused with PIR2 gene coding for cell wall protein that covalently binds to cell wall through the linkage on its N-terminal end. Both constructs were set under the control of an inducible PHO5 promotor. By using two different immobilization systems, the effect of different folding of the recombinant enzyme, caused by immobilization itself, on affecting enzyme activity is reduced. Therefore, the described system for expression and immobilization of recombinant reporter enzyme in the wall was examined in mutants that cannot accomplish endocytosis (end6, end3, vam4) and have lower level of glycosylation (pmt and mnn mutants). Amount of recombinant protein incorporated in cell wall was assessed by measurement of β-lactamase activity using nitrocefin as substrate and in a semi-quantitative manner by western blot.

B12

Molecular and bioprocess studies for superior production of recombinant secretory proteins in *Yarrowia lipolytica*

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Upon overproduction of recombinant secretory proteins (rs-Prots) the yeast producer cell is subjected to multiple stress factors. The metabolic burden imposed by the synthetically forced anabolic process from the inside, and bioprocessing conditions from the outside, both exert strong impact on the producer cell. Investigation into the yeast cell's response awaken by rs-Prots overproduction under different bioprocessing conditions is relevant for both basic and applied research. Answering the following questions: i) how overproduction of rs-Prots having different biochemical characteristics impacts the yeast cell? and ii) how different bioprocessing conditions impact rs-Prots synthesis and the host system? is the subject of our research.

Yarrowia lipolytica is one of the yeast species frequently used as a rs-Prots production host. Considering its favorable characteristics in terms of secretory capacity and robust growth under bioprocessing conditions, we focused our research on this yeast species. To adequately answer the first question, *Y. lipolytica* strains perturbed with high-level expression of different rs-Prots-encoding genes were cultured in steady-state, which was followed by global transcriptome profiling. Careful analysis of the omics data allowed to reveal genes and biological processes responsive to the overproduction of different proteins. The second posed question was addressed in a series of studies on the impact of oxygen provision, thermal treatment and exposure to high osmolarity on the heterologous rs-Prots synthesis. Insight into the interplay between the environmental conditions, the cells' morphotype and efficiency of rs-Prots production is provided. Finally, an impact of repeated heat-shock treatment on *Y. lipolytica*'s global methylome was also investigated.

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B13

Optimization of the recombinant production of *Hermetia illucens* antimicrobial peptides in yeasts

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Antimicrobial peptides (AMPs) play a key role in the innate immunity, the first line of defense against bacteria, fungi, and viruses. Their utilization can contribute to counteract the phenomenon of antibiotic resistance. AMPs are small molecules, ranging from 10 to 100 amino acid residues produced by all living organisms. Because of their wide biodiversity, insects are the richest and most innovative sources of AMPs. *Hermetia illucens*, a bioconverter insect, could be a great source of AMPs because of its extraordinary ability to live in harsh environments. In a previous study, *H. illucens* transcriptome was examined to identify all the sequences putatively encoding AMPs. These analyses allowed to identify 57 putative sequences suitable for subsequent experimental validation studies. Moreover, preliminary data showed that peptide fraction derived from *H. illucens*, whose AMPs production was induced by infection with *Micrococcus flavus*, showed antimicrobial activity against *M. flavus* and mild antimicrobial activity against Staphylococcus aureus and Escherichia coli.
The aim of this work is the optimization of the AMPs production in *Saccharomyces cerevisiae* and *Pichia pastoris* expression systems, in which two putative AMP sequences of *H. illucens* were cloned. Yeasts have been selected as hosts in order to allow the correct eukaryotic post translational processing.
Cloning experiments were performed using vectors with constitutive or inducible promoters suitable for gene expression of AMPs; these systems ensure AMP intracellular production in *S. cerevisiae* (YEp112T) and its secretion by *P. pastoris* (pPIC9K). AMP expression was first verified by real-time quantitative PCR, at different cell growth phases, and the most promising mutants were used to purify the peptides (using a polyhistidine-tag at the C terminus and a nickel-charged resin). Antimicrobial activity of the peptides was tested on *E. coli*, *S. aureus* and *M. flavus* strains using cell extracts or supernatants by the agar diffusion method and bioautography assay.

B14

Codon usage for fungal amylase genes in *Saccharomyces cerevisiae* – to optimise or not?

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Advances in molecular microbiology and synthetic biology have facilitated the genetic engineering and optimisation of DNA molecules for improved expression in foreign hosts. The expression of codon-optimised genes in *Saccharomyces cerevisiae* for heterologous protein production remains an empirical process, leaving much scope to explore the effects of synonymous codon usage. Starch-degrading enzymes are used in a variety of industries, including biofuels that are dependent on amylases capable of hydrolysing raw starch. Although several raw starch-hydrolysing amylases have been reported, amylolytic yeast strains are required that produce recombinant amylases at high levels. In some cases, heterologous enzyme production can be improved by altering the DNA sequence of the recombinant gene to ensure optimal codon usage for the specific host. To develop a high-level amylase expression system, we explored the role of synonymous codon usage by comparing the expression of native fungal amylases with their codon-optimised counterparts using *S. cerevisiae* as the expression host.

Native and codon-optimised variants of the genes encoding the Talaromyces emersonii glucoamylase (temG) and the Aspergillus tubingensis α-amylase (amyA) and glucoamylase (glaA) were expressed in *S. cerevisiae*. Enzyme assays and the comparison of protein concentrations demonstrated that the native variants performed better than the codon-optimised genes, except for the codon-optimised glucoamylase from T. emersonii (temG_Opt) that yielded >3 fold higher extracellular activity than the native variant (temG). The A. tubingensis α-amylase (amyA) and glucoamylase (glaA) genes were redesigned using two different codon-optimisation strategies, but the native genes performed better for both amylases. The results confirm that the codon-optimisation of fungal genes for expression in *S. cerevisiae* does not guarantee improved recombinant protein production. Gene design algorithms may not yet adequately consider the positional effect of rare/abundant codons. Therefore, additional tools are required to more accurately predict the functional impact of synonymous codon usage for a specific host strain.

B15

Modifying the endoplasmic reticulum of *Saccharomyces cerevisiae* for a higher secretion capacity of recombinant proteins

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Saccharomyces cerevisiae is a well-known organism, well suited as a cell factory for producing recombinant proteins like antibodies. Engineering this organism can improve its production capacity significantly, which would increase its potential for industrial applications. Reticulon proteins and Yop1p are known as tubule shaping proteins of the endoplasmic reticulum (ER). It has been previously shown that deletions of reticulon genes and YOP1 create *S. cerevisiae* cells with a higher ratio of sheets compared to tubules. It has been observed that the morphology of the ER has an effect on its functions. Hence, modifying the ER could show an improvement on its secretory capacity. In this work antibody producing *S. cerevisiae* strains with different combinations of deletions of genes determining the ER structure, such as genes of the reticulon proteins, were tested in their effect on secreted IgG antibody titers. Selected modified strains showed improved secreted antibody titers compared to the wildtype strain. These results indicate possibilities that shaping the *S. cerevisiae* ER structure can have for increasing the production capacity of recombinant proteins like antibodies.

B16

Genetic and process optimization strategies to augment laccase production in *Pichia pastoris*

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Laccases are blue multi-copper oxidases that play diverse roles in nature like lignification, delignification, dye degradation and biocatalysis. Selection of suitable host for large scale expression of the enzyme is crucial. Under these circumstance, *Pichia pastoris* (*P. pastoris*) offers excellent benefits over other hosts for recombinant protein production. Widely accepted as an efficient protein production host, P. pastoris offers several advantages like ease of genetic manipulation, requirement of inexpensive media, ability to achieve high protein yield and finally intricate posttranslational modifications. In this study, laccase from *Aspergillus clavatus* was expressed under the control of AOX1 promoter in *P. pastoris*. Expression medium with 0.1mM copper supplementation enhanced laccase activity up to 3.3U/mL in 9 copy GAP laccase clone and 40 U/mL in 1 copy AOX laccase clone. In order to further improve the expression levels, impact of laccase under the control of AOX1 promoter was assessed. Interestingly, AOX laccase (AOXLAC) clone with 0.1 mM copper, 0.5-1% methanol produced nearly 150 U/mL of laccase in reactor studies which was nearly 4 fold higher than control and 44 fold higher compared to GAP laccase clone. Further to enhance laccase expression, several additives targeting the secretion pathway, methanol oxidation pathway, metabolic cost of amino acids, TCA cycle were tested. Among 21 different additives tested, Arginine- Glutamine- Asparagine (RQN) significantly augmented the laccase production to 600 U/mL and productivity to 3.5U/mL/h. Tryptone supplementation however improved the activity to 350 U/mL and productivity to 2U/mL/h. The crude supernatant containing laccase was concentrated by ultra-filtration and later purified up to 5.3 fold by anion exchange chromatography. Kinetic studies showed Km, Vmax, Kcat values to be 2.2 mM, 0.25 µmol min-1 and 28.9s-1 respectively. Effect of pH, temperature, metals and inhibitors on laccase was studied.

B17

Functional characterization of the ATF homologs, encoding putative alcohol acetyltransferases, identified by whole genome sequencing of *Wickerhamomyces subpelliculosus*

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Wickerhamomyces subpelliculosus is a non-*Saccharomyces* yeast widely found in fermented food, such as fermented soybean sauce, cucumber, and coffee as a flavor producer. Our whole genome sequencing and flow cytometry analyses revealed that this yeast has a haploid genome of approximately 16 Mb with 7,615 ORFs. From the obtained *W. subpelliculous* genome, we searched the homologs of ATF genes, encoding alcohol acetyltransferases (AATase) that are responsible for major part of volatile acetate ester production during fermentation and also have potential roles in lipid and sterol metabolism. All five ATF orthologues of *W. subpelliculosus* CBS 5767T (WsATF), identified based on the presence of AATase Pfam domain, have the activation domain (HXXXD) conserved in other AATases. The WsATF genes were expressed under TEF1 promoter in a heterologous host *Saccharomyces cerevisiae* atf1atf2 deletion mutant, but the expression levels of WsAtf proteins were extremely low or undetectable except WsAtf6p. However, the aroma volatile esters from fusel alcohols were not increased even in the recombinant *S. cerevisiae* strain expressing WsAtf6p in the HS-SPME GC/MS analysis. Phylogenetic analysis revealed that WsAtf6p is closely related to *S. cerevisiae* and *Wickerhamomyces ciferrii* (*P. ciferrii*) Sli1 proteins rather than other Atf proteins carrying O-acetyltransferase activity. The WsATF6-expressing *S. cerevisiae* strain showed the resistance to myriocin, as reported in *S. cerevisiae* Sli1p that has N-acetyltransferase activity towards myriocin (ISP-1). The data of thin layer chromatography analysis further indicated that WsAtf6p was not able to acetylate phytosphingosine both in the endogenous host *W. subpelliculosus* and in the heterologous host *S. cerevisiae*. Altogether, our data suggest that the WsAtf6p is a functional N-acetyltransferase with distinctive substrate specificity from W. ciferrii Sli1p, the sphingoid base N/O-acetyltransferases.

B18

Insect sex pheromone production in yeast for use as a bio-insecticide

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Effective insect pest control is necessary for the sustainability of global agriculture under the ongoing climate change. Currently, insect infestation is addressed primarily with chemical insecticides and genetically modified crops. However, the rapid development of resistant strains limits the effectiveness of these applications and underlines the need for alternative approaches. Here, we demonstrate the biological production of insect pheromones by yeast cell factories for use in mating disruption as safe and environmentally friendly substitute of pesticides[1-3]. As a target compound we used the Z9-14:OAc, the main sex pheromone component of fall armyworm *Spodoptera frugiperda*. We first produced the precursor pheromone Z9-14:Me, which can be chemically oxidised to Z9-14:OAc. A mutant library was constructed via SpeedyGenes[4] and GeneGenie[5] for the target enzyme fatty acid desaturase Dmd9 from Drosophila melanogaster, and the different combinations were tested for production of lipids in *Saccharomyces cerevisiae*. Strains producing higher amounts of intracellular lipid bodies were selected as appropriate candidates via Nile Red staining and microscopy and screened using Intellycit iQue screener plus. GC-MS analysis from the best producers revealed the existence of enzyme variants that produce higher amounts of fatty acid methyl esters than the native natural sequence. These findings suggest future rational design strategies to engineer highly active enzymes for moth pheromone production. Future steps include the characterisation of the target regions with improved activity among the identified sequences via Sanger sequencing, and expression of the target compound in the oleaginous yeast *Yarrowia lipolytica*, a platform widely used in industry for high-level production of fatty alcohols.

B19

Novel yeast-based biosensor for environmental monitoring

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Agriculture is a human activity with high impact in the environment. Due to a constant demand for a high and stable crop production, pesticides and other chemicals have been used extensively, but once introduced in the environment these substances can have a negative impact in ecosystems and in humans. Monitorization of agrochemicals in ecosystems is therefore of utmost importance, but unfortunately most of the common analytical methods present complex procedures and have a high economic cost. In this sense, environmental monitoring programs urge for fast, cost-effective and disposable systems, with the ability to monitor the increasing number of relevant analytes as quickly and cheaply as possible. Biosensors have demonstrated a great potential and appear as an alternative or a complementary tool for detection and quantification of environmental contaminants with countless advantages, not only in terms of simplicity, high specificity and sensitivity, but also by bypassing sample preparation. Considering the characteristics of yeast such as its background information, its simple genetic manipulation, its resilience to harsh conditions, and its propensity to fast high-throughput functional genomics to identify specific features/responses of interest, yeast *Saccharomyces cerevisiae* is an excellent choice for use as a biosensor. Since yeast-based biosensors have already been used to detect endocrine disruptive compounds, the main goal of this work was to develop a novel easy-to-use biosensor to detect the presence of azole fungicides in aquatic samples. Its characteristics in terms of selectivity and sensitivity will be presented.

B20

Lactic acid production in the synthetic autotroph *Komagataella phaffi*i

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The methylotrophic yeast *Komagataella phaffi*i was recently converted to a synthetic autotroph by the integration of the Calvin Benson Bassham cycle1. We could already prove that this strain could grow using CO_2 as a carbon source. To follow this up, we want to produce chemicals using this strain.
The chosen product was lactic acid which is a hydroxycarboxylic acid used in food, pharmaceutical and chemical industry. Furthermore, it is the precursor of the biodegradable polymer poly-lactic acid (PLA). Lactic acid can be produced in *Komagataella phaffi*i by the integration of a lactate dehydrogenase (LDH) gene.
In this work we assessed the lactic acid production in the autotrophic strains using CO_2 as carbon source and the LDH gene under the control of the AOX1 promoter. This strain was able to produce up to 150 mg L-1 in approximately 200 hours of cultivation time. We showed that lactic acid can be produced under autotrophic conditions by 13C labeling experiments. Titers were further improved up to 300 mg L-1 by the knock-out of the CYB2 gene which reduced the ability to consume the produced lactic acid. Additionally, we compared the lactic acid consumption kinetics of the CYB2 knock-out strain to its parental strain.

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B21

Technologies for co-production of chitin and chitosan in microbial production hosts

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Chitin and chitosan are gaining interest as an alternative for synthetic polymers to cope with the growing consumer's expectations for sustainable products. Companies such as MycoTech (Bandung, Indonesia) and Bolt Threads Inc. (Emeryville, US) are producing chitinous leather-like products to replace animal-based and plastic-based leathers. A startup in London, the Shellwork, is producing biodegradable chitosan-based plastic bags as an alternative to traditional options. Or in wastewater treatment, chitosan-based flocculant is the potential substitution for currently used synthetic polymers. With increased attention for biopolymers, it is not surprising that the demand for chitin and chitosan has raised significantly. Thus, different sources for them, besides crustaceans, are being investigated to meet the demand.

Fungi and yeast represent renewable sources for animal-free chitin and chitosan that offer many advantages in terms of sustainability, quantity, simple chitin-chitosan extraction process, and environmental independence. However, the low chitin-chitosan content per cell is an economic barrier that prevents the utilization of fungi and yeast in large-scale chitin and chitosan production.

To improve production economics and the sustainability of the manufacturing processes, co-production of value-added products is an emerging strategy. We are developing a disruptive concept for the improved co-production of chitin and chitosan that is applicable to many currently used biotechnologically relevant microbial production organisms. The concept builds on a genetic switch that enables an on-demand induction of chitin-chitosan biosynthesis and can be activated either concomitantly with or after the production of the primary product. Cells with activated production will accumulate higher levels of chitin-chitosan in their cell walls, which can easily be separated from any intra- or extracellular products for chitin-chitosan extraction process.

B22

Production assembly lines in cell factories: Using metabolons to make enzymes and transporters work closely together

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Designing effective and competitive microbial cell factories often requires in-depth pathway engineering and fine-tuning for which numerous tools and analytics are available. Yet, in the whole process, transport over the elementary boundary, the biological membranes, is largely neglected. Since the assembly of compounds usually occurs inside the cell problems regarding cell-toxicity and reduced yields due to inefficient export of the compound of interest or due to low uptake rates of substrates can arise.

In order to overcome these limitations a close connection between the assembly line and the cross-membrane transport will be established. In nature, association of proteins with cellular membranes is crucial for a broad variety of cellular functions. Enzymes can for example be clustered in line to allow smooth and immediate subsequent processing leading to a higher likelihood of an enzyme–substrate encounter. The close proximity in an artificial metabolon of transporters and subsequent corresponding enzymes enhances product formation and prevents e.g. the accumulation of toxic intermediates and feeding alternative metabolic pathways.

Therefore, first, the most optimal ways to anchor part of the assembly line to the cell's membrane will be determined by using a fluorescent reporter protein. Next, the most promising membrane binding strategies will be applied to create an artificial metabolon in a bench-marking scenario. The principle of 'proximity channeling' used in metabolons can be applied broadly in synthetic biology to optimize production yields and is principally applicable to various industrial biotechnological processes.

B23

Use of pectinolytic yeast in wine fermentations

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The use of pectinolytic enzymes in winemaking is state of the art. These enzymes catalyse the degradation of pectic substances through depolymerization (hydrolases and lyases) and de-esterification. As a result, it supports the extraction of juice and facilitates filtration. It has also been shown in winemaking that the presence of pectinolytic enzymes improves the stability, taste, texture, colour and aroma of products.

With regard to enzymes currently applied in winemaking, enzymes derived from filamentous fungi dominate the enzyme industry. Fungal-based pectinolytic enzymes specifically require further purification steps to eliminate unwanted side reactions, which is poorly sustainable. Some non-traditional yeast strains have been reported to exhibit pectinolytic activities. Therefore, the direct use of pectinolytic yeast during wine fermentation process can be an attractive and alternative source for the use of enzymes as input. However, little is known about the effect of non-traditional yeasts with pectinolytic activities on wine fermentation and product quality. In fact, the use of such yeasts can have a very positive effect on the taste complexity and sensory richness of the product.

In this study, from 17 different species more than 500 yeast strains were screened for their polygalacturonase activities (PGA). Enzymatic screening was performed in solid rich medium containing 2% polygalacturonic acid, and the activity of PGA+ strains was separately quantified with a microplate colorimetric test developed in this study. The superior pectinolytic yeasts were dominated by *Kluyveromyces* and *Cryptococcus* sp.. High pectinase producing *Kluyveromyces* sp (intrinsic yeast of grape must) was selected for further winemaking experiments. In particular, we investigated the consequences of the use of *K. marxianus* strains in sequential fermentation with *S. cerevisae* on fermentation kinetics, aroma profiles and more widely sensory properties of wines.

Overall, the use of pectinolytic yeast in wine fermentations has the potential to benefit quality of wine and winemakers.

B24

Non-*Saccharomyces* yeasts: A strategy to improve quality of low altitude Arabica coffee

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The use of yeasts as starter cultures during coffee fermentation result in higher complexity beverages with different and positive sensory characteristics. This work aimed to inoculate two yeasts (*Torulaspora delbrueckii* CCMA 0684 and Candida parapsilosis CCMA 0544) singly and co-inoculation for fermentation using low-altitude coffee processed via dry to improve beverage. Important chemical compounds and groups were analyzed by gas chromatography and Fourier-transform infrared spectroscopy (FTIR). Trained tasters performed the sensory analysis. FTIR detected important chemical groups related to coffee quality, such as carbonyl lipids, aliphatic esters, and carboxylic acids. All samples presented a peak of 1743 cm -1, increasing in roasted samples. 1157 cm -1 identified only in the roasted (associated with C–O ester group stretching and rocking vibration). Alcohols and aldehydes were the main chemical classes found before fermentation. The treatment inoculated with *T. delbrueckii* CCMA 0684 produced a higher concentration of volatile alcohols, acetic acid, and a few pyrazines after 72 h of fermentation. 2-ethyl-5-methylpyrazine (coffee-like taste to a sugar syrup) was a relevant compound in the group of pyrazines. This compound was found in all samples in a lower concentration in spontaneous fermentation. Chemical analyzes such as chromatography and FTIR are essential to determine the formation/loss of metabolites throughout the post-harvest process and after roasting. Yeast inoculation results in different sensory descriptors and differences in the other attributes evaluated. The spice flavor was noticed in all treatments inoculated with *C. parapsilosis* CCMA 0544. Different descriptors were observed in each treatment, and body, flavor, balance, and aftertaste are strongly related to *C. parapsilosis* CCMA 0544. The fermentation process improved the quality of low-altitude coffees, and the combination of non-Saccharomyces yeasts (*C. parapsilosis* CCMA 0544 and *T. delbrueckii* CCMA 0684) was the most indicated as starter cultures, presenting a higher score (85).

B25

Genomic potential of a *Saccharomyces cerevisiae* strain involved in cocoa fermentation through a phylogenomic and pangenomic approach

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The quest to develop a performant starter culture mixture to be applied in cocoa fermentation processes started more than a decade ago. Since then, different yeasts have been proposed as candidate starter culture strains, especially strains of *Saccharomyces cerevisiae*. However, little is known about their genomic potential and the relatedness between *S. cerevisiae* strains isolated from cocoa fermentation processes and other wild and/or domesticated strains from the same species isolated from different sources. In the current study, the genome sequence of a *S. cerevisiae* strain isolated from a spontaneous cocoa fermentation process was unraveled, based on a combination of long-read sequencing using the Oxford Nanopore Technologies' MinION sequencer and short-read sequencing using the Illumina's MiSeq sequencer. Aiming at achieving an accurate representation of the yeast chromosomes, an optimized genome assembly pipeline was established. The high-quality genome assembly was then used for a phylogenomic analysis, together with a set of 105 publicly available *S. cerevisiae* genomes, representing mainly fermented food-derived strains, to shed light onto the relatedness of the cocoa strain genomes. Further, analysis of cocoa-specific metabolic traits was performed to reveal domestication patterns. The phylogenomic analysis showed the overall undomesticated nature of the sequenced strain, which clustered together with other strains from the same geographical origin coming from different ecological niches. A pangenome consisting of 5,193 gene clusters (GCs) was constructed and the core genome (4,701 GCs) and accessory genome (492 GCs) were determined. Manual inspection of the accessory genome led to unravel specific GCs that were enriched in the genomes of the cocoa strains. Furthermore, the sequenced strain contained genetic signatures that could point toward specific niche adaptations, including a higher copy number of invertase genes, the presence of a polygalacturonase gene, loss of aquaporin activity, and conserved amino acid changes in key ester-producing enzymes.

B26

Chemical compounds produced during fermentation and sensory perception in specialty coffee fermented with starter yeasts

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Controlled fermentation using microbial starters has shown different microbial dynamics depending on the processing method, resulting in beverages with different and positive sensory characteristics. This study aimed to demonstrate how the organic acids, volatiles, and sensory profile were impacted by a Canário Amarelo variety fermentation using four starter cultures (*Meyerozyma caribbica* (CCMA 0198), *Saccharomyces cerevisiae* (CCMA 0543), *Candida parapsilosis* (CCMA0544), and *Torulaspora delbrueckii* (CCMA 0684)) inoculated in coffees processed via natural and pulped natural. Real-time PCR (qPCR) monitored the starter cultures population. *T. delbrueckii*, *S. cerevisiae*, and *M. caribbica* had similar dynamics by increasing in natural and decreasing in pulped coffees, while *C. parapsilosis* population decreased in both. Organic acids and volatile compounds were detected by high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC–MS). Among the processes, the highest organic acids final concentration was found in *T. delbrueckii* pulped coffee, reaching 17.39 mg/g of total acids. Isovaleric, citric, and isobutyric (6.63, 6.55, and 3.26 mg/g, respectively) presented the highest concentrations in pulped coffee. In natural coffees, *T. delbrueckii* presented the highest acid total concentration (14.37 mg/g), and succinic acid was the most abundant (13.68 mg/g). In roasted beans, pulped coffees had a higher number of compounds. Alcohol was produced only by *C. parapsilosis* and *S. cerevisiae*. A variety of sensory attributes were perceived in pulped coffees, mainly in *T. delbrueckii*, resulting in a dense and creamy body and lactic acidity beverage. Moreover, the resulting beverage from *C. parapsilosis* presented medium sweetness, acidity, and body and was the only treatment where an intense malic acid was perceived. *S. cerevisiae* presented sensory characteristics of red fruits and dairy notes and medium aftertaste. During coffee fermentation, the dynamic of starter yeasts modifies the chemical composition, becoming an alternative to obtain coffees with different sensory profiles.

B27

Different altitude coffees in anaerobiosis affect the dominant fungal community and biochemical profile

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Microbial communities change in response to the environmental conditions where fermentation occurs, such as temperature, moisture, and altitude, consequently affecting the beverage quality. Moreover, the type of fermentation, be open batch or close batch (SIAF: self-induced anaerobic fermentation), pulped natural or wet. This work aimed to evaluate if coffees from different altitudes cause changes in the dominant fungal community and organic acids, volatile compounds, caffeine, 5-chlorogenic acid (5-CGA). Coffee fruits (20 L) from altitudes 800, 1,000, 1,200, and 1,400 m were fermented under the SIAF method, and sub-samples at 48 h of fermentation were used for analysis. The dominant fungal community was evaluated through ITS-Illumina next-generation sequencing (NGS) and the dada2 pipeline. Organic acids, caffeine, and trigonelline were evaluated through high-performance liquid chromatography (HPLC) and volatiles with gas chromatography-mass spectrometry (GC-MS). Different species were specific for each altitude, and the species abundance change with altitude. Out of the total fungal species identified, yeasts naturally from cold habitats were the most abundant, mainly of *Cystofilobasidium infirmominiatum* at 800 (15.831%), 1,000 (38.218%), and 1,400 m (26.187%), and *Cystofilobasidium ferigula* at 1,200 m (83.857%). Acetic, malic, and citric acid concentrations were higher at 1,400 and 1,200 m. Similar contents of organic acids and volatiles were shared between the high abundance fungal community. 36% (7) of the volatile's alcohols detected characterized low altitudes (1,000 and 800 m), while esters characterized 1,400 m. Different caffeine and 5-CGA values were found in each altitude, presenting the highest at 1,200 m (11.64 g. Kg-1) and 800 m (9.70 g. Kg-1), respectively. Low altitudes presented the highest fungal richness, which probably influenced their strong association with volatile alcohols. Dominant microbiota from different altitudes and controlled conditions under SIAF fermentations are the main drivers of microbial communities and biochemical compounds.

B28

Understanding co-aggregation phenotypes between *Saccharomyces cerevisiae* and *Chlorella sorokiniana* with a focus on cell wall properties

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Autotrophic and heterotrophic partnerships, such as those between microalgae and yeasts, provide an attractive model system for the study of inter-species (kingdom) mutualisms. Of particular interest is the ability of yeast and microalgae to form co-aggregates when co-cultured, an industrially beneficial phenomenon, during microalgae harvesting for human and animal consumption. While the benefits of yeast and microalgae co-aggregation have been well established, the molecular mechanisms which underpin yeast-algae aggregate formation are still largely unknown. Previous work used directed evolution to generate co-evolved strains with improved biomass and aggregation phenotypes. Further investigation of these co-evolved strains revealed distinct difference in cell wall phenotypes in co-evolved strains, relative to the parental strains, indicating changes in cell wall organisation and gene expression. Several co-evolved yeast strains display increased sensitivity to the cell wall damaging agents, Calcofluor White and Congo Red, as well as more pronounced aggregation phenotypes in mono- and co-culture. To further understand how these co-aggregates are formed, this study employed the use of phenotypic screens, cell wall assays (cell surface charge, flocculation) and targeted gene expression analysis to identify key changes in cell wall structure and functioning. This research contributes to an understanding of how cell wall changes lead to improved aggregation phenotypes, thereby enhancing bio-flocculation strategies and design used in microalgae cultivation in the food industry.

B29

Evolutionary enhanced acetic acid stress tolerance and induced glycerol biosynthesis of the probiotic *S. cerevisiae var. boulardii*

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During the environmental stresses, high acid concentration has a negative effect on yeast cell growth and limits biochemical production. Improvement of yeast strains via evolution experiment is an approach to develop robust microorganisms. Here, we aimed to induce adaptive stress tolerance of *Saccharomyces cerevisiae var. boulardii* strain, grown under low pH and high temperature, to investigate inducibility of acetic acid stress tolerance. The evolved strains of *S. boulardii* was tolerated to high levels of acetic acid concentrations than the ancestral strain and the *S. cerevisiae*. Subsequently, using the evolutionary experiments, the evolved yeast strains showed increased survival under high concentration of acetic acid at 8 g/L, when compared with the ancestral strain. This indicated that the evolution strategy could improve the yeast tolerance to acids. Also, the evolved yeast *S. boulardii* strain showed enhanced capacity of glucose utilization and produced high glycerol level at approximately 6.2 g/L higher. The results suggested for reprogramming of stress response mechanism via induction of glycerol as a cellular protectant. Therefore, yeasts were evolved to enhance acetic acid tolerance and induced glycerol biosynthesis for cellular protection. These valuable strains could be employed as probiotics in food and beverage industries.

B30

Kynurenic acid production by *Yarrowia lipolytica* yeast growing on crude glycerol and soybean molasses

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Kynurenic acid (KYNA) is a very valuable compound acting as neuroprotective and antioxidant agent. This acid is credited with an N-methyl-D aspartate receptor antagonist and α7 nicotinic acetylcholine receptor antagonist. The disturbance in KYNA production strongly correlates with various psychiatric and neurodegenerative disorders. The recent data proved the existence of kynurenine biosynthesis pathway also in yeast cells. Due to that, the aim of the presented study was to enhance microbial production of KYNA using glycerol and soybean molasses as carbon and nitrogen sources. Especially soybean molasses as a nitrogen and tryptophan source during KYNA biosynthesis was carefully investigated. *Yarrowia lipolytica* A 101- 1.31 yeast strains as interesting KYNA producer and its glycerol kinase (GUT1) overexpressing derivatives were used in the fermentation experiments. Overexpression of GUT1 was already proved to accelerate glycerol utilization, what may improve also KYNA production in our study. The obtained results showed that *Y. lipolytica* GUT1 transformants were able to produce KYNA in higher concentrations than the parental strain (up to 14 mg/L for *Y. lipolytica* A 101-1.31 GUT1/1 transformant) in a medium with crude glycerol. Moreover, supplementation of the production medium with soybean molasses, the natural source of tryptophan, further increased the concentration of KYNA for all tested strains. Our findings show the potential of *Y. lipolytica* biomass producing KYNA as a functional food ingredient for the treatment of obesity and hyperlipidemia as well as for the modulation of gut microbiota in animals and human.

B31

Characterization of yeast out of fermentations of *Theobroma cacao L*.

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The production of high quality chocolate is an important economic factor worldwide. The seeds of the cacao tree Theobroma cacao L., which is originating from the tropical region of Peru, are used for this purpose. Raw cocoa of high quality is indispensable for the production of high quality, dark chocolates. This depends, among other things, on post-harvest treatment: the fermentation and drying of cacao seeds. Fermentation in particular is essential for the formation of the cocoa aroma, as important aroma precursors are produced. Misfermentation can lead to false flavors and mycotoxin contamination. Nevertheless, the worldwide standard is still a spontaneous fermentation process that does not allow real process control. These circumstances make it increasingly difficult for chocolate manufacturers to procure high, consistent quality raw cocoa.

By using a microbial starter culture, the fermentation process could be controlled better and the formation of certain aroma profiles could be promoted. The development of the taste profile of cocoa is influenced by various factors, such as metabolites formed by microorganisms during fermentation. These can represent flavor precursors with different characters (e.g., fruity, flowery or nutty nuances or also off-flavors), which are involved in the formation of the later chocolate flavor during the further processing of the cacao seeds.

Within the framework of the CORNET project "CocoaChain" funded by the FEI, samples were taken at different locations of spontaneous cacao fermentations in Peru to determine the biodiversity of microorganisms. The isolation and subsequent characterization of yeasts using FT-IR should help to determine the influence of the different isolates on aroma formation during cacao fermentation. For this purpose, the aroma profile of different yeast strains was characterized in submerged fermentation with cacao pulp simulation medium on a laboratory scale.

B32

Biocontrol of postharvest blue and green mould diseases in cv. Starking delicious apples by antagonistic yeasts during ambient and cold storages

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The aim of this study was to control two prevalent postharvest pathogens, blue mould (*Penicillium expansum* DSM62841) and green mould (*Penicillium digitatum* DSM2750), in cv. Starking delicious apples during cold (4°C, 95% RH and 3 months) and ambient storages (20 ± 2 °C, 50 ± 5 % RH and 45 days) by yeast antagonists as an alternative to chemical control method. Epiphytic *Aureobasidium pullulans* GE17 (Y1) and *Meyerozyma guilliermondii* KL3 (Y2) yeast strains were used as microbial biocontrol agents. Quality parameters of apples such as pH, total acidity, brix, weight loss, fruit firmness and browning index were followed during ambient storage, while infection rate, disease severity and lesion diameter values were determined during cold storage. After one-week incubation at 25°C, both yeasts exhibited great colonization ability on wounded parts of apples. Population density present in the apples treated with Y1 alone and its mixture with Y2 reached to 10.14 log cfu/wound and 9.51 log cfu/wound, respectively. Through VOCs produced by mixed culture, radial growth of green mold and blue mold were suppressed by 44.83% and 51.58%, respectively. The results of storage at ambient condition indicated that yeast treatment did not impair any of the quality parameters of apples during storage. After 45 days of storage at ambient condition, the highest natural decay development ratio (28.8 %) was determined in apples belong to the control group. Lower infection values were detected in apples treated with Y1 (8.33%) and mixed culture (11.11%). Yeast application protected to the texture of apples compared to the control. According to the experimentation in cold room, application of Y1 and mixed culture reduced the lesion formation by blue and green molds almost by 50%. Successful biodegradation of patulin by strain Y2 (98.50 %), Y1 (89.57%) and their mixed culture (99.44 %) was proved after 32h of incubation.

B33

Impact of cultivation conditions on organelle-specific redox potentials and stress in recombinant protein producing *Pichia pastoris*

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The methylotrophic yeast *Pichia pastoris* is one of the most effective and established expression host for heterologous protein production. The redox balance of its secretory pathway, which is multi-organelle dependent, is of high importance for increased recombinant protein production. Redox imbalance and oxidative stress are two main factors that can influence protein production and secretion, especially the redox potential of the ER where the protein folding and disulphide bond formation occur. Glutathione is the main redox buffer of the cell and its redox conditions can be determined by the status of glutathione redox couple (GSH-GSSG). In vivo measurements of the redox potential in different subcellular compartments can be achieved by genetically encoded redox sensitive fluorescent probes (roGFPs). However, it is still unclear in what extent the environmental conditions influence the compartment-specific redox potential and glutathione levels and, subsequently, recombinant protein secretion. The aim of this study is to investigate the impact of bioreactor cultivation conditions on oxidative stress, on glutathione pool levels in different subcellular compartments and the generation of reactive oxygen species (ROS).

B34

Nutrient signaling pathways control wine yeast performance

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Wine yeast adaptation to the industrial conditions is key for its biotechnological success. *Saccharomyces cerevisiae* has to be able to have a fully respiratory metabolism during biomass production and a strong fermentation power during winemaking and tolerate many different stress conditions. Nutrient signaling pathways control metabolism and stress response depending on the nutrients present and the environmental conditions. Their function is well known in laboratory conditions, but remain quite uncharacterized in industrial processes and in commercial yeasts. Yeast have favorite carbon and nitrogen sources and repress the use of non-preferred ones, via Protein Kinase A/SNF1 and Nitrogen Catabolite Repression (NCR) pathways respectively. Biochemical analysis of the main pathways revealed unexpected behavior during winemaking. For instance, regarding glucose repression mechanisms, Snf1 kinase was activated only when glucose was exhausted under laboratory conditions, but was active from early fermentation stages when sugar levels are very high. Transcription factor Gln3, which activates genes subject to NCR, was also active for the first hours, even when ammonium and amino acids were still present in media. Deletion analysis of regulatory pathways during winemaking also show that GLN3 and SNF1 share a similar impact in fermentation kinetics and metabolite production, suggesting that there are not isolated carbon or nitrogen signaling, but that both are connected. PKA has also a great impact on fermentation, and deletion on cAMP phosphodiesterase PDE2 by CRISPR-Cas9 increase fermentation speed. Stress response proteins have also an impact on metabolism. Peroxiredoxin Tsa1 acts as a redox sensor, and we have found that its deletion impacts defensive carbohydrate trehalose and glycogen accumulation during biomass propagation in molasses. Therefore, nutrient signaling, stress response and metabolism are all well coordinated in yeast to guarantee a successful biotechnological performance.

B35

Peroxiredoxin Tsa1 plays a role in growth, stress response and trehalose metabolism during wine yeast biomass propagation

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Wine industry is a key economic sector for the European economy. Nowadays, enological industry relies on the use of yeast starters for grape juice inoculation as Active Dry Yeast (ADY). During its industrial use in winemaking, yeasts, and particularly *Saccharomyces cerevisiae*, must withstand several stress conditions. Peroxiredoxins are a family of peroxide-degrading enzymes that challenge oxidative stress. They receive their reducing power from thioredoxins, and these from thioredoxin reductase. The main cytosolic peroxiredoxin Tsa1 acts as a redox switch controlling some metabolic enzymes like pyruvate kinase and the PKA pathway. Hence, Tsa1 is an ideal candidate for studying the control of metabolism by the redox status during yeast performance under industrial processes.

TSA1 deletion in L2056, a diploid industrial wine yeast strain, displays a growth defect during biomass production simulations on molasses, both in flask and bioreactor. Deletion of key player genes for Tsa1 functionality, such as sulfiredoxin SRX1 and one copy of cytosolic thioredoxin reductase TRR1, does not impact growth in molasses. This fact emphasizes that only Tsa1, and concretely its oxidized form, is required for cell proliferation under these conditions. tsa1Δ mutant reveals an alteration in its redox status, showing increased intracellular Reactive Oxygen Species (ROS) and changes in glutathione levels. Strikingly, it also presents a variety of metabolic changes that allow to confer new functions to Tsa1. During growth in molasses, Tsa1 impacts carbohydrate metabolism, repressing early accumulation of trehalose and glycogen, but being required for high trehalose levels during stationary phase. In flasks with sugar beet molasses, *tsa1*Δ mutant shows increased trehalase activities, both acid (Ath1/2) and neutral (Nth1), which do not correlate with trehalose levels. Additionally, TSA1 deletion diminishes yeast fermentative capacity in grape juice fermentation and alters acetic acid production, but the vinification profile does not significantly change.

B36

Deciphering the microbial communities involved in the kombucha fermentation

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Kombucha is an ancestral beverage containing negligible concentrations of alcohol. It is made by the fermentation of sweetened tea with a mixed consortium known as a symbiotic culture of bacteria and yeasts (SCOBY). After centuries of household production, it recently has become a widely commercialized soft drink thanks to the possibility to brew it at large scales while maintaining the traditional process of fermentation.
The SCOBY has the particularity to be distributed between two compartments represented by the cellulosic biofilm synthetized by the bacteria and the liquid. A fraction of both compartments of a previous fermentation is used to initiate the next fermentation.
The SCOBY is a valuable model to study the dynamics and the stability of microbial communities. For this study, we gathered a comprehensive sampling of SCOBY in order to tackle the following issues: what is the diversity in the microbial composition of the SCOBY generated from kombucha of various origins? Does the SCOBY composition evolve over a wide range of successive fermentations? What are the short and long-term impacts of production events and changes in the production process, on the microbial composition?
We used metabarcoding to characterize the microbial compositions in yeast and bacteria of the solid and liquid compartments. Our results show an overall high stability of the SCOBY suggesting an intrinsic high level of resilience.

B37

Copy number variation in Canadian *S. cerevisiae* wine yeast genomes

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Vineyards around the world have proven to be reservoirs of yeast with oenological potential. *Saccharomyces cerevisiae* (*S. cerevisiae*), carries out fermentation of grape sugars to ethanol as well as generating flavour and aroma compounds in wine. We have isolated and genetically screened thousands of *S. cerevisiae* strains from spontaneous fermentations of grapes from the Okanagan Valley (OV). Wineries place a high value on identifying yeast native to their region to develop a region-specific (terroir) wine program. The genomes of 75 candidate OV wine strains were sequenced using Illumina paired end reads and compared to representative genomes from global strain subpopulations. Our phylogenetic analysis shows that OV strains cluster into four clades: commercial wine, Okanagan Valley wine (OKV), North American oak (NAO) and sake. Reports on the genotype-phenotype relationship of *S. cerevisiae* attribute gene copy number variations (CNVs) to a larger portion of trait variations than single nucleotide polymorphisms (SNPs). To find evidence of domestication and wine making traits, CNV profiles were analyzed between four clades using the Kruskal-Wallis test, which discovered that 177 loci had significantly differential CNV within 93 strains (p-value < 0.05). We find that gene loss is prominent in industrial strains having lost on average 493 more genes per genome than OV strains. Strains in the commercial and OKV wine clades have CNV in gene families reflective of wine making traits such as environmental stress tolerance (AAD/ALD, HSP), fermentation (HXT, MAL, FLO), and nutrient requirements (PHO, THI, SNZ). NAO strains demonstrate higher diversity in gene content as evidenced by elevated CNV in genes absent from commercial and OV clades, and this may reflect their generalist lifestyle in the wild. We believe this is the first study to demonstrate the isolation of strains from spontaneous wine fermentations that are genetically similar to a NAO strain.

B38

Ergosterol and fatty acid mixtures impact differently the production of primary fermentation metabolites in wine yeasts

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Lipids are essential energy storage compounds and are the core structural elements of all biological membranes. During wine alcoholic fermentation, the ability of yeasts to adjust the lipid composition of their plasma membrane partly determines their ability to cope with various fermentation-related stresses, including elevated levels of ethanol and the presence of weak acids. In addition, the lipid composition of the grape juice also impacts the production of many wine-relevant aromatic compounds. Several studies have evaluated the impact of lipids and of their metabolism on fermentation performance and aroma production in the dominant wine yeast *Saccharomyces cerevisiae*, but limited information is available on other yeast species. Thus, the aim of this study was to evaluate the influence of various specific fatty acid and sterol mixtures on the fermentation rates of various non-*Saccharomyces* yeasts and the production of primary fermentation metabolites. The data show that the response to different lipid mixtures is species-dependent. For *Metschnikowia pulcherrima*, an increase in carbon dioxide production was observed in medium enriched with unsaturated fatty acids whereas *Kluyveromyces marxianus* fermented further in synthetic medium containing a higher concentration of polyunsaturated fatty acids than monounsaturated fatty acids. *Torulaspora delbrueckii's* fermentation rate increased in medium supplemented with fatty acids and ergosterol present at equimolar levels. The data indicate that these different responses may be linked to variations in the lipid profile of these yeasts and divergent metabolic activities, in particular the regulation of acetyl-CoA metabolism. Finally, the results suggest that the yeast metabolic footprint and ultimately the wine organoleptic properties could be optimized via species-specific lipid adjustments.

B39

Whole genome sequencing and functional SNP analysis of Canadian *S. cerevisiae* wine yeast strains

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The application of next generation sequencing and high-throughput genomics to oenological science has great potential for the winemaking industry. Vineyards host a diverse community of *Saccharomyces cerevisiae* (*S. cerevisiae*) yeast strains with unique genomic characteristics that can enhance both the fermentation and style of wine. Here, we describe an investigation into the genomic characteristics of *S. cerevisiae* strains isolated from spontaneous grape fermentations from the Okanagan Valley (OV) in British Columbia, Canada. Over a five year period, thousands of *S. cerevisiae* strains were isolated from spontaneous fermentations conducted in OV wineries and vineyards. Whole genome sequencing of 75 candidate OV *S. cerevisiae* strains and single nucleotide polymorphism (SNP) analyses has placed them into 4 separate phylogenetic clades: Commercial Wine (CW), North American Oak (NAO), Sake (SK), and Okanagan Valley (OKV). Both OKV and NAO clades demonstrate decreased heterozygosity and an overall increase in genetic diversity, signatures that these strains are wild and not domesticated. Strains in the OKV and NAO clades contain ten to twenty thousand more SNPs, respectively, than strains within the CW clade. Triploidy is prevalent in the SK clade and aneuploidy is present in both SK and CW clades, indicating likely domestication of these strains. Genes with predicted loss of function (LOF) were identified based on frameshift mutations, loss of start codons, or premature stop codon introduction. Clustering analysis of genes with LOF predicts divergent functionality across the different clades and gene ontology enrichment identifies predicted LOF of metabolically relevant transporters and transcription factors. Most notable are transporters involved in iron homeostasis, hexose transport, and ion uptake. Notable transcription factors include those involved in maltose and sulfur metabolism. These findings provide OV winemakers with a genomic account for regional wine characteristics and may identify strains with potential for commercialization.

B40

Adaptative laboratory evolution to reduce acetic acid yield of *Saccharomyces cerevisiae* wine yeast strain under aerobic condition

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The use of respiratory metabolism has been proposed as a tool to decrease the alcoholic content in wines. *Saccharomyces cerevisiae* strains -despite the Crabtree effect- can significantly reduce their ethanol yield under aerobic conditions, compared to standard fermentation. However, aerobic conditions cause an exacerbated acetic acid production, limiting their usefulness for this application.
In previous works, recombinant wine yeast strains defective for some carbon catabolite repression (CCR) related genes showed a reduced acetic acid yield under oxygen presence, (Curiel et al., 2016), although the GMO status precludes their applicability in winemaking.

Here, we present a methodology to obtain industrial strains able to carry aerobic fermentation of grape must with lower acetic acid production than their parental strains, using adaptative laboratory evolution to alleviate CCR.
We evolved four *S. cerevisiae* -three commercially available-by repeated batch subculture for ~100 generations. The selective pressure was established by 2-deoxy-D-glucose (2-DG), in a media with galactose as the only carbon source.
All the evolved populations showed improved growth on different carbon sources (fermentable and non-fermentable) under CCR control and all of them showed a significant acetic acid yield reduction. Almost all showed a good sugar consumption and a slight increase in glycerol production. Clones isolated from vinification were tested for the most common wine-industry factor stresses. Selected candidates were confirmed for acetic acid production under aerobic conditions.

The shift between aerobic and anaerobic conditions has been reported as a trigger of acetic acid production, (Tronchoni et al. 2021). Colonies isolated from evolved populations were tested under a first aerobic phase (4d) followed by an anaerobic phase (7d), obtaining a significant decrease in acetic acid compared to parental. However, the conditions shift was still having an impact. We finally present results of process optimization to solve this issue with one of those improved strains.

B41

Selection of indigenous non-S*accharomyces* strains for formulation of mixed starter cultures aimed to ethanol reduction in wine

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The interest on mixed fermentations by S*accharomyces*/non-*Saccharomyces* has greatly increased in the last years due to the reduction in microbial diversity and consequent flattening of wine sensory characteristics determined by the common practice of inoculated fermentation with commercial *Saccharomyces cerevisiae* strains. The strategy of using selected mixed cultures is believed to be the key to produce wines with desirable characteristics that meet changing market demands with less ethanol but still with balanced aromatic quality. This work is addressed to the characterization of indigenous non-*Saccharomyces* strains by physiological and biochemical analysis, in order to identify the strains suitable as mixed starter. For this purpose, numerous indigenous non-*Saccharomyces* strains, belonging to *Hanseniaspora* guilliermondii, *H. osmophila*, *Torulaspora delbrueckii, Metschnikowia pulcherrima*, *Saccharomycodes ludwigii* were tested for parameters of oenological interest, such as tolerance to different concentrations of ethanol and SO2 and production of extracellular hydrolytic enzymes, such as β-glucosidase and esterase. On the basis of the results, some strains were chosen and tested in mixed fermentations trials with the commercial *S. cerevisiae* strain EC1118. The fermentation process was monitored daily by evaluating the consumption of sugars and the microbiological evolution to assess starter fermentative performance and persistence of non-*Saccharomyces* strain during the process. The experimental wines were analyzed for the content of ethanol, volatile acidity and main secondary compounds influencing wine aroma. A selected mixed starter, composed by *S. cerevisiae* and *H. osmophila* strains, was tested in microvinification trials, by using *H. osmophila* strain as free and microencapsulated cells.

The different formulation of the non-*Saccharomyces* strain influenced the mixed starter performances and wine aromatic composition, indicating a potential influence of *H. osmophila* formulation on interaction mechanisms between the strains in mixed culture.

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B42

Torulaspora delbrueckii phenotypic and metabolic profile towards biotechnological exploitation

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Non-*Saccharomyces* yeasts were considered, for many years, as a source of contamination during wine production and conservation. More recently, the use of these unconventional microorganisms for wine fermentation has been widely investigated and discussed, due to their impact on the complexity of wine organoleptic profile. Among this group, *Torulaspora delbrueckii* gained prominence in the winemaking sector owing to its contribution with low volatile acidity, the release of sweet compounds, colour intensity and sensory perception of wines. *T. delbrueckii* is also frequently found in home-made bread doughs, such as sourdoughs, and has attract interest for bread fermentation, due to its organoleptic impact and high resistance of some isolates. In this work, a collection of 44 *T. delbrueckii* strains with different geographical and technological origins was constituted, and their phenotypic behaviour was evaluated through a battery of tests with biotechnological relevance, including from an oenological point of view. Subsequently, the fermentative character of the isolates with greater phenotypic heterogeneity was evaluated, through individual fermentations in synthetic grape must. These analyses showed that temperatures above 37 °C and 14 and 18% (v/v) ethanol concentrations, significantly affected *T. delbrueckii's* growth. On the other hand, the majority of the strains shared a great resistance to stress environments, including the presence of fungicides. HPLC analysis reflected variable results for ethanol production, glycerol and citric acid concentrations contributing the most for inter-strain variability. Our results disclosed some *T. delbrueckii* strains, isolated from natural environments, that combine a great fermentative power, the release of high contents of glycerol, and a high resistance and production of ethanol, highlighting their biotechnological potential.

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B43

Isolation *Torulaspora Delbrueckii* from honey associated with osmotic stress and acid production for industry application

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Sugar-tolerant yeast are very important for the foods and beverage fermentation application. The purpose of this study is screening natural yeast isolates from honey samples by using molecular biological and physiology of osmoadaptation. The intergenic transcribed spacer region for sequencing of rDNA (ITS1-5.8S rDNA-ITS2) nucleotide sequence alignment method was used to identify yeast strain by PCR-amplified. Five different yeast strains were identified representing 3 different genera, *Saccharomyces cerevisiae*. *Torulaspora delbrueckii* (identity 99.76%), and *Candida etchellsii* (identity 99.18%) by comparison with the sequence on GenBank database (NCBI-BLAST). The occurrence of strains with special sugar tolerant with 800 g/L glucose can be beneficial for industrial fermentation. Organic acids compounds were produced by the yeast strain which were analyzed by HPLC methods. The major organic compound included citric acid (11.02 g/L), malic acid (16.62 g/L), succinic acid (4.17 g/L), formic acid (1.70 g/L) and acetic acid (6.18 g/L) were detected at 12 h, 24 h and 48 h of fermentation. The diversity of yeast species is valuable with potential for industrial applications such as in fermentation of food and beverages.

B44

Recovering wine yeast biodiversity: how the breeding of a single strain led to improved offspring

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Biodiversity loss in winemaking environment is becoming an alarming phenomenon (Renouf et al., 2007). Several reasons account for this loss, i.e., the use of standardized industrial yeasts or the search and the massive employment of specialized wine strains possessing optimized oenological properties. Several biological surveys aimed at the discovering of the hidden oenological potential of the yeast biodiversity in our wine-producing regions are however underway (Pretorius et al., 1999). Many intrinsic characteristics of autochthonous strains can be selected and improved through targeted development protocols (Rodríguez et al., 2010). The auto-diploidization ability of natural homotallic strains is one of such properties that can be used in classical breeding projects on yeast spores, in order to obtain a population of homozygous at all loci individuals (Solieri et al., 2015). These strains can be screened for particular oenological traits and therefore selected for a wider application, not only in food related environments. In our study, 31 monosporal cultures were obtained from a classical breeding project, inducing the sporulation, followed by micromanipulation and growth of the isolated spores, of a *Saccharomyces cerevisiae* strain usually employed in our region in white winemaking. These monosporal cultures were screened for their technological characteristics and compared to the parental strain, to highlight any improvement obtained by breeding. An FTIR analysis was also performed on all the samples, to further characterize the existing differences with the parental strain. The genomes of all the monosporal cultures, together with the one of the parental strain, were then sequenced using an NGS technology. The resulting reads were then assembled and the genomes of the spores were compared with the parental strains, in order to try to highlight the differences obtained with the breeding.

B45

Response of one *S. cerevisiae* strain to addition of argan oil during propagation and dehydration: preliminary results

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Saccharomyces active dry yeasts (ADY), commonly used as starters in winemaking, have to tolerate oxidative stress, caused by biomass propagation and dehydration. In the last years, some studies have investigated the use of natural antioxidants as protective agents against oxidative cellular damage. In this study the effect of argan oil was evaluated both on yeast propagation phase and as a protective agent before desiccation. In the first phase, a *Saccharomyces cerevisiae* strain was produced in molasses supplemented with different concentrations of argan oil (8, 12, 16 ml/L). The biomass produced was dried by spray drying and cell viability was evaluated after rehydration at three different temperatures (20, 26 and 37°C). The second phase was focused on the use of argan oil as a protective agent before drying. Different concentrations of argan oil (8, 12, 16 ml/L) were added to the biomass submitted to spray drying and the cell viability was evaluated after rehydration at two different temperatures (20 and 37°C). The samples of this phase were tested in fermentation trials carried out at 20°C in two different laboratories (Fermenting Yeast Laboratory of Basilicata University and AEB SPA). The fermentations were monitored daily by weight loss determination and sugar consumption. Fermentations were stopped when weight and °Brix reductions were constant for three consecutive days. The experimental wines were analyzed for the content of main secondary compounds affecting wine aroma and for conventional chemical parameters of oenological interest. The results obtained have shown a potential positive effect of argan oil on the increase in biomass yield, whereas less influence was found as protective agent during dehydration. Good results in cell viability were obtained by rehydration at low temperatures (20°C).

B46

Using non-conventional yeasts to produce low alcohol beers

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Beer is one of the most consumed alcoholic beverages. But in the last years, a slight reduction of global beer consumption has been observed. Besides, growth of low-alcohol beer is expected at 2.8 % and no-alcohol at 8.8 % in the next five years. These variations have been observed due to consumer´s trends towards healthier behaviors. The physical dealcoholisation methods that are usually used in industry as reverse osmosis, dialysis and osmotic distillation produce also the removal of other volatile compounds which are essential for the organoleptic properties of beer as higher alcohols and esters. One alternative to reduce the ethanol content is the use of non-conventional Saccharomyces yeast in brewing. In this project, we have selected around 300 representative strains covering the different species of Saccharomyces and their corresponding subpopulations. We included representatives of the 26 subpopulations of *S. cerevisiae*, 4 subpopulations of S. kudriavzevii, 5 subpopulations of *S. uvarum*, 3 subpopulations of *S. eubayanus*, 6 subpopulations of *S. paradoux*, 1 strain of *S. jurei*, 2 subpopulations of *S. mikatae* and 2 subpopulations of *S. arboricolus*. We also collected strains from Naumovozyma and Kazachstania genera, which can be potentially used to hybridize with *S. cerevisiae*. We also added genetically distant species as *Candida tropicalis*, *Candida shehetae*, *Pichia kluyverí*, *Saccharomycodes ludwigii*, *Torulaspora delbrueckii*, *Williopsis saturnus* and *Zygosaccharomyces rouxii* which have been already described as low ethanol yield producers in beer fermentations. We are now developing a metabolic study of these yeast strains to select the strains with lower ethanol yield which will be used to develop an analysis with transcriptomic and metabolomic techniques. The great genetic diversity that we have achieved in our collection will be key to selecting candidates to produce hybrids or develop co-culture systems to finally produce reduced alcohol beer with organoleptic quality.

B47

Coding microsatellite sequences in non-*Saccharomyces* wine yeast

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Yeast microsatellite loci consist of short tandem-repeated DNA sequences of variable length useful for strain differentiation, population genetics, and evolutionary biology. Coding microsatellite sequences have been recognized in the yeast *Saccharomyces cerevisiae* and *Candida albicans*. These particular loci are dynamic components of yeast genomes and play a relevant role in gene evolution. We have previously shown that indels and non-tandem repeat variations are frequent among microsatellite loci of the non-*Saccharomyces* wine yeasts *Starmerella bacillaris*, *Hanseniaspora uvarum*, *Saccharomyces uvarum*, *Torulaspora delbrueckii*, *Brettanomyces bruxellensis*, and *Meyerozyma guilliermondii*. Our results indicated that, besides a variable number at their originally defined tandem-repeated motifs (TRM), allelic variants for some microsatellite loci of these yeast species were also dependent on SNPs and/or indels flanking their TRM. In this work, we explored the possibility that non-Saccharomyces wine yeast microsatellite sequences are located within protein-coding regions. In silico analyses revealed that some microsatellite loci from *H. uvarum*, *T. delbrueckii*, *B. bruxellensis*, and M. guilliermondii localize into protein-coding sequences, many of them corresponding to proteins having regulatory activities. The coding microsatellite sequences involve trinucleotide repeats, which result in allelic variants that do not disturb their open reading frames. Coding TRM show a remarkable bias in nucleotide composition, most of them corresponding to codons for either highly acidic or basic residues (i.e., Glu, Gln, Asp, Asn, and Lys). In addition, sequence analyses of allelic variants revealed that many of the SNPs in regions flanking the TRM result in silent mutations that do not affect the protein sequences. Sequence variations in coding microsatellite loci may reveal specific adaptive profiles of wine yeast strains.

B48

Natural polymorphism of pectinase PGU genes in the genus *Saccharomyces*

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Pectins are polysaccharides of plant origin, consisting of galacturonic acid residues, linked by α (1–4) - glycosidic bonds, present in the form of methyl esters. Even low levels of pectin substances in wine can produce colloidal turbidity and cause filter stoppages. Degradation of plant pectin substances is a complex process involving several enzymes; the major one is pectinase (endo-polygalacturonase, EC 3.2.1.15).

Based on strains of various origin, we investigated distribution and properties of PGU genes in the genus *Saccharomyces*. According to molecular karyotyping and Southern hybridization *S. arboricola, S. cariocanus, S. cerevisiae, S. kudriavzevii,* and *S. paradoxus* have a single PGU gene located on chr. X. The other three species carry polymeric PGU genes of different chromosomal localization: *S. mikatae* and *S. jurei* (chr.X and VIII), S. bayanus (X, I, XIV). According to phylogenetic analysis the highest similarity of endo-polygalacturonases (>95%) was in *S. cerevisiae*, *S. paradoxus*, and *S. cariocanus*, and in *S. mikatae*/*S. jurei*. The most divergent were endo-polygalacturonases of S. arboricola and *S. bayanus*: the level of their similarity to each other and to the Pgu proteins of the other species did not exceed 89%.

Our results demonstrate considerable intraspecific polymorphism of endo-polygalacturonase secretion in Saccharomyces species, except *S. bayanus*. The ability to secrete active endo-polygalacturonase is apparently a specific feature of the species. Relatively high pectinolytic activity was also exhibited by *S. paradoxus*, inhabiting mostly natural sources worldwide. Apparently, the polygalacturonic acid component of pectins represents an important carbohydrate nutrient for yeasts of natural habitats.

Considering our results along with the previously published data on high pectinolytic activity of some *S. paradoxus* strains (Eschstruth and Divol, 2011), it seems reasonable to employ the gene pool of this species in wine yeast selection research.

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B49

Evaluating the heterogeneity of lactic acid production by *Lachancea* species and strains.

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Lachancea species, in particular *Lachancea thermotolerans* are frequently encountered in grape juice and fermenting must. Several studies have shown that strains of this species produce lactic acid which acidifies wine while enhancing colour, aroma, and mouthfeel. But there is large strain variability in the production of lactic acid, which is driven by genetic diversity in this species. Furthermore, lactic acid production by *L. thermotolerans* is easily affected by environmental conditions. However, the underlying molecular mechanisms of this phenomenon are poorly understood. This study aimed to assess the heterogeneity in lactic acid production among *L. thermotolerans* strains and strains of the other *Lachancea* species viz. *L. fermentati* and *L. lanzarotensis* in synthetic wine fermentations under oxygenated (5% dissolved oxygen) and anaerobic conditions. In addition, the relative expression of genes associated with lactate biosynthesis i.e., three lactate dehydrogenase (LDH1, 2 and 3) paralogous genes, lactate dehydrogenase (cytochrome) (LDH Cyto), pyruvate kinase (PK) and NADPH dependent methylglyoxal reductase (NADPH-MGR) was examined. The levels of lactic acid produced in this study ranged from 0.06 g/L to 2.1 g/L with the highest production from *L. thermotolerans* strains. Aeration had minimal impact on lactic acid production in some strains while in others there was a significant decrease in the levels quantified. Our results suggest the possible implication of LDH1 and LDH2 as well as LDH Cyto in the higher production of lactic acid in some strains under anaerobic conditions. This study provides insights on the intra- and inter- species variation of lactic acid production and the impact of oxygenation on production levels and genetic regulation.

B50

Bioprospecting thermotolerant yeasts from distillery effluent and molasses for high-temperature ethanol production

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Efficient bioethanol production from lignocellulosic biomass requires yeasts, which are capable of utilizing a broad substrate range, tolerating multiple inhibitors, and yielding a high amount of ethanol at elevated temperatures. Molasses and distillery effluent have been underexplored niches for isolation of thermotolerant yeasts; therefore, we isolated 98 thermotolerant yeasts from 7 different distillery effluent and molasses samples. Post-MSP-PCR screening, 34 yeast isolates were identified by D1/D2 region sequencing. These yeasts belonged to 8 genera and 9 different species viz. *Candida blankii, C. ethanolica, Kluyveromyces marxianus, Millerozyma farinosa, Ogataea polymorpha, Pichia kudriavzevii*, *Saccharomyces cerevisiae*, *Torulaspora indica*, and *Wickerhamiella shivajii*. After screening these 34 yeasts for their ethanol production potential at 40oC using 5% glucose, we selected 7 high ethanol-yielding yeasts (ethanol titre 22.31 – 24.5 g l-1 with fermentation efficiencies of 87.3 – 95.7 %.) for high-temperature ethanol production and assessment of inhibitor tolerance. At 45°C, *P. kudriavzevii* DSA3.2 and *K. marxianus* MSS6.3 yielded the highest ethanol titers 22.3 and 23.0 g l-1 with 87.4% and 90% efficiency, respectively. Among the selected strains, *P. kudriavzevii* DSA3.2, DSA3.1, and *K. marxianus* MSS6.3 exhibited significant tolerance against multiple pre-treatment generated inhibitors (e.g., furfural, 5-HMF, and acetic acid). The selected yeasts were also assessed for their ethanol production potential from alkali-treated rice straw, wherein, at 45°C, *K. marxianus* MSS6.3 and *P. kudriavzevii* DSA3.2 produced 10.5 and 10.3 g l-1 of ethanol with 84.5 and 80.4 % efficiency respectively via separate hydrolysis and fermentation (SHF). While during simultaneous saccharification and fermentation (SSF) at 45oC, *K. marxianus* MSS6.3 and *P. kudriavzevii* DSA3.2 produced 10.9 and 10.5 g l-1 of ethanol with 85 and 84.5 % efficiency respectively. Therefore, with further optimization and scale-up, *P. kudriavzevii* DSA3.2 and *K. marxianus* MSS6.3 can be considered as potential candidates for efficient ethanol production from lignocellulosic biomass through SSF approach.

B51

Construction and analysis of a yeast for the simultaneous release and esterification of the varietal thiol 3-sulfanylhexan-1-ol

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Polyfunctional thiols like 3-sulfanylhexan-1-ol (3SH) and its ester 3-sulfanylhexyl acetate (3SHA) are important aroma determinants in wine with exceptionally low odour thresholds. 3SH is largely found in grape must bound to glutathione and cysteine and requires enzymatic action to be perceived sensorially. The wine yeast *Saccharomyces cerevisiae* is ineffective in releasing volatile thiols from their precursor configuration. For this purpose, a yeast strain was constructed that expresses the carbon-sulfur lyase encoding tnaA gene from Escherichia coli and overexpresses its native alcohol acetyltransferase encoding genes, ATF1 and ATF2. The resulting yeast strain, which co-expresses tnaA and ATF1, showed elevated 3SH-releasing capabilities and the esterification of 3SH to its acetate ester 3SHA. Levels of over 7000 ng/L of 3SHA in Sauvignon blanc wines were achieved. Enhanced release and esterification of 3SH was also shown in the fermentation of guava and passionfruit pulp and three varieties of hops. This study offers prospects for the development of flavour-enhancing yeast strains with optimised thiol-releasing and esterification capabilities in a diverse set of beverage matrices.

B52

4-VG production by *S. cerevisiae* ethanol red mutant strain expressing phenolic acid decarboxylase from lignolytic yeast

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Due to an increase in consumers' demand for natural products, lignocellulosic biomass appears as a sustainable alternative to produce aroma compounds from natural feedstocks using microorganisms and enzymes. Among the high-value molecules that can be obtained from biomass, ferulic acid (FA) represents an interesting candidate, not only because it is a high-value molecule itself, but also because it is a platform molecule for the production of other chemicals, such as 4-vinylguaiacol (4-VG). The latter presents broad applicability in medicine, food, perfumery, and cosmetic industries. The most feasible process for biocatalytic synthesis of 4-VG is the decarboxylation of ferulic acid by a cofactor-free enzyme, phenolic acid decarboxylase (PDC). In addition, the heterologous expression of this enzyme improves 4-VG production in *Saccharomyces cerevisiae*, since its decarboxylase requires a cofactor regenerating system. To develop a robust yeast with optimized decarboxylation activity on phenolic acids, two PDC genes (PDC_A and PDC_B) from a wild yeast isolated from a lignin-degrading consortium, were separately integrated in *S. cerevisiae* ethanol red strain under the control of the constitutive gene promoter pTDH3 and terminator TPS1 sequences. Firstly, the decrease of FA in the cultivation medium was measured by spectrophotometry (absorbance at 325 nm) in high-throughput screening method during 96h. Although the PCD genes have 76% of identity, the clones with PDC_A expression did not present a FA consumption. The strains, with PDC_B expression, were micro fermented with YPD medium containing 1mM of FA for 96h, followed by 4-VG quantification by GC-MS. As a result, all the mutant strains showed high production of 4-VG, increasing at least 5 times comparing to the wild type. In brief, this study offers prospects for the production of aroma compounds from molecules derived from lignocellulosic biomass.

B53

Engineering the glucose responsive promoter PG1

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The methylotrophic yeast *Pichia pastoris* (syn *Komagagtaella* spp) is a highly valuable organism for heterologous protein production due to its high product yields, its ability to grow in high densities, and to perform post-translational modifications. Separation of the growth phase and protein production is desirable and has been achieved in P. pastoris with the utilization of AOX1 promoter, that is strongly induced by methanol. Nevertheless, methanol has also some disadvantages for the industry, as it is a hazardous and flammable compound. Therefore, identification of novel promoters that do not require methanol for their induction has been pursued over the last years. For this purpose, a set of glucose induced promoters were identified by Prielhofer et al., 2013 (1). Among these, the PG1 promoter is induced at low glucose concentrations and repressed in glycerol or glucose excess. This promoter offered elevated protein yields and the separation of the growth and the production phase. Further modifications of the promoter led to a variant, the PG1-3, that achieves higher productivity than the AOX1 promoter (2).

To further increase protein production, we used the PG1 as a model for promoter engineering. On one hand, we used a random approach where PG1 was subjected to error prone PCR, and subsequent selection of the best performing single copy clones through cell sorting. On the other hand, we used a rational approach where segmental deletions of the promoter were done, allowing us to elucidate the most relevant regions on the promoter. Combining the information obtained with these two methodologies, we were able to successfully construct a set of promoter variants that achieved higher protein production than the previously described PG1-3 promoter.

1. Prielhofer R., et al. 2013 Microb Cell Fact 12:5
2. Prielhofer R., et al. 2018 Biotechnol Bioeng 115(10):2479-2488

B54

Challenges and prospects in production of therapeutic proteins with an expanded genetic code in *Pichia pastoris*

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Expansion of the genetic code is a powerful tool for manipulation of protein properties by reassigning a natural triplet codon with another amino acid or utilization of quadruplet codons assigned to a specific amino acid. To achieve the codon reassignment, genetic introduction of a part of translation machinery responsible for the specific amino acid recognition into a host cell is needed. Such a system is named to be orthogonal in the host cell since it must not interact with the equivalent endogenous translation machinery. Up to now, the method has been broadly applied in engineering and research of biological molecules even beyond proteins. This work focuses on production of full-length IgG and its Fab fragment with the incorporated unnatural amino acid p-azidophenylalanine (pAzF) on a place of an internal amber stop codon. A pair of suppressor tRNA and modified tRNA synthetase originating from Escherichia coli that specifically recognize pAzF (Young et al., 2009) is used for amber codon reassignment. The aim of producing IgG with pAzF is to subsequently use it for conjugation reactions with biologically active molecules (i.e., payloads) carrying reactive groups specific for the azido moiety of pAzF. Thus obtained IgG conjugates would have enhanced therapeutic properties compared to the payload alone. In addition, Fab fragments are considered due to their production simplicity and potential to replace full-length IgG for certain applications.
The industrial yeast *Pichia pastoris* is used as the expression system due to its strong methanol inducible promoters, high secretion capacity and ability to grow to high cell densities in inexpensive media. It is a valuable screening platform for antibody production and pairing it with the genetic code expansion has a potential for rapid development of antibody-drug conjugates. This work addresses some of the main obstacles in optimizing *P. pastoris* for this purpose.

B55

Flo8 – a versatile regulator for improving recombinant protein production in *Pichia pastoris*

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Flo8 is a main transcriptional regulator of pseudohyphal growth in yeast. Recently, it was shown that disruption of *FLO8* in the popular recombinant protein production host *Pichia pastoris* (syn *Komagataella* spp) abolishes pseudohyphal growth and significantly reduces cell-to-surface adherence, making the mutant an interesting base strain for research and industry [1,2]. However, knowledge on the physiological impact of the mutation remains scarce and comprehensive studies employing *FLO8*-deficient strains for recombinant protein production are lacking. Here, we re-analysed published RNAseq data of the *P. pastoris* wildtype and Δ*flo8* mutant cultivated in glucose-limited chemostats at a "fast" (0.1 h^{-1}) and "slow" (0.05 h^{-1}) growth rate setpoint [2], revealing that Flo8 affects the expected flocculation targets, but also mating, respiration, cell cycle genes as well as catabolite repression and that its actions are specific to the respective growth condition. Furthermore, we tested the Δ*flo8* mutant in combination with the strong glucose-regulated (methanol-independent) *GTH1* promoter (P_{G1}) [3] and its engineered derivative $P_{G1\text{-}3}$ [4] for recombinant protein production in small scale screenings and bioreactor cultivations. It was demonstrated that P_{G1} and $P_{G1\text{-}3}$ expression strength was significantly elevated in the Δ*flo8* mutant, resulting in substantially enhanced recombinant protein titers.

[1] Rebnegger C., et al. 2016 Appl Environ Microbiol 82(15):4570-4583

[2] De S., et al. 2020 FEMS Yeast Res 20(5):foaa044.

[3] Prielhofer R., *et al*. 2013 Microb Cell Fact 24;12:5.

[4] Prielhofer R., *et al.* 2018 Biotechnol Bioeng 115(10):2479-2488.

B56

Direct proof of the amyloid nature of yeast prions [PSI+] and [PIN+] by the method of immunoprecipitation of native fibrils

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Prions are proteins that can exist in several structurally and functionally distinct states, one or more of which is transmissible. Yeast proteins Sup35 and Rnq1 in prion state ([PSI+] and [PIN+], respectively) form oligomers and aggregates, which are transmitted from parents to offspring in a series of generations. Several pieces of indirect evidence indicate that these aggregates also possess amyloid properties, but their binding to amyloid-specific dyes has not been shown in vivo. Meanwhile, it is the specific binding to the Congo Red dye and birefringence in polarized light after such staining that is considered the gold standard for proving the amyloid properties of a protein. In this work we applied a new methodology that allowed us for the first time to isolate native fibrils of yeast prion proteins and analyze their key amyloid properties. We used immunoprecipitation to extract native fibrils of the Sup35 and Rnq1 proteins from yeast strains without artificial overproduction of prion proteins. These fibrils from prion-containing strains are detected by electron microscopy, stained with Congo Red and exhibit yellow-green birefringence in polarized light after such staining. All these data show that the Sup35 and Rnq1 proteins in prion state form amyloid fibrils in vivo. The technology of fibrils extraction in combination with standard cytological methods can be used to identify new pathological and functional amyloids in any organism, to analyze the structural features of native amyloid fibrils, and to study the interaction of amyloids with other proteins.

Room C – Café Central

C01

Treatment with surfactants enables quantification of translational activity by O-propargyl-puromycin labelling in *P. pastoris*

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Protein synthesis is regulated at several cellular levels, starting with transcription of the gene of interest and subsequent translation of the mRNA. To better understand cellular processes of protein production, rate measurements at limiting steps are necessary. But, while abundant methods are available to measure transcription levels, measuring translation is still a challenge. The recently established usage of O-propargyl-puromycin (OPP), which incorporates itself into nascent polypeptide chains, made simple measurements of global translation rates possible in mammalian cells however, unmodified yeast cells are unsusceptible to puromycin and therefore this method has not been used in yeast, so far. Using a *Pichia pastoris* (syn. *Komagataella phaffi*i) strain with an impaired ergosterol pathway (*erg6*Δ), we could increase susceptibility to puromycin, but translation measurements were then restricted to this strain background, which displayed growth deficits. Using surfactants like Imipramine, instead, proved to be more advantageous and circumvented previous restrictions. The Imipramine-supplemented OPP-labelling with subsequent flow cytometry analysis, enabled us to distinguish actively translating cells from negative controls, and to clearly quantify differences in translation activities in different strains and growth conditions. Specifically, we investigated *P. pastoris* at different growth rates, verified that methanol feeding alters translation activity, and analysed global translation in strains with genetically modified stress response pathways.

In conclusion, we set up a simple protocol to measure global translation activity in yeast on a single cell basis providing us with a valuable tool for observation and manipulation of *P. pastoris* cells during conditions relevant for protein production. The use of surfactants poses a practical and non-invasive alternative to the commonly used ergosterol pathway impaired strains and thus would be beneficial to a wide range of applications where increased drug and dye uptake is needed.

C02

Improving CRISPR\Cas9-mediated genome editing technique in *Saccharomyces cerevisiae*

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The CRISPR/Cas9 system allows to achieve highly efficient simultaneous marker-less genomic integration of multiple DNA fragments in different loci of the *S. cerevisiae* genome [1, 2]. However, the method requires a number of time-consuming preparatory steps. Thus, one of the most common ways to introduce multiple modifications using CRISPR/Cas9 technique requires creation of intermediate strains expressing Cas9 as well as assembling of donor DNA fragments containing long homologous flanks (500 bp) at their ends, which facilitate homologous recombination in vivo [1, 2]. Vectors containing an appropriate number of gRNA sequences are used for multiple genetic modifications [3]. For integration into other loci a new vector(s) for expression of appropriate gRNAs should be additionally assembled. Thus, the time savings in multiplex genomic engineering can be lost due to the need to obtain intermediate strains and many genetic constructs. Here, we demonstrated the possibility of several time-saving modifications of the standard CRISPR/Cas9 based genome editing technique:

1. Editing of single locus without preliminary introduction of Cas9 expression plasmid, using fragment of donor DNA with short length (70bp) homologous flanks;
2. imultaneous editing up to three genomic loci without preliminary introduction of Cas9 expression plasmid, using donor DNA fragments with short length homologous flanks and gRNA sequences cloned into one or separate plasmids.

These modifications were made possible by the use of electroporation for DNA transfer instead of the standard LiAc-based yeast transformation method.

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C03

Pat1 participates in the adaptive transcriptional response mediated by the CWI MAPK pathway in yeast under cell wall stress conditions.

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Pat1 is an RNA binding protein present in all eukaryotic organisms which mainly plays a role during the cytoplasmatic mRNA degradation as a decapping activator. Interestingly, this protein has been found bound to some promoters of constitutive genes, suggesting it could participate in transcription. During the last decades, our lab has worked on the characterization of the transcriptional program triggered by the cell wall stress in *S. cerevisiae* mediated by the MAPK Cell Wall Integrity (CWI) pathway through the MAPK Slt2 and the transcription factor Rlm1, in coordination with the recruitment and activity of the chromatin remodeling complexes SWI/SNF and SAGA

In previous work, the *pat1*Δ strain was selected in a large-scale transcriptional screening to identify deletion mutants in non-essential genes affected in the induction of gene expression under cell wall stress.

Thus, the principal aim of this work has been to characterize the functional role of the protein Pat1 in the transcriptional response under situations that affect the *S. cerevisiae* cell wall integrity. Genome-wide transcriptional studies using DNA Microarrays revealed that out of 87 genes induced upon treatment with Congo Red (CR, a chitin binding dye that interferes with the cell wall construction) in a WT strain, almost 65% of them were Pat1-dependent. These results were confirmed by RT-qPCR experiments. Remarkably, no defect in the CWI signaling (Slt2 phosphorylation/activation) was detected in a *pat1*Δ strain. Furthermore, through ChIP experiments, we have been able to detect in a WT strain the presence of Pat1 at the coding region of some prototypical CWI-dependent genes under stress conditions in an Slt2-dependent manner. In addition, in a *pat1*Δ mutant, both the recruitment of Rlm1 and RNA polymerase II was partially impaired. All this data suggest that Pat1 plays a role in the transcriptional response mediated through the CWI pathway.

C04

Synthetic genetic interactions of non-coding RNA in *Saccharomyces cerevisiae*

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Over the past years evidence has been emerging for non-coding RNAs (ncRNAs) to play essential roles in the cell. Recent studies have identified a diverse catalogue of ncRNAs in yeast with only a handful ascribed a function. Previously, we generated and performed a large scale environmental phenotypic screening of the single ncRNA deletion mutant collection in *Saccharomyces cerevisiae* and identified ncRNAs responsible for significant fitness changes. Here, a double ncRNA deletion mutant library was generated in order to study ncRNA genetic interactions. The synthetic genetic array (SGA) methodology was used to shed a light on their functions by detecting mutant colonies with phenotype different than expected. Data was analysed in 5 different experimental conditions to reveal environmentally dependent functions. Following SGA, over 1000 significant ncRNA epistatic interactions were observed mostly displayed positive interactions (~90.2%), with only ~5.1% and ~4.7% being synthetic sick or lethal, respectively. An increase of negative interactions was recorded when epistasis was scored on different media and stressors, suggesting that these ncRNAs may have environmentally dependent functions or may be differentially transcribed in various conditions. The vast majority of ncRNA-ncRNA interactions did not correlate with the epistasis of their neighboring genes, suggesting that the ncRNA SGA network is independent from the protein one. We also focused on the two U3 paralogues SNR17A and SNR17B responsible for processing of 18S rRNA. As expected, they share the majority of genetic interactions in standard rich media, however a large number of unique epistatic interactions were found in other conditions, suggesting that SNR17A and SNR17B may have sub-functionalised under different environmental pressures.

C05

Exploring the effect of genetic background on suppression interactions in natural yeast isolates

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Genetic suppressors are mutations that confer a fitness gain to a cell carrying a deleterious, or even lethal, mutation in another gene. Suppression interactions often identify previously unknown connections between functionally related genes, making suppressor mutations a powerful tool to study gene and pathway function. However, for the budding yeast *S. cerevisiae*, most suppressor mutations have been described in the lab reference strain S288C, which could potentially limit the diversity of discovered suppressors since these could be specific to the genetic background in which they occur. To gain an understanding of the effect of the genetic background on the identified suppressors, we are isolating spontaneous suppressor mutations in genetically diverse *S. cerevisiae* strains. To do this, we are introducing temperature-sensitive alleles of the essential genes IDI1, TAO3, SEC17, GLN1, and RRB1 into three natural yeast isolates and selecting suppressor colonies able to grow at the restrictive temperature. After identification of the suppressor genes by whole-genome sequencing, we will introduce the "wild" suppressors in the lab strain S288C to assess the specificity of the suppressor to its wild genetic background. If the wild suppressor is indeed specific to its original genetic background, we will use bulk segregant analysis and quantitative-trait locus mapping to identify the genetic variants in the wild genome that are required for the suppression of the temperature sensitive mutants. Determining the frequency and mechanisms of genetic background-dependent suppression will improve our understanding of how diverse backgrounds can affect genetic traits.

C06

Engineering of genetically encoded fluorescent calcium and pH sensors targeted to the Golgi apparatus in *Saccharomyces cerevisiae*

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Cation regulation processes are crucial for cell operation. Among those ions, Ca2+ is an essential intracellular messenger, Mn2+ is required as a cofactor for many enzymes and pH needs to be tightly regulated to ensure proper enzymatic activity. A complex network of transporters act in concert to maintain the homeostasis of these cations. However, some actors of this network are still poorly characterized or even unknown. In this context, we are studying the yeast ortholog of the GDT1 family, a group of calcium/manganese transporter [1]. This protein of interest is localized at the Golgi apparatus membrane and is important for protein glycosylation [2]. In the last few years, several groups working on GDT1 family members showed that those proteins are involved in the homeostasis of Ca2+, Mn2+ and H+, using phenotypical assays, glycosylation analysis, cytosolic ion concentrations measurements, transport assays in heterologous expression system, etc. However, their precise role within cell and transport mechanism is still debated.
In order to better understand their physiological function, we try to get in vivo measurements of calcium and protons directly in the yeast secretory pathway. For this purpose, we are addressing genetically encoded indicators to the Golgi lumen. Those probes are fluorescent proteins derived from the Green Fluorescent Protein to be sensitive to specific ion concentrations. For calcium, we are currently testing the CEPIA and the GAP probes, as well as the FRET sensor D4cpv, while pHluorin is used for pH variations measurements. At this stage, the latter has been correctly targeted to the Golgi membrane and used to show that Gdt1p is involved in pH regulation of yeast Golgi [3].

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C07

CYCLIC: a combinatorial library of cyclic peptides as a flexible tool to discover metabolic modulators and enzyme inhibitors.

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Combinatorial libraries are well established molecular biology tools which enables the screening of several millions of sequences toward a molecular target in protein-protein interaction (PPI) assays. In this work, we adapted a previously published cyclic peptide-encoding library (Barreto and Geyer, 2014) to the classical GAL4-based yeast two-hybrid system and we named it CYCLIC (Combinatorial librarY of cyCLIC peptides). Peptide cyclization is obtained by inserting the peptide-encoding oligonucleotide in an engineered split intein from *Synechocystis s*pp.. We addressed the potentiality of this tool in the identification of peptides capable to modulate or inhibit enzymes and transcription factors activities. In the first case, peptide-based enzyme inhibitors were identified to block the activity of essential cell wall biosynthetic enzymes in oomycetes, which are devastating plant pathogens. In the second case, GAL4-DNA Binding Domain was directly used as target protein to isolate peptides that could induce perturbations in the galactose metabolism in yeast acting directly on the endogenous GAL4 protein. Here, we identified a peptide which is able to reduce GAL4 activity, as highlighted by both phenotypical and molecular analyses. Taken together, our combinatorial library is an effective tool to identify small peptides capable to interfere with the activities of distinct protein families and with cellular processes.

C08

Quantitative genetics underlying co-effects of Matrine and Doxorubicin on growth in *Saccharomyces cerevisiae*

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Doxorubicin has been wildly used as a highly effective chemotherapy for decades but with serious side effects. Matrine has been reported to have combined anti-cancer effects with other chemotherapy drugs. Here, we aim to assess the potential combined effects of Doxorubicin and Matrine, and the genes responding to treatment, via yeast quantitative trait loci mapping. A panel of F1 and F12 yeast segregants from a four-parent Saccharomyces cerevisiae cross, which have been genotyped, were used for phenotype measurements. The growth of each segregant was recorded by Optical density in a platereader using PHENOS (a pipeline for high throughput phenotyping of many strains). Subsequently, rQTL mapping was performed to identify specific regions of the genome, with variation, that affect response to the treatments. Candidate genes were confirmed by reciprocal hemizygosity. There were no significant growth differences between controls and 4mM Matrine. However, Matrine enhanced the growth inhibition caused by Doxorubicin. Yeast cells stopped growing under Doxorubicin treatment but were elongated and alive. The death rate was significantly increased when Matrine and Doxorubicin were used to treat the cells together. Hundreds of QTL intervals were identified that had genetic variation underlying this response. By overlapping analysis between F1 and F12 generation, 12 regions were chosen as candidate regions. RHA verification indicates that the SUMO-mediated DNA repair pathway plays a crucial role in responding Doxorubicin and Matrine. Using the yeast QTL mapping system to explore the underlying pathway of chemotherapy agents gives us another perspective on potential treatments. The results not only suggest DNA damage is caused by these drugs, as in previous studies, but also indicate a more precise bioprocess. Matrine could have a co-effect with Doxorubicin potentially mitigating the side effects of Doxorubicin by enhancing the chemotherapy effects at lower doses.

C09

“Characterization of the nuclear morphology in a mid-M block-and-release experiment using nocodazole.”

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Lower eukaryotes, such as yeast and most protists, are characterized by having a closed mitosis, which means they undergo the mitotic cell division without dismantling the nuclear envelope. Cells need to reshape the nucleus to split the genetic material into each daughter cell. An equal and faithful distribution of the DNA is essential to avoid genetic imbalances that endanger the stability of the progeny. We hypothesize that abnormal nuclear morphology can affect genome stability, as chromosomes are specifically located and tethered to different sites at the inner nuclear membrane, and this could be used as an antifungal and antiparasitic strategy.

Using *Saccharomyces cerevisiae* as a model organism, we and others have observed that the characteristic spheric shaped nuclear morphology is lost during mid-M blocks. In this work, we have characterized the nuclear shape, along with nuclear components such as the spindle and the nucleolus, in a mid-M block-and-release experiment using the microtubule depolymerizing drug nocodazole (Nz). We will present data on changes in morphology, viability and genetic stability, as well as how they relate with each other, during both a normal Nz block-and-release and when nuclear shape is previously altered by either genetic or environmental means.

C10

Cas9 as a tool to generate directed DSBs in anaphase bridges in *Saccharomyces cerevisiae*: an initial approximation.

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CRISPR/Cas9 technology is widely known as a tool to edit the genome using a small guide RNA (sgRNA) that directs an endonuclease Cas9 to cut at the specific place in the genome that we want to modify. At this point, the cut ends can be joined generating small mutations, or DNA templates can be introduced to replace the endogenous or own sequence for the one introduced synthetically giving rise to the editing of the genome.

Although this system was originally designed for gene modification, it presents, as an intermediary in the gene editing process, the selective generation of a DNA Double Strand Break (DSB). Thus, CRISPR/Cas9 can also be used to generate sequence dependent DSBs through the synthetic design of a sgRNA, so that it is possible to direct Cas9 to cut "anywhere" in the genome that interests us.

Little is known about cellular responses to DSBs when they occur over DNA bridges in anaphase (AB). In these cases, if the progression of the cell cycle is not stopped, the breakage of the DNA leads to the irreversible migration of the ends to the daughter cells. We want to study how cells repair DSBs when they occur in an AB. Now, we are evaluating the feasibility of the CRISPR/Cas9 system as a tool for this purpose by creating strains of *Saccharomyces cerevisiae*, in which Cas9 is directed to DNA regions frequently found in AB.

C11

The effect of ascus length on meiotic outcome in the fission yeast *Schizosaccharomyces pombe*

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Nitrogen starvation triggers mating and subsequent meiosis in the fission yeast *Schizosaccharomyces pombe*. Following fusion of the two parental nuclei, the telomeres of all chromosomes attach to the spindle pole body at the nuclear envelope and the process of nuclear oscillation (horsetailing) begins. During this process, the nuclear material travels up and down the length of the zygote to facilitate the correct pairing of homologous chromosomes, thus permitting recombination to occur. This event is followed by two consecutive meiotic divisions, resulting in the production of four gametes (spores) enclosed in a single sac (ascus). Previous work has shown that a wild isolate of *S. pombe* can produce asci that are two to three times longer than those produced in the lab isolate. We hypothesized that this increased length may influence meiotic outcome by impacting the horsetailing process. Using various markers to visualize spore number we discovered that longer asci were less efficient at gamete production. We will also utilize a recombination assay employing fluorescent markers under the control of spore-specific promoters, to measure the recombination rate in long versus short asci. In addition, integrating fluorescent nuclear markers will allow us to visualize and measure how different horsetailing parameters e.g., speed and distance travelled, are impacted by ascus length. Findings from these studies will further aid our understanding of the features required for successful gamete formation and recombination in fission yeast.

C12

The influence of lncRNAs on yeast cell wall proteins

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Yeast *Saccharomyces cerevisiae* cell wall is an extracellular organelle important for shape, protection, and communication with the environment during growth and cell division. In the cell wall structure, mannoproteins are covalently or noncovalently attached to a polysaccharide network that usually consists of three main groups of polysaccharides: mannoproteins, β-glucan and chitin. Two types of cell wall proteins (CWPs) covalently bound to β-glucan are GPI-CWPs bound via GPI-anchors and Pir-CWPs bound via alkali labile ester bonds. The third type of proteins is noncovalently bound to β-1,3-glucan. The cell wall is continuously remodeled as yeast cells undergo life cycle such as mating, meiosis and sporulation which are expected to be coupled with changes in the expression of genes encoding cell wall proteins. Advanced RNA sequencing methods provide insight into the yeast transcriptome and reveal pertinence of long non-coding RNAs (lncRNAs) that play important roles in various cellular processes such as transcription, translation and cell growth. Information on differences in cell wall protein levels during mitosis and meiosis is limited. We first performed relative quantification of yeast cell wall proteins in whole protein extracts using cell wall proteins, Bgl2 and Pir4. It enables us to further explore other cell wall proteins focusing on Pir5 and Spr1, which are important for sporulation. We investigated whether there is a correlation between transcription level and protein level, using the wt strain and the rrp6 mutant whose known targets are lncRNAs such as cryptic unannotated transcripts (CUTs) or meiotic unannotated transcripts (MUTs). This may indicate that sense/antisense transcripts form a double-stranded RNA (dsRNA) that may directly or indirectly prevent translation of sense mRNA.

C13

Chemogenomic profiling of N-nitrosamine toxicity in yeast *Saccharomyces cerevisiae*

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N-nitrosamines (including N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA)) are a class of compounds that cause cancer in many experimental animals and are considered carcinogenic in humans. They have been detected in cooked foods, treated water and recently, have been found as a contaminant in diverse medications. Starting in July 2018, drug regulators have recalled hundreds of lots of sartan drugs, as well as in diabetes medications and proton pump inhibitors due to high levels of NDMA and NDEA. Despite its known toxicities, our understanding of how N-nitrosamine exerts their biological effects is limited. To address this knowledge gap and to characterize genes and pathways that are perturbed by N-nitrosamine exposure, we applied an unbiased yeast chemogenomic assay that quantifies the requirement for each gene (through their deletion mutants) for resistance to a compound in vivo. We found that several evolutionarily conserved pathways were perturbed by N-Nitrosamines including arginine biosynthesis, mitochondrial genome maintenance and DNA repair. We further tested several of metabolic intermediates of Nitrosamines (including methylamine and ethylamine) and other compounds related to their synthesis, (N,N-dimethylformamide and formamide) and found that they also affected the arginine biosynthetic pathway. Follow-up screens and single strain analysis allowed us to gain additional insights into why these particular pathways are affected by these pervasive contaminants. To visualize our data, we developed a queryable web application https://ggshiny.shinyapps.io/2020NitrosoMechanisms/ that offers a resource for further analysis. Our current efforts are focused on examining the transcriptomic profile of effects of these compounds in yeast, as well as overexpressing the arginine biosynthetic proteins to further explore their role in N-nitrosamine toxicity.

C14

Evaluation of the biocontrol potential of a commercial yeast starter against fuel-ethanol fermentation contaminants

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According to the 2030 Agenda for Sustainable Development it is urgent to minimize the application of hazardous chemicals by industries in order to avoid environmental degradation. Fuel-ethanol industries apply chemical treatments, namely acid washing and antibiotics to prevent economic losses owing to microbial contaminations. In fact, the regular use of acid washing treatments and antibiotics in fuel-ethanol fermentations increases environmental risks and the rise of antibiotic-resistant bacteria, which justifies the search for less toxic antimicrobial methods.

Previously, we found that *Saccharomyces cerevisiae* wine strains secrete antimicrobial peptides (AMPs) derived from glyceraldehyde-3-phosphate dehydrogenase (GAPDH) that are active against several wine yeast and bacteria. In the present study, we investigated the capacity of a fuel-ethanol commercial starter, *S. cerevisiae* Ethanol-Red, to secrete similar AMPs. Results showed that indeed Ethanol-Red secretes the same AMPs, what was confirmed by performing immunological tests with a specific polyclonal antibody against the GAPDH-derived AMPs. Then, we evaluated the bioactivity of those AMPs against several Lactic Acid Bacteria (LAB) and *Brettanomyces bruxellensis* fuel-ethanol contaminants. Bioactivity assays showed that their minimal inhibitory concentration against six strains of *B. bruxellensis* and six LAB species varied from 1500 to 2000 μg/mL, depending on the strain. Finally, the biocontrol potential of Ethanol-Red against those fuel-ethanol contaminants was assessed by performing alcoholic fermentations with each of those contaminant strains in mixed-culture with Ethanol-Red. Results showed that Ethanol-Red exerts an antagonistic effect against all *B. bruxellensis* strains and LAB species tested, inducing a decrease in their culturability of approximately 3 orders of magnitude within 11 days and 7 days, respectively. Thus, the present study demonstrates that it is worth to explore the biocontrol potential of Ethanol-Red as a natural strategy to combat microbial contaminants of fuel-ethanol fermentations, thus allowing to minimize the application of chemical treatments.

C15

Heterologous expression of *Marchantia polymorpha* Acr3 in budding yeast reveals novel features of plant arsenite transporters

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Acr3 is the ubiquitous family of membrane transporters that confers high-level resistance to arsenicals in bacteria and fungi. Putative Acr3 orthologues have been also identified in green algae and lower plants, although their function in these organisms remains largely uncharacterized. The *Saccharomyces cerevisiae* Acr3 (ScAcr3) is the best studied member of the Acr3 family. ScAcr3 displays the ten-transmembrane topology and localizes to the plasma membrane. In contrast to bacterial Acr3 proteins, ScAcr3 transports not only arsenite but also antimonite by exchange with protons. In order to better understand the function of plant members of the Acr3 family, we cloned and expressed Acr3 orthologue from the common liveworth *Marchantia polymorpha* (MpAcr3) in *S. cerevisiae*. We found that MpAcr3 is able to fully complement arsenite, arsenate and antimonite sensitivity of the yeast acr3 mutant cells by transporting trivalent forms of these metalloids out of the cells. Similar to the yeast orthologue, MpAcr3 acts as a metalloid/proton antiporter using highly conserved cysteine and glutamic acid residues located in the transmembrane regions. Unexpectedly, we discovered that MpAcr3 possesses a metalloid sensor region, which is unique for plant members of the Acr3 family, that regulates its subcellular localization. Our work proves that *S. cerevisiae* serves as an excellent model to study transport properties and trafficking of plant membrane proteins.

C16

Stress conditions optimization for enhanced protein production in *Yarrowia lipolytica*

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Bioprocessing conditions strongly impact overall performance of a microbial producer cell of proteins. Multiple environmental stress factors, such as limited oxygen availability, local fluctuations in pH and temperature or high osmolarity in the terminal stage of the culture, are known to exert their adverse effects on the production cultures. Likewise, excessive overproduction of a heterologous protein is known to awake endogenous stress in the host cell. On the other hand, increasing evidence suggests that careful optimization of the stress factor administration in terms of time and intensity can exert positive effect on the bioprocess. In this study, we subjected a recombinant *Y. lipolytica* strain to optimization of thermal treatment conditions (20-42°C, 5 to 300 min) to maximize the overproduction of a heterologous enzyme. Statistical design of experiments (DoE) and response surface methodology (RSM) approaches were adopted to systematically address the problem. Optimized thermal conditions were then administered in combination with another stress factor – hyperosmotic conditions induced by osmoactive compound addition. The cultures were analyzed for the heterologous enzyme productivity, viability of the cells and synthesis of metabolites typical for *Y. lipolytica*. Gene expression profiling targeting a gene encoding target protein and a set of genes related to stress response in *Y. lipolytica* was conducted to get an insight into awaken molecular response to the treatment conditions.

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C17

Urm1 (ubiquitin related modifier 1) – not as ubiquitin-like as supposed?

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Protein conjugation by ubiquitin, so called ubiquitination, is widespread among eukaryotes and regulates numerous vital biological processes. Through a three-step enzymatic cascade, ubiquitin is covalently bound to lysine residues in substrate proteins. With the discovery of Urm1 (ubiquitin related modifier 1) by Nobel Prize laureate Professor Ohsumi, a unique ubiquitin-like protein was found in yeast. Urm1 also functions as a lysine-directed protein modifier in a process termed urmylation. However, it additionally acts as a sulfur donor for tRNA thiolation. Interestingly, both functions depend on the initial sulfur transfer onto Urm1, generating a thio-activated intermediate. Regarding the protein modification pathway of Urm1, this step is clearly different to canonical ubiquitination. Investigating the conjugation of Urm1 to its well-known substrate and antioxidant enzyme Ahp1 in yeast, we found that several lysine residues are able to serve as Urm1 acceptors. Moreover, urmylation probably involves oxidation of a transient non-lysine site for primary transfer to Ahp1 from which Urm1 is distributed to lysine residues eventually forming a covalent isopeptide bond. The functional duality of Urm1 and the apparent deviation of urmylation from canonical ubiquitination led us speculate that in the end, conjugation by Urm1 may not be as ubiquitin-like as originally anticipated.

C18

Transcriptional heterogeneity in metabolically cooperating communities

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Cell to cell variability within microbial communities can lead to resistance to environmental stresses, including treatment with antimicrobial drugs. One important source of variability in microbial communities is a cell's metabolic state. Complimentary metabolic states can underpin cooperation in interspecies and synthetic communities of microbes, however the extent of metabolic variation between individual cells in isogenic communities is not well understood. We gathered single cell gene expression data in yeast for isogenic cells, as well as in synthetic Self-Establishing Metabolically Cooperating Communities (SeMeCos) composed of cells with different metabolic capabilities (metabotypes). In the SeMeCos, where metabolic heterogeneity is known to occur, we see metabotypes cluster together based on their transcriptional signature. We have begun to characterize the transcriptional signatures of metabotypes in these clusters and compare them to previously collected data for metabotypes growing in isolation. We search for these and similar metabolic signatures in the data from isogenic cultures to characterize the extent and nature of metabolic heterogeneity in isogenic yeast cultures.

C19

Phenotypic response to stress conditions of xylitol-producing yeasts isolated from the sugarcane mill and sugarcane derivatives in Colombia

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The capacity to assimilate D-xylose in 50 yeast strains isolated from different substrates inside a sugarcane mill in Puerto Lopez (Meta, Colombia), as well as fermented beverages in Paipa (Boyacá, Colombia) were evaluated. Only 12 yeasts belonging to the genus *Candida* (*Lodderomyces/Spathaspora*), *Meyerozyma*, and *Wickerhamomyces* were able to transform D-xylose. All the yeasts evaluated have as their main product xylitol, being the strain *Meyerozyma caribbica*, which presented the highest yield (YP/S= 0.795 g xylitol/g xylose) and conversion efficiency (η= 87%).

Strain IBUN-090-03558 identified as *Meyerozyma caribbica* and IBUN-090-03565 identified as *Candida tropicalis* were evaluated under conditions of acid stress, in a medium with xylose (5%) as a carbon source and containing acetic acid (one of the main inhibitory compounds present in lignocellulosic biomass hydrolysates), evidencing the ability of *Candida tropicalis* to maintain xylitol production (24.39 g. L-1 ±0.26) in the presence of 2g.L-1 of the acid. In contrast, *Meyerozyma caribbica* decreased its xylitol production by 16.9%. Additionally, a culture strategy was implemented in the preparation of the inoculum exposing the cells to low (10°C) and high (37°C) temperatures. In the phenotypic response, *Meyerozyma caribbica* was able to significantly increase the production of xylitol (26.67 g.L-1 ± 0.63) in the presence of 6 g.L-1 of acetic acid, when the inoculum was exposed to low temperature compared to the inoculums prepared at higher temperatures. *Candida tropicalis* was not affected by the temperatures tested for inoculum preparation, but its xylitol production (18.50 g.L-1 ± 0.62) decreased by 24.8 % at this acid concentration. Fermentation tests on sugarcane bagasse hydrolysates were also carried out.

The results contribute to the description of cross-stress resistance and demonstrate the ability of non-conventional yeasts to improve their response to stress factors.

C20

Hsp31p, a budding yeast protein protecting against external stresses, is specifically secreted to periplasm via unconventional pathway

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Saccharomyces cerevisiae Hsp31p protects cells against various environmental stresses. It is described as a chaperone and as glyoxalase III that oxidizes methylglyoxal (MG), a toxic by-product of glycolysis, but the exact biological role of this protein is unknown. We have recently found that Hsp31p is secreted to periplasm. Since it bears no associations with endoplasmic reticulum and Golgi apparatus, the components of typical eukaryotic cell secretory system, Hsp31p is probably secreted by unconventional pathway. This pathway, although ubiquitous among Eukaryotes and in case of humans also medically important, is still poorly characterized. Numerous proteins are secreted via this pathway, among them those that, similarly to Hsp31p, protect cells against environmental insults. To get an insight into the mechanism of unconventional secretion we analyzed Hsp31p polypeptide to identify amino acid residues that are responsible for its secretion. Two residues: Ser209 and Phe17, as well as C-terminus of Hsp31p polypeptide turned out to be indispensable for its targeting to the periplasm. Noteworthy, the modified, not secreted variants of Hsp31p were unable to protect the cell against MG stress. Identified regions of Hsp31p polypeptide were found to be located on the surface of natively folded molecule as determined by X-ray crystallography, suggesting that Hsp31p secretion is driven by interactions through these regions with some other proteins or by posttranslational modifications of the respective residues. Our results indicate that Hsp31p secretion is a specific process and that its periplasmic localization is inherent to its protective function against environmental stresses. This finding together with the easily distinguishable phenotype of Hsp31p non-secretion makes this protein a convenient model for studies of the unconventional protein secretion mechanisms.

C21

Cellular stress vs cellular response: using yeast to study the PCD-necrosis continuum

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Traditionally, the relationship between cellular stress and response has been modelled as a continuum where the intensity dictates the response. Briefly, at low levels of stress, cells may adapt to slight shifts in their environment to maintain normal cellular functions. As the level of stress increases, cells may also respond by inducing genetically encoded cell death processes. At even higher levels, there is loss of control as cells undergo a rapid and unregulated form of death – necrosis.

The yeast *Saccharomyces cerevisiae* has long been a choice model to study cellular stress responses as many of the key pathways that regulate them in humans are conserved in yeast. We have previously shown that the point at which yeast undergo PCD in response to exogenous copper stress can be delayed by the heterologous expression of pro-survival sequences but is paradoxically enhanced under exogenous iron stress. Interestingly, when cells are exposed to several-fold higher concentrations of copper stress, cell death is neither rapidly observed nor is viability completely lost. Based on these findings, we hypothesize that there are multiple stress-dependent responses. To further investigate this, we have tested the response of yeast to different PCD-inducing stresses including lithium and ethanol. Like copper, lithium does not induce rapid nor complete levels of cell death in yeast at levels several-fold beyond the minimum concentration needed to induce PCD. In contrast, ethanol induces apoptotic-like cell death and necrosis in yeast. The differences in the way that yeast respond to increasing concentrations of copper, lithium and ethanol suggest that necrosis is not a response that ubiquitously occurs at high levels of stress. These findings contrast the traditional model in which we relate cellular stress to cellular response. Ultimately, a newer model may be needed to describe how cells arrive at their fates under different stress contexts.

C22

Uncovering non-canonical functions of Mec1/ATR and Tel1/ATM in yeast cells: phosphorylation of Snf1/AMPK

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The DNA damage response (DDR) pathway is highly conserved in eukaryotic cells. In *S. cerevisiae*, it is activated by two protein kinases, Mec1 and Tel1, and responds to replication stress and/or DNA damage in the nucleus. Upon the DDR activation, Mec1 and Tel1 phosphorylate several kinase mediators (eg. Rad53) which subsequently phosphorylate many effector proteins to initiate cell cycle control and DNA repair, but also modify a considerable part of the cellular proteome and reprogram transcription of hundreds of genes. However, there are already several reports suggesting that the apical DDR kinases, Mec1 in particular, function also outside of DDR. To name just a few instances: Mec1 has been shown to promote replication and suppress rearrangements of the nuclear genome independently of the downstream checkpoint kinases (Lanz et al, 2018); Mec1 and several downstream kinases have been shown to promote survival in response to various proteotoxic stresses (Corcoles-Saez et al., 2018); Mec1 forms a complex with two kinases, Snf1 and Atg1, that localizes in close vicinity to mitochondria, presumably to regulate mitophagy (Yi et al., 2017). Consequently, the current evidence suggests that Mec1 or Tel1 kinases have other substrates than the canonical ones within DDR and probably they function also outside the nucleus. In our study we verify if Mec1 or/and Tel1 phosphorylate directly mitochondrial proteins. Our inquiry is based on the census of yeast proteins possessing characteristic amino acid motifs that may be phosphorylated by Mec1 and Tel1 kinases. In the list of those putative Mec1/Tel1 substrates there are about 40 mitochondrial proteins or proteins that are functionally linked to mitochondria. We present our results indicating that the Snf1 kinase is phosphorylated by Mec1/Tel1 kinases. In addition, our experiments show that Snf1 has also a yet unknown moonlighting localization inside mitochondria.

C23

Clarification of metabolic regulation model with nitric oxide via translational modifications

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Nitric oxide (NO) is an important signaling molecule in many organisms. In mammals, NO functions mainly through signal transduction mediated by the soluble guanylyl cyclase (sGC)-dependent system or protein post translational modifications. PTMs are involved in the regulation of functions and/or stabilities of proteins. S nitrosylation and S glutathionylation in which cysteine residues on the protein are modified dependent on NO, has been reported to regulate activity and function of enzyme. Our previous studies showed that NO was involved in cell protection or cell death under high temperature or H2O2 stress conditions, respectively, in the yeast *Saccharomyces cerevisiae*. *S. cerevisiae* cells do not possess the gene encoding a mammalian sGC orthologue on its genome, suggesting that NO functions mainly through PTMs including SGA in yeast. However, there has been little reports about comprehensive analysis of NO mediated PTMs in yeast. Here, we identified proteins with SGA and analyzed their physiological functions in yeast.

First, we performed proteomic analysis to identify proteins with SNO using yeast cell lysate treated with NO Our result indicated that many proteins including glycolytic enzymes were S-nitrisylated. On the other hand, further analyses using proteins extracted from yeast cells treated with NO detected only SGA proteins but not SNO proteins. These results suggest that proteins are S-glutathionylated rather than S-nitrosylated in yeast cells. Interestingly, we revealed that the fructose-1,6-bisphosphate aldolase Fba 1 in the glycolytic pathway was S-glutathionylated under NO treatment conditions. Moreover, the enzyme activity of Fba 1 decreased and several metabolites in the pentose phosphate pathway, such as 6-phoshogluconate and NADPH, were accumulated in response to NO. From these results, we hypothesize that SGA of the Fba 1 protein is important for regulating the glycolytic and pentose phosphate pathways in yeast cells.

C24

Dosage sensitive JDPs: a case study on Caj1 overexpression mediated growth phenotypes in budding yeast

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J-domain protein (JDPs) are the obligate co-chaperones of HSP70 machines. Multiple JDPs work with the same HSP70, driving them to perform diverse cellular functions. In *Saccharomyces cerevisiae*, there are 22 JDPs, out of which 13 share the nucleo-cytoplasmic compartment. Expression levels of these JDPs are highly variable, ranging from the least abundant JDP, Apj1 (~125 molecules/cell) to the most abundant, Ydj1 (~120000 molecules/cell). While most JDPs are highly specialized, being in the same subcellular compartment they show functional redundancy making their functional analysis difficult. Higher than optimal levels of some JDPs can result in dominant-negative phenotypes, in extreme cases, lethality. Caj1 is one of class II, non-essential JDPs localized in the nucleo-cytoplasmic compartment of *Saccharomyces cerevisiae*. Deletion of Caj1 alone has no obvious growth phenotype; however, overexpression of Caj1 results into pleiotropic growth phenotypes. At lower temperature, Caj1 overexpression rescues the cold sensitivity of tryptophan auxotrophic yeast strains by stabilizing the tryptophan permeases, Tat1 and Tat2 at plasma membrane. However, at elevated temperatures, Caj1 overexpressing cells grew slowly and displayed increased plasma membrane damage, possibly due to the stabilization of heat-labile plasma membrane proteins, such as Lyp1. In addition, higher levels of Caj1 also causes cell cycle defects, resulting in filamentous growth phenotype. Our results show that Caj1 overexpression causes both Hsp70 dependent and independent dominant negative effects in budding yeast. Apart from loss-of-function studies, understanding the mechanisms of dosage sensitivity is equally informative and may contribute to our current understanding of the functionality of JDP networks in the cell.

C25

Role of erythritol in osmoprotection in yeast *Yarrowia lipolytica*

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Erythritol is produced and released to the environment by un-conventional yeast *Yarrowia lipolytica* during growth in the hyperosmotic conditions. It seems to contradict the well-known mechanism of hyperosmotic stress response, which is based on the accumulation of osmolytes inside the cell. Yet, the presence of erythritol in the medium partially rescues the phenotype of the osmosensitive strain *yl-hog1*Δ. High production of this polyol is dependent on the HOG pathway, but when erythritol is artificially added to the medium it provides protection even when HOG is damaged. Thus, secretion and further utilization of erythritol might be an element of auxiliary, prolonged stress response mechanism.
Erythritol-based osmoprotection is dependent on transcription factor EUF1, which might be a key to understanding the role of erythritol in yeast metabolism and stress response. So far EUF1 was known to control the expression of genes involved in the utilization of erythritol, however transcriptome analysis revealed numerous other target genes.

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C26

Poacic acid, a beta-1,3-glucan-binding antifungal agent, activates transcriptional responses regulated by the cell-wall integrity and high-osmolarity glycerol pathways in yeast

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Yeast cell integrity depends on the cell wall, an essential structure necessary not only for maintaining morphology but also for protecting cells against environmental stress conditions. For all these reasons, this structure constitutes an ideal target for antifungals discovery. The cell wall in yeast is a macromolecular complex mainly composed of β-1,3-glucan, β-1,6-glucan, chitin and mannoproteins. The most abundant component of the yeast cell wall is the β-1,3-glucan which serves as a backbone to which the other cell wall components are linked. Yeast cells growing under cell wall damage conditions elicit a transcriptional reprogramming, mainly governed by the Cell Wall Integrity (CWI) MAPK pathway, to support cellular integrity. Accumulated evidence during the last decades demonstrated the existence of both specific and common transcriptional profiles potentially associated with the mode of action of different cell wall interfering agents. In this work, we characterized the transcriptional response triggered by the plant natural product poacic acid (PA) which inhibits β 1,3 glucan synthesis in *Saccharomyces cerevisiae* and has antifungal activity against a wide range of plant pathogens. Regarding the cellular response to PA, we uncover a transcriptional co-regulation mediated by parallel activation of the cell-wall integrity and high-osmolarity glycerol signaling pathways. Strikingly, activation of the CWI by PA is not dependent on a single cell-surface sensor. We carried out RNA-seq using a WT strain grown in the presence or absence of 100µg/mL of PA for 1 h. At this concentration, 137 and 17 genes were significantly twofold up- or down-regulated, respectively. Out of 137 genes induced by PA in the WT strain, 88 were dependent on the CWI MAPK Slt2. Moreover, we observed that despite targeting β 1,3 glucan remodeling, the transcriptional profiles and regulatory circuits activated by caspofungin, zymolyase, and PA differed, indicating that their effects on cell-wall integrity have different mechanisms

C27

The MAP kinase Slt2 plays a role in iron homeostasis through regulation of the transcription factor Aft1

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Iron is an essential metal involved in a number of biological processes common to all eukaryotic cells. Cells develop mechanisms to tightly regulate its homeostasis, in order to avoid abnormal accumulation and the consequent cell toxicity. In budding yeast, the high affinity iron regulon is under the control of the transcription factor Aft1. In this work, we present evidence demonstrating that the MAPK Slt2 of the cell wall integrity pathway (CWI), phosphorylates and negatively regulates Aft1 activity upon the iron depletion signal, both in fermentative or respiratory conditions. Slt2 physically interacts with Aft1 suggesting that it is a direct regulation, since we have observed that the iron scarcity is not transmitted to Slt2 through other signaling pathways such as TOR1, PKA, SNF1 or TOR2 / YPK1. Furthermore, our results also demonstrated that slt2 mutant has a shorter chronological life span as a consequence of the deregulation of Aft1.

C28

Purine deprivation resembles nitrogen starvation in budding yeast

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When starved for “natural” nutrients, such as nitrogen, budding yeast induce specific stationary phase phenotype, and this is why cells become extremely resilient to environmental stress and can survive for a long time period. In contrast, when an auxotrophic element (uracil, leucine) is scarce, cells don't stop their metabolism and culture loses viability rapidly. Intracellular metabolites are typically synthesised in multi step reactions where loss of any component evokes auxotrophy.

We explored effects of purine auxotrophic starvation in each purine synthesis pathway gene knock-out mutant. WT and knock-outs were cultivated in (1) full SD media, without (2) nitrogen or (3) adenine. Cell cycle dynamics, sublethal stress resistance and transcriptomic data were analysed. Phenotypically, adenine starved cells have similar or even higher stress resistance than nitrogen starved. Interestingly, purine starvation ensured higher desiccation tolerance than nitrogen starved cells. In transcriptomic level, however, downregulated gene groups are similar across starvations and knockouts, while upregulated gene groups differ. We think that purine deprivation is at least partly signalized through pathways usually exploited during natural starvations.

Purine synthesis pathway is highly conserved across eukaryotes and auxotrophic organisms are found in natural habitats - these are intracellular parasites which rely on purine supply from the host cell. Probably, the giving up of purine synthesis together with the ability to survive without purines is an old trick of evolution. In this research we have created a solid basis of observations that can help to understand reactions of eukaryotic cells to purine deprivation and reasons why this auxotrophic starvation strongly differs from other auxotrophic starvations.

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C29

Production, characterization and antifungal application of biosurfactant sophorolipids from *S. bombicola* and *C. riodocensis*

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Sophorolipids are biosurfactants composed of a disaccharide sophorose linked by a hydroxyl group of fatty acids. Due to their surface-active properties, sophorolipids have a great potential for using in various industrial applications. The aim of this study is to characterize the functional property and production of sophorolipids by yeasts using a combination of glucose and palm oil as a hydrophilic or hydrophobic substrate, respectively. *Starmerella bombicola* has been known as an attractive yeast species for biosurfactant production while Candida riodocensis has not been much explored. *S. bombicola* and *C. riodocensis* were used as a host for production of sophorolipids from vegetable oil which is generally consisted of C16-18 fatty acids and thus is ideal for sophorolipids production. Here, the results showed that *C. riodocensis and S. bombicola* produced biosurfactants with emulsification activity against kerosene at the highest activity of 54.62% and of 60.23% after 4 or 7 days of cultivation, respectively. According to oil displacement assay, *S. bombicola* displayed slightly better activity to displace oil (1.17 cm) when compared to *C. riodocensis* (1.08 cm). Then, characterization of extracted sophorolipids was determined by fourier transform infrared spectroscopy (FTIR) which is confirmed as sophorolipids containing both lactonic and acidic forms. Palm oil appeared to promote cell growth of yeast cells as apparent for *C. riodocensis*. Yields of sophorolipids was obtained for *C. riodocensis* (45.70 g/L) and *S. bombicola* (39.36 g/L). Lastly, treatment with sophorolipids at 15-30 µg/ml also inhibited *Candida albicans* hyphal growth; thereby demonstrating their potential application in biomedicals.

C30

Yeast as a platform for organic acids production – exploitation of *Cyberlindnera jadinii* carboxylate transporters for microbial cell factory improvement

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The production of fine chemicals, including organic acids, is mainly supported by fossil-based processes. Novel technologies derived from renewable sources are required, as the current model of natural resource exploitation is unsustainable. In recent years, several strategies were developed for the bio-based production of organic acids including the expression of carboxylate transporters in microbial cell factories. In this work, we focused on the identification and characterization of new carboxylate transporters from the yeast *Cyberlindnera jadinii*. Its predicted transportome was analysed using two approaches. Initially, the *C. jadinii* homologs of the carboxylate transporters Jen1p (SHS family) and Ady2p (AceTr Family) were expressed in *Saccharomyces cerevisiae* W303-1A *jen1*Δ *ady2*Δ strain for functional characterization. In a parallel approach, a pipeline of bioinformatics tools was used to retrieve putative carboxylate transporters from the *C. jadinii* NRRL-1542 inferred proteome. This method considered the following criteria to retrieve data from a specific database: a) a single representative genome/proteome on the species level; b) multiple matches reflecting the presence of homologs within a species directly reflects the presence of orthologues, and c) e-values from BLAST searches that are statistically reliable; d) proteins displaying a topology of 4-20 transmembrane segments; e) removal of the partial proteins; f) search for conserved motifs involved in the transport of carboxylate and g) identification of sequences with homology to known di- and tricarboxylate transporters.
The putative transporters selected using this tool were cloned and expressed in *S. cerevisiae*. Transporter activity was determined through growth on different carbon sources and measurement of the uptake of radiolabelled carboxylic acids, such as acetate, lactate, succinate, and citrate. A phylogenetic study of the most promising transporters was carried out.

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C31

Studying native *Kluyveromyces marxianus* transporters to reprogramme the balance between hexose and pentose transport

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Efficient use of lignocellulosic hydrolysates in yeast-based biorefineries requires the simultaneous consumption of hexose and pentose sugars. This remains a key bottleneck because of the low number of specific pentose transporters identified and the slow rate of pentose uptake when glucose is present. To aid the identification of novel pentose transporters, we recently developed a *K. marxianus* screening platform by deleting 12 genes that encoded transporters of the major facilitator superfamily (MFS). Using this ΔPT platform, we identified several native *K. marxianus* transporters that recovered the native strain's ability to grow on either or both xylose or arabinose. We then carried out kinetic studies on the most promising candidates using14C -labelled sugars. One member of these transporters showed high affinity for both xylose and glucose, though the affinity for glucose was still 50-fold higher than for xylose. We chose this as a candidate for engineering in an attempt to convert the protein into a specific xylose transporter. We used in silico analysis to select targets for site-directed mutagenesis and made and characterized several mutants. One of these mutants lost the capacity to transport glucose but still transported xylose with medium affinity kinetics. This successful reprogramming of the transporter to increase its preference for xylose over glucose forms the basis for our continuing work to develop transporters that may facilitate efficient co-consumption of glucose and xylose by cell factory strains.

C32

Peroxisomes and yeast anhydrobiosis

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Peroxisomes could be defined as highly dynamic, DNA free, single membrane surrounded organelles. Cell can contain from 1-2 peroxisomes to several dozen peroxisomes. Yeasts are convenient model to study peroxisomes because cell transfer from a glucose containing medium to a medium containing a peroxisome proliferator (oleate, methanol) induces synthesis of peroxisomal enzymes and the growth and division of peroxisomes.

Plasma membrane and other intracellular membranes are one of the most important yeast cell structures affected by dehydration and subsequent rehydration. It is supposed that maintenance of cell's plasma membrane and other intracellular membranes molecular integrity is one of the most important factors determining yeast cells viability during their transition into the state of anhydrobiosis and subsequent reactivation from this state. Nothing is known at the moment about the stress response of peroxisomes in yeast cells during their transition into the state of anhydrobiosis. In this study we continued investigation of yeast anhydrobiosis. The aim of this study was to begin new additional experiments to obtain the information concerning the role of these organelles in the transition of yeasts into the state of anhydrobiosis and following reactivation.

In this study we used *Saccharomyces cerevisiae* wild-type strain (BY4742) and four deletion mutants with affected peroxisome division (*pex11*Δ), inheritance (*inp1*Δ), biogenesis (*pex3*Δ) and peroxisomal matrix protein import (*pex6*Δ).

Our primary results showed that yeast with intact peroxisomes after their induction are able to withstand better the dehydration-rehydration stress. On the contrary strains with impairment in peroxisome division, biogenesis and matrix protein import showed lower viability than cells from media without peroxisome inducer after dehydration and subsequent rehydration. Overall, our results showed for the first time that if yeast cells were incubated in a medium with a peroxisome inducer before transfer into state of anhydrobiosis, it gave advantage of better withstanding dehydration-rehydration stress.

C33

Volatile fatty acid utilization for lipid production by species belonging to *Yarrowia* clade

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Microbial production of lipids became a attractive alternative to production of biofuels from pants. Some advantages include space-saving and weather independence; however, the most important issue concerns the lack of competition of microbial lipids with food production. Among many oleaginous microorganisms, *Yarrowia lipolytica* became the most studied and engineered species for improved lipid production. This species belongs to a larger group of yeast called *Yarrowia* clade. They accumulate large amount of lipids in lipid bodies and are able to utilize wide spectrum of different substrates. A suitable carbon source should be cheap and renewable. One of such substrates are Volatile Fatty Acids (VFAs) produced for example by microbial fermentation of municipal wastes. The most abundant VFAs are acetic, propionic and butyric acid, which although being toxic at higher concentrations, can be used by *Yarrowia* clade members as substrates for intracellular lipid production.

In the current study we analyzed growth and VFAs utilization by 15 *Yarrowia* clade species using minimal media dedicated to lipid production. Single VFAs were used as carbon sources and their utilization by the analyzed species were compared. Based on the microcultures, 8 species were chosen for further analysis: *Yarrowia lipolytica*, *Yarrowia bubula, Yarrowia keelungensis, Yarrowia brassicae, Yarrowia hollandica, Yarrowia paraphonii, Yarrowia phangngaensis, Candida hispaniensis*. Among them, the fastest utilization of VFAs in shake flask cultures was observed for *C. hispaniensis* for acetate and *Y. keelungensis* for propanoate and butyrate. Furthermore, lipid biosynthesis from VFAs were also analyzed. The highest yield of intracellular lipids was observed for *Y. hollandica* and *Y. phangngaensis* which reached 14,26% and 13,03% respectively. Currently, bioreactor cultures and optimization of culture conditions are under intensive investigation. Our preliminary results proved that VFAs, a cheap and renewable carbon source, can be used to produce lipids using the *Yarrowia* clade members.

C34

Membrane stress caused by short chain fatty acids in *S. cerevisiae*

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The viability and competitiveness of microbial-based biotechnological processes are often limited due to harsh conditions such as: the presence of feedstock-derived inhibitors including organic acids (OAs), non-uniform nature of the substrates, osmotic pressure, high temperature, extreme pH. OAs play a unique role in these processes since they are often released from biomass treatments and, may also be products of interest obtained from microbial factories, with applications in several industrial sectors Yeast inhibition by OAs is more pronounced at low pH as, when in their undissociated form, they can cross the plasma membrane and once inside the cell, in a higher pH environment, OAs dissociate releasing H+ and the corresponding anion. The accumulation of both species has detrimental effects on cells, decreasing the intracellular pH, interfering with the lipid organization and membrane permeability and possibly inducing cell death. Therefore, controlling the cellular inward/outward flux of OAs can improve fermentation performances. The plasma membrane plays a pivotal role in this process since it is the physical barrier that separates the extracellular environment and intracellular components, is responsible for maintaining the correct ion homeostasis and is the sensor of the overall cellular environment, rearranging its composition in response to the presence of OA(s) Nonetheless, it is difficult to predict which membrane composition can be advantageous for the cells under certain conditions. By the modulation of the transcription factor ECM22, involved in the regulation of ergosterol biosynthesis, we aim at evoking a rewiring of the membrane system. Sterols abundancy can affect membrane properties like fluidity and permeability. We show how changes in the content of sterol can have different effects on yeast robustness, depending on the molecular structure of the OAs.

C35

Trk1, the main potassium importer in yeast responds to decrease in intracellular potassium concentration by increasing affinity and maximum velocity

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One of the key prerequisites for yeast survival and growth is uptake of essential compounds, such as potassium. Potassium is a vital cation and its sufficient intracellular concentration is crucial for various processes, for instance: regulation of membrane potential, cell turgor, activity of enzymes and protein synthesis. At the same time, excess of internal potassium is potentially toxic for yeast cells and can lead to loss of membrane potential, deacidification of vacuoles and cell death. It is therefore of vital importance for the cells that the import of potassium is kept within precisely defined boundaries, regardless of its external concentrations. In *Saccharomyces cerevisiae*, transporter Trk1 is considered to be a key player in potassium uptake. The most distinctive feature of Trk1 is its alleged ability to switch between two affinity modes (low and high-affinity mode), as a response to changes in external potassium concentration. The precise nature of the Trk1's affinity changes remains unclear.
We focus our study on dependence of kinetic parameters of Trk1 on changes in external and internal potassium concentrations, as well as characterization of specific mutations that abolish proper switching between affinity modes, to elucidate, in more detail, the potential mechanism, dynamic and regulation of affinity changes of Trk1.
We found a significant correlation between gradual loss of intracellular potassium and continual increase in both affinity and maximum velocity of Trk1-mediated transport, suggesting the possibility that rather than switching between two affinity modes as a reaction to changes in external potassium concentration, Trk1 precisely adjusts its kinetic parameters as a response to changes in internal potassium content. Additionally, based on previously known mutation (L949P) we identified and characterized new mutations (L81P, F820P and L1115P) with distinct effects on function, localization and affinity changes of Trk1.

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C36

Antifungal activity of *Xylaria sp*. BCC1067 extract against *Candida albicans* and ability to interfere drug efflux pump transporter

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Fungal co-infections, especially *Candida* species, are prevalent in critically ill COVID-19 patients. The *Candida* spp. are common opportunistic fungal pathogens, among which *C. albicans* is commonly found. Up-regulation of the MDR1and CDR1genes associated with drug efflux transporters represents an important mechanism of antifungal drug resistance. Currently, effective antifungal agents from natural sources are increasingly used to fight drug-resistance. In this study, we found antifungal activity of natural extract from fungal Xylaria sp. BCC 1067 against yeast *Saccharomyces cerevisiae* mutants overexpressing drug efflux transporters CaMDR1. Moreover, plasma membrane perturbation leading to improper localization of CaMdr1p was observed after treatment. Drug efflux pumps have been reported as mechanisms responsible for the antifungal resistance. The potential of the Xylaria extract was further investigated and found antifungal activity against *C. albicans*.

C37

Regulation of phospholipid metabolism in terms of phosphatidylcholine synthesis in *Yarrowia lipolytica* yeast

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Phospholipids (PL), the most abundant components of membrane lipids, are critical to maintaining membrane structures and homeostasis in numerous cellular processes. As amphipathic molecules, they form a bilayer to which integral and peripheral membrane proteins and other complex components are bound. In addition, they are reservoirs of secondary messenger molecules, provide precursors for the synthesis of macromolecules, serve in the membrane association modifications and act as molecular chaperones. In the yeast *Yarrowia lipolytica*, containing full set of organelles, phospholipids are synthesized via pathways that are common to those found in higher organisms. However, phospholipid biosynthesis in yeast is an intricate process regulated by genetic and biochemical mechanisms, and its regulation is related to the synthesis of other major classes of lipids, including fatty acids (FA), di- and triacylglycerols (DAG/TAG), sterols and sphingolipids. The key intermediate in the biosynthesis of phospholipids is phosphatidic acid (PA), which in *Y. lipolytica* is mostly directed to the production of TAG and in smaller amount to phospholipids.

In this study, different *Y. lipolytica* transformants with increased production of phospholipids, with a particular attention paid to phosphatidylcholine, were constructed. The obtained strains were tested in a minimal medium with nitrogen limitation. Using glucose as the carbon source, the highest phospholipid titers were obtained with the strain overexpressing lysophospholipid acyltransferase (SLC) and CDP-diacylglycerol synthase (CDS), reaching 13 mg/g of PL (12-fold higher than the wild-type) and 7 mg/g of PC. When glucose was replaced by glycerol, a substrate preferred due to its low cost and rapid conversion by *Y. lipolytica*, the best results, 12 mg/g of PL and 6 mg/g of PC (7-fold higher than the wild-type), were obtained in a strain co-expressing CDS and phospholipid methyltransferase (OPI3).

C38

Heterologous expression in *Saccharomyces cerevisiae* cells reveals unique properties of Trk proteins from non-conventional yeast species

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In the model yeast *Saccharomyces cerevisiae*, Trk1 is the main potassium importer. It is involved in many important physiological processes, such as the maintenance of ion homeostasis, cell volume, intracellular pH or plasma-membrane potential. ScTrk1 protein can also be of a great interest for industry, as it was shown that changes in its activity influence ethanol production by *S. cerevisiae* cells.
Non-conventional yeast species attract attention due to their unique properties concerning either metabolism or ability to cope with different environmental conditions. Thus, they represent a large potential to be used in food industry, biotechnology or agriculture.
In this work, we aimed to study Trk proteins from *Debaryomyces hansenii* and *Hortaea werneckii*, two organisms known for their ability to cope with high-salt stress, *Yarrowia lipolytica*, a halotolerant yeast able to grow in hydrophobic environments and used to produce several types of enzymes or metabolites, and *Kluyveromyces marxianus*, a thermotolerant and fast-growing species emerging for its industrial potential.
Trk proteins originating from these four non-conventional yeasts were studied upon heterologous expression in *S. cerevisiae* cells lacking their own Trk importers, and their properties were compared to the properties of ScTrk1. Our experiments reveal differences in studied Trk proteins´ abilities to support the growth of cells under various cultivation conditions such as low K+ or the presence of toxic cations. The potential of various Trk proteins to support stress resistance of *S. cerevisiae* wild-type strains was also examined.

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C39

Eisosome membrane domains are essential for the long-term survival of Quiescent yeasts

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Quiescence is the most common, but poorly studied, cellular state of microorganisms in nature, essential for resistance and long-term survival under nutrient-limiting and stress conditions. The Plasma Membrane (PM) of yeast shows high degree of compartmentalization into several distinct domains, whose physiological role remains poorly understood. The Membrane Compartment of Can1 (MCC) or eisosome is the most studied domain and corresponds to furrow–like PM invaginations. We have previously shown that eisosomes expand, in number and size, at the beginning of the stationary phase, and at this stage they protect a sub-population of transporters from endocytosis, allowing efficient growth recovery after transient nutrient starvation. However, the physiological role of MCCs upon long-term starvation at the stationary phase and the entry in / survival during Quiescence remains unknown. In this work, we show that MCCs expand specifically in quiescent cells and are required for their long-term survival. More precisely, we provide evidence that MCCs expand, following glucose exhaustion, only in respiratory-active cells possessing cortical mitochondria. This MCC expansion requires not only glucose depletion by also active respiration and is deficient in rho0 cells and mutants of the respiratory chain. Additionally, we show that MCC assembly in quiescent cells requires Lsp1, a protein previously considered inessential for MCC organization at the exponential phase. Most importantly, we provide evidence that strains lacking MCCs reach lower cell densities and show defective long-term survival of quiescent cells, phenotypes related to defective function of mitochondria. More specifically, in the absence of MCCs yeasts do not consume the ethanol produced by fermentation and display defective mitochondrial membrane potential. The molecular mechanisms provoking the above defects are under investigation. Our results uncover the dynamic changes in PM compartmentalization occurring during Quiescence and indicate the importance of this re-organization for respiration and long-term survival of quiescent yeasts.

C40

Determination of total carotenoid and lipid content and their composition in *Rhodotorula toruloides*

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The aim of this study was to investigate the production of carotenoids and lipids by the oleaginous yeast, *Rhodotorula toruloides* CBS14, cultivated on wheat straw hydrolysate and to develop and validate a method in Ultra-high-performance liquid chromatography (UHPLC) to quantify the different carotenoids produced. The study was conducted in two parts. In the first part, the yeast cells were cultivated in shake-flasks and the carotenoids from the samples were extracted using an acetone-extraction method. The UHPLC and spectrophotometer methods were applied to compare total carotenoid content in saponified and unsaponified extracts. From UHPLC analysis, five major carotenoids were determined: β-carotene, γ-carotene, ε-carotene, torularhodin and torulene. Among these, β-carotene was the major carotenoid produced with a total amount of 1.48 mg/100 g dry weight followed by γ-carotene and ε-carotene. Torularhodin and torulene were identified and quantified for the first time using individual commercial reference standards. The carotenoids were extracted with recoveries between 66 and 76%, except for torulene and torularhodin, which had lower recoveries as they were significantly affected by saponification. This effect of saponification on individual carotenoids has not been described before. Total carotenoid content in the saponified yeast extract analyzed by UHPLC, and in unsaponified yeast extract analyzed in the spectrophotometer, was 2.06 mg/100g and 4.02 mg β-EQ /100 g dry weight, respectively. In the second part, the yeast cells were cultivated in fermenters to investigate the lipid and carotenoid accumulation at different time points during the cultivation. Among the carotenoids, similar to the results from first part, β-carotene was observed to be in abundance at all time points and among the various fatty acids produced, analyzed by gas chromatography (GC), oleic acid was the major fatty acid followed by palmitic acid and linoleic acid. The lipid concentration at the end of the cultivation was 9.24 ± 0.28 g/L.

C41

Screening for kinase genes regulating COPII vesicle trafficking in *Saccharomyces cerevisiae*

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Each organelle in a cell has their own function that is maintained by unique proteins and lipids. To maintain organelle homeostasis, the transport of proteins and lipids between organelles must be tightly regulated. Newly synthesized proteins and lipids in the endoplasmic reticulum (ER) are delivered through the Golgi apparatus to their final destination. The ER-to-Golgi transport is mediated by coat protein complex II (COPII) vesicles, which is formed by activation of Sar1 by Sec12 and subsequent assembly of inner coat complex Sec23/Sec24 and outer coat complex Sec13/Sec31 at the ER exit sites where cargo proteins exit the ER. These coat proteins undergo phosphorylation, one of the post-translational modifications, suggesting a regulation of COPII vesicle formation by kinases. However, kinases involved in COPII vesicle-mediated transport have not been identified.

In this study, we carried out a screen to identify kinase genes involved in COPII vesicle formation using a yeast knockout strain collection. Cargo proteins are matured during transport from the ER to the plasma membrane or the vacuole and a defect in COPII vesicle-mediated transport leads to the accumulation of immature cargo proteins. Therefore, we analyzed the accumulation of immature proteins in 100 kinase gene knockout strains. We found that 4 knockout strains accumulated immature ER form of cargo protein, carboxypeptidase Y. We also screened multicopy suppressor kinase genes that suppress the temperature-sensitive growth defect of the sec 12-4 mutant cells, and obtained 7 genes encoding protein kinases. These results imply that post-translational modification by kinases plays an important role in COPII vesicle formation.

C42

Dissecting the role of succinate dehydrogenase assembly factor Sdh5 in mitochondria dynamics of yeast cells

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Mitochondria are highly dynamic organelles with different morphological transitions and diverse functional status. Mitochondrial network changed by fission and fusion mechanisms that are responded to physiological conditions. Disrupting mitochondria dynamics often causes activity alteration. However, less was discussed about how disrupting mitochondria activity affects dynamics. Succinate dehydrogenase has dual roles in both oxidative phosphorylation system and tricarboxylic acid cycle. Its abnormality was reported in several diseases, such as familial paraganglioma syndrome and Leigh disease, with abnormal mitochondrial functions. SDHAF2/Sdh5 is an assembly factor required for the function of succinate dehydrogenase. Succinate dehydrogenase is the only mitochondrial oxidative phosphorylation components encoded by nuclear genome. Here we used budding yeast as model organism to clarify the effects of disrupting Sdh5 in mitochondria in replicative senescence stress and diauxic shift phase. We found that log phase Δ*sdh5* cells exhibited higher mitochondrial respiration compared to wildtype. However, replicative senescent Δ*sdh5* cells possessed lower mitochondrial maximal respiration rate and reserve capacity compared to wildtype. Additionally, deletion of SDH5 led to different mitochondrial network morphology. For cells undergoing replicative senescence stress and diauxic shift, mitochondrial morphology transformed from tubular to fragmented form in wildtype cells, whereas Δ*sdh5* cells had the transition from tubular form to globular form. Furthermore, ectopically expressing attenuated Sdh5 in Δ*sdh5* cells was unable to compromise the irregular mitochondrial morphological transition. In summary, our results demonstrated that nuclear encoded SDH5 affects mitochondrial dynamics and functions in a mitochondria-targeted manner. In addition, these results indicate mutual effects between the regulation of mitochondrial dynamics and function.

C43

The SRS2 mRNA colocalizes with P-bodies and stress granules during genotoxic and replication stress.

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Srs2 is a multifunctional protein involved in the regulation of homologous recombination (HR) and in resolving of an unusual DNA structures that might arise during replication and transcription processes. Srs2 acts dually, promoting or limiting HR at different steps of this process. This complex Srs2 behavior relies on its dual activity, helicase, and translocase. Helicase Srs2 activity is important for promoting error-free sub-pathways of HR. Srs2 capacity to remove proteins from DNA and its translocase activity acts anti-recombinogenic by disassembling Rad51 presynaptic filaments that enable HR initiation. Due to the important role of Srs2 as an HR regulator, the level of this protein has to be tightly regulated. Either removal of Srs2, or increase of Srs2 level sensitize cells to genotoxic stresses and leads to genetic instability.

We demonstrated that increased transcription of SRS2 gene expressed from ADH1p promoter did not result in a proportional increase of protein level. We also observed low level of Srs2 protein despite an increase in the SRS2 mRNA level, in cells lacking G1/S transcription regulator Swi6, which, as we have shown previously, suffer from constant replication stress due to insufficient level of factors involved in DNA replication and repair. These data suggested post-transcriptional regulation of SRS2. Such conclusion was strengthened by the accumulation of mRNP granules in *swi6*Δ cells, which we observed using fluorescently tagged P-body marker. These data implicate a new regulation step in the control of Srs2 expression: the presumed sequestration of SRS2 mRNA into granules. Employing fluorescence microscopy and MS2-tagging methodology we were able to show that SRS2 mRNA colocalizes with both, P-body and stress granule markers. We also showed that the number of such colocalization events increased after genotoxic stresses caused by HU or zeocin, as well as in *swi6*Δ strain.

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C44

Tolerance to isobutanol toxicity is mediated by a transcriptional factor Znf1 of pentose phosphate pathway

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The production of biofuels from renewable biomass such as grain and wood are facilitated by microbes including bacteria or yeast. Bioethanol is regarded as one of the carbon-neutral products of useful biofuel; however, its low energy content and high hygroscopicity are viewed as disadvantages. In terms of fuel quality, biobutanol is more preferable to ethanol because of various significant advantages associated such as its higher energy density and lower hygroscopicity. Moreover, the branched-chain structure of isobutanol provides a higher octane number than the isomeric n-butanol. It is naturally produced in the low amount in the cytosol by the Ehrlich pathway by the yeast *S. cerevisiae*. Isobutanol toxicity to host has still limited the yield of product. In this study, we aimed to address the role of transcriptional regulators of stress response in mediating expression of genes in the pentose phosphate pathway to enhance yeast tolerance to isobutanol. We also attempted to identify key factors involved in reduction of isobutanol toxicity using several deletion mutants of *S. cerevisiae* including Δ*tal1*, Δ*tkl1*, Δ*rpe1*, Δ*zwf1*, Δ*gnd1* strains. Deletion of Znf1 significantly deceased cell dry weight under high concentrations of isobutanol. Our results implicated the zinc cluster Znf1 as a key transcription factors of these genes. Thus, the engineered strain with overexpression of ZNF1 gene could potentially improve production of isobutanol as well as enhance isobutanol tolerance what is possible for bio-based isobutanol production.

C45

Investigating the mechanism of the Tup1-Cyc8 (Ssn6) co-repressor complex in the yeast *Saccharomyces cerevisiae*

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The Tup1-Cyc8 (Ssn6) co-repressor complex is a powerful epigenetic repressor of genes in the yeast *Saccharomyces cerevisiae*. The highly conserved complex brings about a repressive chromatin structure at regulatory regions of its target genes or prevents the recruitment of the factors needed for activation of transcription. A gap in the current understanding is if either of the Tup1 or Cyc8 subunits contribute independently to the mechanism of repression. To investigate this, I am studying the phenotypic differences between a *tup1*Δ, a c*yc8*Δ and a *tup1cyc8*Δ strain. The FLO family of genes are repressed by the Tup1-Cyc8 complex, these genes encode the proteins required for flocculation, a stress response in yeast where the cells aggregate, or form flocs, to protect cells within the floc. Interestingly each mutant strain has a distinct flocculant phenotype. The *tup1*Δ strain displays large, dense flocs compared to smaller, more dispersed flocs associated with the *cyc8*Δ strain. RT-qPCR showed that FLO1 is highly de-repressed in the *tup1*Δ strain whereas it is de-repressed to a significantly lower level in the *cyc8*Δ strain. However, this pattern is not seen at all target genes. For instance, SUC2, which encodes invertase which hydrolyses sucrose, is also repressed by the complex. RT-qPCR showed that this gene is significantly more highly de-repressed in the *cyc8*Δ strain compared to the tup1Δ strain. The results of RNA-Sequencing show that distinct sets of genes are upregulated in each mutant strain, which indicates that subunits of the complex may be dominant in bringing about repression at different sets of genes. Additionally, the results show a core set of 429 genes significantly upregulated in all three mutant strains. Interestingly, this cohort of genes are upregulated to different extents in each mutant strain, again, indicating that each of the subunits make distinct contributions to repression at different genes.

C46

Assembly of RNA polymerase III complex in yeast: novel layer of regulation.

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The biogenesis of eukaryotic RNA polymerases is poorly understood. The present study used a combination of genetic and molecular approaches to explore the assembly of RNA polymerase III (Pol III) in yeast cells. We identified a regulatory link between Rbs1, a Pol III assembly factor, and Rpb10, a small 70 amino acids subunit that is common to all three RNA polymerases. Overexpression of Rbs1 increased the abundance of RPB10 mRNA which correlated with suppression of Pol III assembly defects. Rbs1 is a poly(A) mRNA-binding protein and mutational analysis identified R3H domain to be required for mRNA interactions and genetic enhancement of Pol III biogenesis. Rbs1 also binds to Upf1 protein, a key component in nonsense-mediated mRNA decay (NMD) and levels of RPB10 mRNA were increased in a upf1Δ strain. Interestingly, we identified extension of reading frame behind the stop codon in RPB10 mRNA that may potentially be used for the synthesis of an elongated protein of 120 amino acids. Overproduction of Rbs1 enhanced level of the canonical form of Rpb10 protein, whereas the stop codon read-through and increased synthesis of longer form is observed in upf1Δ mutant. In addition, Rbs1 is involved in controlling steady-state levels of yeast transcripts that are regulated by Upf1 protein via their 3' regulatory sequences. Genome-wide RNA binding by Rbs1 was characterized by UV cross-linking based approach (CRAC). We demonstrated that Rbs1 directly binds to the 3' untranslated regions (3'UTRs) of many mRNAs including transcripts encoding Pol III subunits Rpb10 and Rpc19. Likely Rbs1 protects RPB10 mRNA and other transcripts subjected to NMD degradation mediated by their 3' ends. Orthologues of Rbs1 protein are present in other eukaryotes, including humans, suggesting that this is a conserved regulatory mechanism.

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C47

In search for reliable reference genes in comparative gene expression analysis – ACT1, GAPDH and beyond

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The most common practice to reduce the impact of uncontrolled variation in comparative gene expression analysis (CGEA) is normalization to a reference gene. The reference gene is assumed to be stably expressed in a biological object of interest, and thus – to reflect the amount of sample within the CGEA reaction. Therefore, it is of fundamental importance to determine reliable reference genes for a given biological system, and also specifically for a given experimental scheme. So far, in the CGEA conducted with *Y. lipolytica*, only a single gene ACT1 was routinely used as the internal calibrator. However, its adequacy in this regard has never been systematically tested. In the present research, usefulness of the ACT1 gene along with twenty other genes as the internal calibrators was examined. Their validation was conducted by determining their expression level and expression stability under various experimental conditions, including variable composition of the culturing medium, growth phase and strain - wild type and a recombinant, burdened with heterologous protein overexpression. Extensive statistical testing and analyses with dedicated computational tools (geNORM and NormFinder) indicated the genes that remained unaffected by ongoing overexpression in the recombinant strain. In this case TPI1 and SEC62 were ranked as the most stable genes in the exponential growth phase. IPP1 was stably expressed irrespectively of the growth phase or the used culture medium. SEC62 was indicated as the most accurate internal calibrator for the wild-type strain, whilst SEC61 - for the recombinant strain. TPI1 was not affected by any introduced variable but growth phase. ACT1 was ranked in the top positions but only for the wild-type strain in the stationary growth phase. This study contributes to better understanding of basic metabolism in *Y. lipolytica*, but also provides practical hints for appropriate design of CGEA experiments in this species (Project NCN, DEC-2019/03/X/NZ1/01128).

C48

Understanding the role of J domain and RRM of Cwf23 in RNA splicing

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Each round of splicing requires the spliceosome to undergo extensive remodeling and exchange of spliceosomal components. Spliceosome disassembly requires NineTeen complex-Related (NTR) complex which is composed of Cwc23, Ntr1, Ntr2, and Prp43. Cwc23 is an essential J domain protein in *Saccharomyces cerevisiae*, whose J domain, unlike other JDPs, is completely dispensable for its essential cellular functions. Cwc23 interacts with Ntr1 and is required for its recruitment to the spliceosome during spliceosome disassembly. Cwc23 is ubiquitous as its orthologs have been identified in several eukaryotic species, suggesting involvement of Hsp70:JDP machinery in regulating spliceosomal dynamics. Cwf23, the Cwc23 ortholog in *Schizosaccharomyces pombe*, presents a fascinating example of protein evolution. Although the J domain is conserved, Cwf23 also has an RNA recognition motif (RRM) at its C-terminus. In vitro RNA binding and yeast two-hybrid assays using mutants showed that besides having a role in RNA binding, RRM of Cwf23 is also important for interaction with spNtr1. Specific mutations in RRM affected the growth of fission yeast cells. Interestingly, mutations in the N-terminal J domain caused lethality. Based on our results, we propose that Cwc23 orthologs have a role in spliceosome remodeling and compared to budding yeast, in more complex and "intron-rich" eukaryotes, the J domain and hence the Hsp70 co-chaperone function is essential. Additionally, our data also shows that the C-terminal region of Cwc23 orthologs in complex eukaryotes has acquired novel moonlighting functions to deal with increased spliceosomal complexity.

C49

Malfunctions of the vesicular trafficking affect the post-translational modifications status, activity, and localization of DNA repair proteins

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The results of our genome-wide studies brought our attention to vesicular trafficking as a process contributing to genome preservation. The 63 strains lacking various vesicular trafficking genes were found to be sensitive to DNA double-strand break (DSB) stress, among them, strains lacking SYS1, RVS161, RVS167, VPS63, YPT6, and genes. These strains exhibited various phenotypes suggesting the instability of their genomes. They showed increased susceptibility to zeocin and MMS. The recruitment of Rad52-YFP to DNA repair foci was decreased in these strains, which suggests impaired DSB recognition or defects in DNA repair. The frequency of Rfa1-YFP spontaneous foci formation was increased in these strains, which indicates enrichment of ssDNA regions in their genomes.
Here we show, that in the absence of RVS161, RVS167, VPS63, YPT6 or SYS1 genes the post-translational modification pattern of several proteins engaged in DNA repair, e.g., Rfa1, Rad51 or Cdc13 is changed in cells exposed to genotoxic stress. We showed that this post-translational modification is phosphorylation. The phosphorylation status affects the molecular functions of these DNA repair proteins and influences their recruitment propensity to the repair foci. Furthermore, we show that in the tested vesicular trafficking mutants, the telomere length is increased compared to the WT strain. We conclude that disturbances in vesicular trafficking affect the usage of different DNA repair pathways at the telomeric regions.

C50

Enhancement of acetic acid tolerance in *Komagataella phaffii* by overexpression of transcriptional factor Haa1

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Lignocellulose-derived inhibitors represent one of the major drawbacks in the production of fuels and chemicals from biomass. Weak acids such as acetic acid are released and formed during biomass hydrolysis. Acetic acid is known to inhibit microbial growth and impair fermentation by decreasing intracellular pH and therefore deregulating transmembrane ion balance. Tolerance mechanisms in *Saccharomyces cerevisiae* are relatively well elucidated and englobe the expression of genes involved in drug transport, sugar and amino acid metabolism, protein folding, and other functions. The transcriptional factor *Haa1* regulates the yeast response towards acetic acid and its overexpression increases yeast performance in presence of such acid. In this study, we identified and evaluated the effects of *Haa1* overexpression in *Komagataella phaffii*. First, the *Haa1* gene was overexpressed in *K. phaffii* under control of the constitutive promoters pGAP and pPRPP1b. Then, tolerance of recombinant strains was evaluated in YPD medium without or supplemented with 2, 4, 6, and 10 $g \cdot L^{-1}$ of acetic acid and in sugarcane hydrolysate. Results showed a similar physiological response of *K. phaffii* to acetic acid in the absence and presence of 2 $g \cdot L^{-1}$ of the acid. In presence of 4 $g \cdot L^{-1}$ of acetic acid, the glucose consumption and yeast growth were reduced for all strains, but overexpression of *Haa1* allowed the recombinant strains to reach higher biomass formation and fermentation rates. The *Haa1* overexpression also improved *K. phaffii* tolerance towards sugarcane hydrolysate. The results demonstrate that acetic acid inhibition can be reduced by homologous *Haa1* overexpression in *K. phaffii* until a threshold concentration of the inhibitor is reached.

Room D – Donauinsel

D01

Evolution of mutualistic behaviour between *Chlorella sorokiniana* and *Saccharomyces cerevisiae* within a synthetic environment

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Yeast and microalgae are microorganisms with widely diverging physiological and biotechnological properties. Evolutionary experiments can be applied in a wide variety of areas and have been successfully used for strain improvement strategies and for the generation of desired phenotypes. More importantly, it allows an understanding of the evolutionary trajectory of microorganisms and sheds light on adaptive mechanisms which can be complex to unravel in the natural environment. We describe an entirely novel synthetic ecology-based approach to evolve co-operative behaviour between winery wastewater isolates of the yeast *Saccharomyces cerevisiae* and microalga *Chlorella sorokiniana*. These data describe improvements to co-evolved strains over time through a comparison of parental strains and isolates from generations 50 and 100. The data show that biomass production and mutualistic growth improved when co-evolved yeast and microalgae strains were paired together in co-evolution conditions. Monoculture growth of co-evolved strains was investigated using semi-selective conditions and phenotypic changes were observed in both yeast and microalgal species. It was hypothesized that a metabolic trade off takes place during evolution as species adapt to the conditions in which they are co-evolved. Combinations of co-evolved strains displayed a range of phenotypes, including differences in amino acid profiles and targeted gene expression data. Taken together, the results demonstrate that biotic selection pressures can lead to rapid adaptation of mutualistic growth phenotypes.

D02

Assessing fermentative traits across the population spectrum of *S. cerevisiae*

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Microbe domestication has led to profound genetic and phenotypic changes in the lineages that underwent artificial selection. For *Saccharomyces cerevisiae* these changes were critical for the production of fermented foodstuffs and beverages. The availability of domesticated and wild populations of this species allows for detailed comparisons, aiming for a better understanding of domesticated phenotypes and to rationally increase the diversity and attributes of domesticates. The strains from the wine population, the most well-studied domesticated population, metabolize efficiently the sugars present in grape must (mostly glucose and fructose) to ethanol, in the particular environmental conditions of wine fermentation. This results in the complete exhaustion of glucose and fructose and in a final ethanol content between 9 – 15% (v/v). Taking advantage of a high-resolution population map of *S. cerevisiae*, we investigated the distribution of relevant traits for wine fermentation across a diverse range of populations, including wild - mostly isolated from oak trees - and domesticated ones. We observed that ethanol resistance is not particularly selected for in the wine population and a considerable variation among strains of this lineage was observed. Interestingly, the wild population from the Mediterranean region showed a similar pattern but other wild populations from China / North America were, on average, more resistant to ethanol. However, during synthetic grape must fermentations, wine strains were able to exhaust glucose and fructose, while this property was not mimicked by the other populations that nevertheless showed considerable variation between them. Overall, a considerable phenotypic variation was detected among wild populations. These results also showed that ethanol resistance predates the domestication of wine yeasts. These yeasts have an evolutionary relationship with Mediterranean oak yeasts that is closer than with any other known wild population. The phenotypic properties investigated here support this assessment.

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D03

Quantitative genetics of yeast hybrids: harnessing the power of biodiversity for industrial applications

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Hybrids between different *Saccharomyces* species are commonplace in nature and in industrial settings, where they underwent centuries of selection in harsh fermentative conditions. Through hybridization we are able to combine advantageous traits and increase genetic diversity without resorting to GMO products. However, the study and the development of inter-species hybrids has been hindered by both their sterility and their genetic makeup. Thus, to accelerate evolution, it is of the foremost importance to untangle the genomic complexity of the industrial hybrids.
In this study we generated a novel platform to study inter-species hybrids by crossing geographically distant strains from different species. The allotetraploids generated exhibited high spore viability, allowing continuous multigenerational breeding and generated a phenotypically diverse progeny.
Pooled F12 segregants exhibiting extreme traits were then tested as a platform to perform large scale mapping of QTL, thanks to their varied genomic heritage. Through state-of-the-art genetic techniques we identified species-specific and hybrid-specific features responsible for traits of biotechnological interest such as maltose, low temperature, and acetic acid. Furthermore, we investigated the effect of the mitochondria on the QTL landscape, by comparing QTL regions mapped in hybrid progeny derived from parental lines with different mitochondria.
Our platform has proven successful in both harnessing the power of biodiversity for industrial application and in bringing yeast hybrids in the realm of quantitative genetics.

D04

New insights into the evolution of members of the drug: H+ Antiporters Gene Family 1 (DHA1) in *Hemiascomycete* Yeasts

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The transporters of the Major Facilitator Superfamily (MFS) involved in Multidrug Resistance (MDR) catalyze the active cellular expulsion or partition of unrelated cytotoxic compounds [1,2,3]. In the *hemiascomycete* yeasts, the MFS-MDR transporters divide into two different Gene Families, the Drug:H+ Antiporters Family 1 (DHA1) and the DHA2-ARN-GEX Family (DAG) [1,2,3].

In this work, we reconstruct the evolution of the DHA1 genes, expanding the number of yeast species under analysis in two previous studies [1,2] (from 25 to 63), many belonging to taxonomic families not sampled yet. Two databases were developed in-house by our research group. The first database, henceforth designated as GenomeDB, compiles the available biological information of the translated ORFs encoded in 94 hemiascomycetous strains. The second database, henceforth designated BlastDB, compiles the amino acid sequence similarity of all possible pairwise combination between the translated ORFs comprised in the GenomeDB. A network traversal approach using as starting nodes known members of this gene family allowed the identification of 1382 bona fine DHA1 proteins. The construction of a phylogenetic tree representing the DHA1 proteins recovered the 22 clusters previously reported [1,2], and allowed the identification of two additional clusters. Gene neighborhood analysis allowed the detection of ten main DHA1 gene lineages in the evolution of the hemiascomycete yeasts. Multiple gene duplication events and genome rearrangements were found in the lineage at the origin of the *Saccharomyces cerevisiae* FLR1 gene, originating a strong loss of synteny between the FLR1 homologs encoded in the post-WGD species. Five independent Horizontal Gene Transfer (HGT) events were also detected in the lineages at the origin of the *S. cerevisiae* QDR3, HOL1 and YHK8 genes and in genes classified in clusters D and K2.

[1] Dias PJ, et al. (2010). OMICS.
[2] Dias PJ, Sá-Correia I (2014). Genomics.
[3] Dias PJ, Sá-Correia I (2013). BMC Genomics.

D05

Finding the mechanistic basis of thermotolerance in evolved thermotolerant strains.

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When cells are grown under a selection pressure in laboratory conditions, it is termed as adaptive evolution experiments (ALE). It is a method used extensively to answer questions on evolution. ALE is also a promising strategy for industrial application, metabolic engineering and exploring mechanism for drug resistance for antibiotics. Microorganisms are the preferred choice for their shorter generation time and selection pressures such as nutrient-deficient media and heat stress are extensively used for ALE. Heat stress affects protein folding, organization of cytoskeletal structure, reaction kinetics of several proteins and enzymes, alteration of metabolites, and the integrity of organelles. Growing cells in challenging heat stress condition from generation to generation lead to adaption of cells under the selection pressure. In this process of natural selection, the cells accumulate certain fixed beneficial mutations that lead to better fitness at higher temperature. Possibly, these adapted cells rewire their regulatory network and solve some other challenges posed by heat stress.

We adapted 55 *Saccharomyces cerevisiae* strains for 600 generations in ALE experiments followed by their phenotypic and genotypic characterization. The genome of thermotolerant (TT) strains were sequenced. Gene duplication events, chromosome duplication events and mutations accumulated during adaption were found and properties of thermotolerance of few nonsense mutations were validated by phenotypic characterization. From the analysis, we got few strains have lost the mitochondrial DNA content completely. Still, we are exploring the mechanism of mitochondria DNA loss and their role in thermotolerance. The study on molecular mechanism of thermotolerance exploring novel mutations will be helpful in answering evolutionary questions as well as explore the pathways that may be able to solve the cellular challenges due to heat stress.

D06

Comparative analysis of cell wall-related proteins of different yeasts

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The yeast cell wall is an extracellular structure with a complex role in preserving cell shape and integrity, and in interaction with different molecules and cells in its surrounding. Fact that 1200 *Saccharomyces cerevisiae* genes directly or indirectly affect cell wall formation and regulation (de Groot et al., 2001) clearly speaks of the importance of the cell wall. Here we present results from our recently published manuscript of in silico analysis comparing 187 *S. cerevisiae* proteins involved in cell wall biogenesis and function with cell wall proteomes of 92 different yeast species. The main goal of the research was to estimate the evolutionary conservation of proteins involved in protein glycosylation, cell wall synthesis and remodeling, and of cell wall proteins with still unknown functions. As expected, proteins involved in processes of great importance for cell wall integrity and its protective function were highly conserved. However, GPI anchored proteins involved in flocculation, aggregation, cell separation, and those with still unknown functions were not highly conserved. This analysis was coupled with analysis of proteins located in the cell wall by protein biotinylation and western blotting. Results of this analysis show distinctive protein patterns and significant differences in the overall amount of cell wall proteins for different yeast genera, and even for different species of the same genera.

de Groot, P.W. et al. 2001. Comp. Funct. Genomics, 2(3), pp.124-142.

D07

YMR003w, a gene of unknown function, is required in spontaneous and UV induced mutations

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We identified YML003w, a putative protein of unknown function in a screen for variation in response to Doxorubicin. In the reference strain, S288C, a frameshift relative to all other strains sequenced, results in a truncation and delimination of the next gene, YML002w, which is not expressed in S288C. The null mutant, yml003wD is sensitive to DNA damaging agents, Doxorubicin and MMS but not to UV. As it might be involved in a DNA damage repair process, the spontaneous and UV-induced mutation frequency of yml003wD in S288C and a lineage used in the mapping of variation underlying phenotypic response to Doxorubicin were measured. No mutation events are seen indicating a significant reduction in mutations in the absence of this protein. Overexpression of this truncated protein results in an increase in spontaneous mutation frequency. A series of double deletions with several other mutagenesis related genes were created. The spontaneous mutation frequency is reduced in most strains tested (rad18Δ, rev1Δ, ubc13Δ, ccr4Δ, mms2Δ, rev3Δ, rad5Δ) but not in yml003wD rad5D or yml003wD mms2D. The deletion of yml003w can also suppress the UV sensitivity of some strains (rad18Δ, ubc13Δ, rev3Δ) but not in rad5Δ. A GFP-fusion of YML003w locates to the mitochondria. Our findings suggest that YML003w plays an important role in mutagenesis and UV sensitivity and potentially interacts with RAD5 mediated pathways.

D08

Horizontal acquisition of a betaine biosynthetic pathway in osmotolerant yeasts

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The osmotolerant *Wickerhamiella* and *Starmerella* yeast genera form a lineage, the W/S clade, which is notable for carrying numerous genes that were acquired through horizontal gene transfer (HGT). Yeasts belonging to this clade are usually found in the floral niche (1) and are thus expected to experience osmotic stress associated with high sugar concentrations found in floral nectar and honey.
One strategy employed by microorganisms to deal with low water activity environments is the accumulation of compatible solutes, which are used to adjust intracellular water activity and to protect biomolecules from denaturation. Bacteria and plants usually synthesise and accumulate glycine betaine (or betaine) in response to extreme abiotic stresses. Exogenous betaine is also known to improve growth in yeasts exposed to osmotic stress, but de novo betaine biosynthesis has not been reported in yeasts so far.
Recently, we found in W/S clade species two genes (betA and betB) required for de novo betaine biosynthesis, which were likely acquired from filamentous fungi. Some species also harbour a bacterial version of betA (2), suggesting that synthesis of betaine might be relevant for osmotolerance in W/S yeasts.
Here we studied the role of HGT-derived bet genes in betaine synthesis and osmotolerance by constructing bet deletion mutants in W/S yeast *Starmerella bombicola*. We found that both betA and betB genes are expressed in the wild type strain and confirmed that this strain is able to synthetize betaine de novo. The bet deletion mutants are unable to synthetise betaine and show a decreased tolerance to osmotic stress. Altogether our results support the current hypothesis that HGT was a major driver of the adaptation of W/S yeasts to the floral niche.

1. Gonçalves P,et al. (2020), Yeast. DOI:10.1002/yea.3463
2. Shen XX, et al. (2018), Cell 175(6):1533-1545. DOI:10.1016/j.cell.2018.10.023

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D09

Endophytic yeasts in domestic and imported agricultural crops.

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During the two years of the study were examined 1,885 samples of internal tissues for 81 agricultural cultures from 38 countries, including Russia as domestic origin. Purchases of agricultural products of Russian and foreign origin were made in Moscow retail stores. The sampling was carried out on the basis of visual characteristics reflecting ripeness and the absence of spoilage. The study of endophytic yeast communities was carried out according to the standard scheme; surface sterilization – sampling of internal tissues – suspension preparation – plating – creation a collection of endophytic yeasts – species identification.

The median abundance of endophytic yeasts in the studied fruits and vegetables was 2.35×10^3 CFU/g. In the process of the study, we isolated 102 species of yeasts: 52 species of ascomycetes and 50 species of basidiomycetes. Ascomycetes yeasts were predominant in almost all studied groups of agricultural products; their average share was about 75%. The data analysis showed noticeable difference between domestic products and imported ones, as well as, difference between fruit and berry products from vegetables. The maximum species diversity of endophytic yeasts was registered for tropical fruits. The following species were found both in domestic and imported products with a high frequency of occurrence: *Candida zeylanoides* (18 %), *Aureobasidium pullulans* (16 %), *Debaryomyces fabryi* (16 %), *D. hansenii* (16 %), *Meyerozyma caribbica* (15 %), *Metschnikowia pulcherrima* (13 %), *C. parapsilosis* (13 %), *Hanseniaspora u*varum (12 %), *Rhodotorula mucilaginosa* (10 %), *Mey. guilliermondii* (10 %), *Rh. babjevae* (6 %). *C. parapsilosis* as clinically significant species of BSL-2 level deserve a special attention with further monitoring. The created collection of endophytic yeasts will make it possible to assess the plant growth-promoting properties and select biocontrol yeasts for plant protection.

This work was supported by the Russian Science Foundation (grant No 19-74-10002).

D10

The beauty of the yeasts: diversity and functions in the wheat phyllosphere

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Microorganisms inhabiting the phyllosphere (aerial surfaces of plants) include bacteria, fungi, viruses, archaea among others. The majority of studies on the phyllosphere has focused on pathogenic microorganisms, however, many microorganisms, such as yeasts, can provide beneficial functions to the plants by promoting growth and inhibiting pathogens. Although yeasts are abundantly found on leaf surfaces and have been described to degrade fungicides and mycotoxins, the underlying molecular mechanisms for plant growth and protection against pathogens remain largely unknown. This project aims at investigating the taxonomic and functional diversity of yeasts colonizing the surface and internal tissues of wheat flag leaves (the last leaf before the ear emergence). Taxonomic identification by partial ITS sequencing of 176 yeast isolates revealed a diverse composition consisting of 13 different genera. These isolates were functionally characterized based on their carbon utilization, biofilm formation and antifungal activity, traits which play an important role in survival in the phyllosphere. Overall, the yeast isolates were able to utilize 16 out of 31 carbon sources tested. Isolates belonging to the genera *Vishniacozyma* and *Holtermanniella* showed the most flexible carbon utilization. Biofilm formation was observed for members of the *Metschnikowia*, *Vishniacozyma* and *Aureobasidium* genera. Out of the 176 isolates, 50 were active against *Fusarium graminearum* and four against *Zymoseptoria tritici*, a total of 27% of the collection. Additionally, a number of isolates also showed antifungal activity via volatile compounds. Synthetic communities designed based on the results of these different assays will be used to test their ability to protect plants against the fungal pathogens using detached leaf and in planta bioassays. To further explore the genetic potential of the phyllosphere yeasts comparative genome analysis will be performed. These findings will contribute to develop a new microbiome-based strategy to improve tolerance of wheat plants to fungal foliar infections.

D11

Evaluating the competition capacity of *S. cerevisiae* strains with different environmental origins and their sugar consume profile

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The wine industry has implemented co-culture fermentations as an efficient method to achieve the wine properties demanded by the consumer. The strain proportion and the nutrient consumption, defined by the competition capacity, determine the final products in wine. The aim of this study is to evaluate the competition capacity of yeast strains with different environmental origins and to evaluate their sugar consume profiles in mono- and co-culture fermentations.
Sixty *S. cerevisiae* strains from different environments (wine, traditional ferments, wild, etc.) were analysed. The competition capacity of each *S. cerevisiae* strain was measured by the cell proportion during co-culture fermentations with CR85 (*S. kudriavzevii*). Ten *S. cerevisiae* strains (with high, medium, and low competitor capacity) were selected to compare their nutrient consume profile in single and co-cultures fermentations by measuring the sugar consume. ANOVA and Tukey HSD tests were carried out to analyse the statistical differences among groups.
Significant differences ($p<0.05$) were found between wine strains and wild, bioethanol producing, and traditional ferments strains. The wine strains were the best competitors with a median *S. cerevisiae* proportion of 0.5 (range: 0.99-0.06) vs. the global median proportion of 0.34.
Sugars consumption patterns differ according to the competitive capacity of the strain: In high competitor strains, the consumption slows down in competition in comparison with monocultures. In medium strains, the pattern is strain-dependent (slowing down in competition or no difference). In low competitors, due to the low proportion of *S. cerevisiae*, consumption follows the *S. kudriavzevii* monoculture pattern.
In conclusion, wine strains, as a group, are shown as the best competitors although they have a wide range of competition capacity. Additionally, the sugar consumption patron in co-culture fermentation is related to the strain´s competition capacity.

D12

Evidence for higher-order ecological interactions in *Saccharomyces cerevisiae*

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The use of microbial ecosystem-based biotechnology faces significant challenges because of our lack of functional and mechanistic understanding of these biological systems. Non-linear ecological interactions within microbial ecosystems are arguably the main contributors to the infamous unpredictability of ecosystem-based bioprocesses. Higher-order interactions, or interactions in systems comprised of more than two members that cannot be explained by pairwise interaction contribution, are particularly significant and understudied in this context. Wine fermentation presents an excellent model to study yeast ecosystem establishment and functioning. Wine yeasts are known to have undergone adaptive evolution to fermentation-specific abiotic stressors, and recent data suggest the evolutionary relevance of biotic stressors, i.e., co-habitant species, in the functioning of this ecological niche. While some progress has been made in characterizing pairwise ecological interactions between wine yeast, very little is known about how more complex, multi-species systems function – an important endeavour, given that this is more relevant to their natural ecological state. Here, we sought to evaluate emergent non-linear ecosystem properties by determining the transcriptomic response of *Saccharomyces cerevisiae* to pairwise versus tri-species consortium culture. mRNA sequencing revealed that genes expressed during pairwise co-culture were enriched in the consortium dataset, and that just under half of the dataset represented expression of genes unique to consortia growth. Through interactive protein-association network visualizations, we were able to provide a holistic cell-wide view of the gene expression data, which highlighted known stress response mechanisms to be specifically activated during growth within the consortium. This provides exciting new evidence that shows the presence of higher-order interactions within the simplest of synthetic wine yeast ecosystems. The findings are significant both in terms of providing further evidence for the importance of biotic stress in the eco-evolutionary development of wine yeasts, but also in bringing us closer to more predictable, efficient yeast ecosystem-based bioprocessing.

D13

Catalogue of yeasts from Columbia

Mr. Mauricio Ramirez-Castrillon[1], Ms. Anabel Barona-Colorado[1], Ms. Maria Camila Bados-Lopez[1], Ms. Dahyana Bolaños-Burbano[1]

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Colombia is recognized for its great biodiversity. Is the first country in birds and orchids biodiversity, second place in amphibians, butterflies, and freshwater fish, third place in palms and reptiles, and fourth place in mammals. However, yeast diversity has been little explored and little is known about the sampling effort. This work aimed to review all publications, repositories, databases, and public collections that reported isolation and identification of yeasts in Colombia, between 2000 and 2020. Some of the combinations to search in each database were: "yeast AND Colombia", "yeasts AND diversity AND Colombia OR yeasts AND species AND Colombia", in English and Spanish. All clinical isolations (human or related to zoonotic diseases) were excluded. We found at least 112 yeasts, identified at the level of species, located in 11 departments. 75% of the yeasts belong to Ascomycota, and 25% to Basidiomycota. Several publications reported only genera or no identification (31 manuscripts), with a total of 21 different morphotypes. The most common primary environments were associated with different parts of plants, air, and soil. Secondary environments that reported yeasts were fermented fruits, fermented beverages, soils surrounding sugarcane culture, and soils contaminated with hydrocarbon. The most representative species were *Saccharomyces cerevisiae* (154 reports), *Hanseniaspora uvarum* (61), *Candida tropicalis* (59), *Pichia kluyveri* (56), *Meyerozyma guilliermondii* (53) and *Candida albicans* (53). There is evidence of a lack of sampling effort and the results suggested the potential to find and describe new species.

D14

Uncovering the hidden diversity of *Torulaspora*

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The genus *Torulaspora* presently accommodates nine species. The type species is *T. delbrueckii*, which is also the most well-known due to its frequent occurrence in artisanal wine and sour bread fermentations and to its biotechnological relevance for increasing aroma complexity in wines and for leavening pre-frozen bread dough. Exploring the genomic and phenotypic diversity in other *Torulaspora* species might be of relevance for the design of novel fermentations and the mapping of fermentation-relevant attributes. Here, circa 100 genomes of *Torulaspora* spp. were analysed, including all the established species in the genus, three of them sequenced here for the first time. The sister genus, *Zygotorulaspora*, was also included in the analysis. A high-resolution phylogeny based on single-copy core genes and the determination of average nucleotide diversity (ANI) between clades allowed a reassessment of the species currently considered in *Torulaspora*. For *T. pretoriensis* and *T. globosa*, a strong heterogeneity was detected, which suggests that each of these includes a cryptic species. Moreover, another four isolated clades appear to correspond to undescribed species, thus expanding considerably the species diversity of the genus.

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D15

The evolution and ecological relevance of MEL genes in *S. cerevisiae*

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Melibiose assimilation is rarely observed in *Saccharomyces cerevisiae* and for many years this trait was used as a marker to discriminate Mel negative *S. cerevisiae* wine and top-brewing yeasts from Mel positive *Saccharomyces pastorianus* bottom-brewing yeasts. In *Saccharomyces*, melibiose assimilation is possible due to the presence of the MEL gene that codes for an alpha-galactosidase, which converts melibiose to glucose and galactose. In this study we surveyed the distribution of MEL genes and their allelic diversity at a population level in *S. cerevisiae* and found that this gene is fixed only in a few populations. One of them is ecologically associated with processed olives, like olive brine, olive oil, and olive mill wastewater (alpechin). An intriguing observation is that melibiose or chemically similar oligosaccharides are absent from those environments. Moreover, we detected that these strains have multiple MEL copies, which suggests that these environments confer a selective pressure to keep and even to expand this gene although the ecological relevance of this gene is presently unclear. To shed light on this subject we used the CRISPR-CAS9 system to inactivate all the copies of the MEL gene in one of the strains from this population. As expected, the transformant strain could not grow on melibiose but, more importantly, it showed a growth defect in olive brine when compared to the wild type strain. These results suggest that the alpha-galactosidase activity might be involved in the assimilation or even detoxification of other alpha-galactosyl containing compounds. Our results suggest that MEL genes are crucial for fitness in these niches.

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D16

Yeasts associated with wild plants of the *Fabaceae* family inhabiting meadows in Slovakia

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The family *Fabaceae* includes a number of important agricultural plants. They also play a role in honey production, because as melliferous plants, they produce nectar which is collected by bees and other insects. For this plant family, a root nodule symbiosis with the nitrogen fixing bacteria (rhizobia), is typical and, therefore, the diversity and beneficial role of belowground microbiota to plants have been studied. However, a little is known about the microbial community that inhabit phyllosphere of the *Fabaceae* plants.

In this study, we focused on diversity of yeasts associated with meadow plants of the *Fabaceae* family. From May till August 2017, we collected samples from the flowers and leaves of red clover (*Trifolium pratense*), field clover (*Trifolium campestre*), spring vetchling (*Lathyrus vernus*) and cow vetch (*Vicia cracca*) inhabiting meadows in two localities: Malé Leváre (southwest of Slovakia) and Martin (northern Slovakia). Identification of yeast strains was performed by the MALDI-TOF MS biotyping in combination with sequence analysis of the D1/D2 domain of the LSU rRNA.

Together, we identified 21 taxa, 19 of them were isolated from the leaves and only 8 taxa originated from the flowers of the *Fabaceae* family plants. *Aureobasidium pullulans* and *Filobasidium vieringae* were the most widespread yeast species, they were present on all collected plants of the Fabaceae family. *Cystofilobasidium macerans* was isolated from tufted vetch, red clover and field clover. *Vishniacozyma victoriae*, *Holtermaniella festucosa*, *Holtermaniella takashimae* and *Metschnikowia pulcherrima* inhabited two *Fabaceae* species, while the majority of the yeast taxa identified (14) were detected only individually on the phylloplane of the monitored plants.

The most diverse yeast community was found on the surface of cow vetch, (14 species), followed by red clover (10 taxa), field clover (6 taxa) and spring vetchling (3 taxa).

D17

Termite gut: an under-explored niche for diverse and novel yeast species

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The insect gut is a specialized habitat harbouring various symbiotic microbes, especially yeasts. The gut of beetles has already been established as a hyperdiverse source of yeasts, including many undescribed taxa. The termite gut also represents a similar niche that has not been thoroughly investigated for its yeast diversity. Thus, our study aimed to uncover the yeast community inhabiting the gut of different termites from the Western Ghats of India. We collected 7 termites samples and identified the termites by COI gene sequencing. We isolated 103 yeasts from the gut of these seven different species of termites. Post-MSP-PCR screening, the selected isolates were identified by sequencing the D1/D2 region. These yeasts belonged to 21 different species distributed under 16 genera viz *Pichia, Millerozyma, Meyerozyma, Papiliotrema, Rhodotorula, Debaryomyces, Kazachstania, Metschnikowia, Aureobasidium, Yamadazyma, Saitozyma, Cyberlindnera, Nakazawaea, Candida, Vishniacozyma,* and *Kwoniella*. Amongst these isolates, yeast strains ATS2.16 and SMT1.3 were considered to represent two novel ascomycetous yeast species of the genera *Metschnikowia* and *Nakazawaea*, respectively. Strain SMT1.3 differed from closely related *Nakazawaea siamensis* DMKU RK467 by 11 substitutions (2.09%) in D1/D2 region and 54 substitutions and 29 deletions (12.3% variation) in the ITS region. While the species delineation in *Metschnikowia* ATS2.16 was achieved by D1/D2 domain and whole genome-based phylogenetic analyses. Whole-genome sequencing of ATS2.16 was performed using Illumina HiSeq 2500, and a genome size of 10.93 Mb was obtained. Species tree inference from all genes (STAG) analysis based on 959 orthologous genes confirmed the species novelty and placed our strain ATS2.16 next to *Metschnikowia proteae*. The average nucleotide identity (ANI) between the genomes of ATS2.16 and closely related species *Metschnikowia proteae* was 79.74%, which suggests that it is a distinct species. These results supported the claim that the termite gut is an underrated source of diverse and novel yeast species

D18

Spring tree fluxes are refugia of psychrophilic yeasts

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Fluxes resulting from tree injuries naturally occur in early spring. They result from winter damages occurring through freeze-thaw cycles and injuries caused by birds and animals as well as from an anthropogenic intervention. The sugar-rich ephemeral substrate occur on bark where tree sap is leaking through damages or on surfaces of pruned branches. Tree sap infested with microorganisms has been the source of isolation of many species, including the biotechnologically relevant carotenoid yeast *Phaffia rhodozyma*.

Tree fluxes caused by tree injuries sampled during 2013–2020 in Braunschweig, Lower Saxony, Germany. Yeast communities developing in tree fluxes were strongly dominated by basidiomycetous yeasts. Ascomycetous yeasts were rare and associated with the activity of insects feeding on thick microbial (including yeasts) mats developed on fluxes. A stump of a chopped birch tree was visited by Drosophila melanogaster, which vectored *Hanseniaspora osmophila*, *Kazachstania servazzii, Pichia fermentans*, and *Zygotorulaspora florentina*. Another visited by *Drosophila* tree flux on a sycamore tree yielded *Komagataella pastoris*. Basidiomycetous yeasts were represented by (i) typical phylloplane species, e.g., *Filobasidium magnum, Rhodotorula babjevae, Rhodosporibiobolus colostri, Sporobolomyces roseus, Vishniacozyma victoriae;* and (ii) psychrophilic and psychrotolerant yeasts, e.g., *Cystofilobasidium spp. (C. capitatum, C. infirmominiatum), Goffeauzyma gastrica, Holtermanniella wattica, Leucosporidium creatinivorum, Mrakia spp. (M. blollopis, M. gelida, M. fibulata),* and *Tausonia pullulans*. Repeated attempts to isolate *Phaffia rhodozyma* were not successful.

Global warming has already significant effects on our lives shrinking and vanishing cold habitats on the planet. Because spring sap fluxes usually occur early in spring when temperatures are still low, yeast communities were dominated by psychrophilic and psychrotolerant yeasts. Whether or not cold-adapted yeasts survive warm temperatures in a dormant state is yet unknown.

D19

Is chlamydospore formation of the yeasts indispensable for adaptation to live in the gut of lacewings?

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Various insects harbor the yeasts in their digestive tracts. In the guts of lacewings (Neuroptera: *Chrysopidae*) the specific yeasts of the genus *Metschnikowia* has been known. This interaction is suspected as cultivation mutualism. As previous studies focused on only one genus of the host, trends and relationships across the *Chrysopidae* have not yet been clear. Then we start clarifying the yeast flora of the *Chrysopidae* intestines for the first time in Japan. We also aimed at understanding how yeasts behave through their host guts.
In 2018-2021, we collected 80 individuals of lacewings in Japan, and isolated yeasts from their intestines. In total, 79 isolates were established from the guts of 10 spp. of 6 gen., 2 sub-fam., fam. *Chrysopidae*. Among them, 68 isolates were sequenced, all of which were identified as *Metschnikowia* by BLAST search. As a result of phylogenetic analysis, the obtained isolates were divided into 5 undescribed species belonging to 3 clades (I-III). In order to clarify the relationships between these yeasts and their hosts, yeasts were fed to the yeast-free host animals, their excrements were examined every 2 days for 2 weeks, and finally dissected hosts to observe the yeast cell behaviors in various parts of the intestines. In the foregut including diverticulum, active budding cells were observed in all species. In the midgut and hindgut, destroyed cells were found in all species. But only for the yeasts of the clade I and II, thick-walled chlamydospores were recognized in the midguts and culturable yeast cells were recovered from the excrements. These results suggest that chlamydospore formation must be indispensable for adaptation of the yeasts to the *Chrysopidae* gut environment as they can resist against digestion by their hosts.

D20

Genome, transcriptome and secretome analyses of the antagonistic, yeast-like fungus *Aureobasidium pullulans* to identify potential biocontrol genes

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Fungi of the genus *Fusarium* are among the most important soilborne plant pathogens, they threaten crop production worldwide, and their management by traditional methods is inconsistent. Some filamentous fungi (e.g*., Trichoderma*) and bacteria have been described to inhibit Fusarium. However, yeasts are underexplored for the application against plant pathogens despite being ubiquitous in all environments, showing remarkable stress tolerance and harboring great potential as plant protection agents.

A quantification of antagonistic activity from 40 naturally occurring yeasts against a broad range of saprophytic, beneficial and pathogenic fungi in vitro reveals strong activity for six isolates. Biochemical assays to measure enzymatic activity highlight one isolate of *Aureobasidium pullulans* that has increased protease activity in co-culture with *Fusarium* oxysporum. To elucidate potential biocontrol mechanisms, we have assembled a reference genome of this strongly antagonistic *A. pullulans* strain, performed RNA-seq experiments, and analyzed proteins secreted during the interaction with the pathogen *F. oxysporum*. These analyses defined a subset of 79 A. pullulans genes (among the 10,925 annotated genes) that were transcriptionally upregulated or only detected at the protein level during the competition. The potential biocontrol genes comprised predicted secreted hydrolases such as glycosylases, esterases, and proteases, as well as genes encoding enzymes involved in the synthesis of secondary metabolites. This work highlights the value of a sequential approach starting with genome mining and consecutive transcriptome and secretome analyses in order to identify potential target genes for detailed, functional analyses.

D21

Saccharomyces cerevisiae discrimination by metabolomics

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Alcoholic fermentation is known to be a key step in the winemaking process that directly impacts the composition and quality of the final product. Microorganisms are the main players, the major ones being *Saccharomyces cerevisiae*. The impact of different strains of *S. cerevisiae* on the composition and diversity of volatile compounds in wine is well described in the literature. However, little is known regarding the impact of non-volatile metabolism of different strains of *S. cerevisiae* on the wine matrix. Ultra-high-resolution mass spectrometry (uHRMS) and multivariate analysis were used for an un-targeted metabolomics approach. Results from fermentations conducted by twelve different strains of *S. cerevisiae* on the same must, showed that, despite similar fermentation kinetics, wines made by different yeast can be discriminated with this original approach based on their specific metabolomic signature. Markers were extracted and annotated expressing numerous metabolic differences within the same yeast species. Our study revealed significant biomarkers to each strain of *S. cerevisiae*, and more generally, a chemical composition specific for each wine. This indicates that despite their closeness, the metabolisms of these strains are associated to distinct chemical fingerprints and reveal differences in some metabolic pathways.

It was possible to point out the specific production of one or more metabolites in each strain. We demonstrate that uHRMS and multivariate analysis are perfect tool to emphasize intraspecies differences among *S. cerevisiae*.

D22

Genetic suppression analysis links riboflavin biosynthesis to glycerol metabolism

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Genetic suppression is the process by which a mutation in a suppressor gene can rescue the phenotype caused by a mutation in another gene. Suppressor genes often encode for proteins involved in the same pathway or in the same complex as the mutant they are suppressing. However, while isolating bypass suppressors of the essential gene RIB3, which encodes for an enzyme involved in riboflavin biosynthesis, we found suppressor mutations in DAK1 and GPP1, which belong to the seemingly unrelated glycerol metabolic pathway. In this project, we aim to understand how changes in glycerol biosynthesis can suppress riboflavin mutants. Our hypothesis is that the suppressor mutations lead to overproduction of the metabolite diacetyl and that this allows riboflavin synthesis to occur in the absence of the Rib3 enzyme. To test this hypothesis, we are investigating the effect of various metabolites on the fitness of *rib3*Δ, *rib*3Δ *gpp1*Δ, and *rib3*Δ DAK1-overexpression strains. We observed that the presence of riboflavin or glycerol in the media improves the fitness of the strains. The effects of other compounds that might affect diacetyl production are currently being tested. We will also investigate genetic factors that can influence the fitness of the various *rib3*Δ mutant strains by combining RIB3, GPP1, and/or DAK1 mutant alleles with overexpression or deletion of other genes involved in glycerol degradation or diacetyl synthesis. Thus, we aim to find chemical and genetic factors that strongly improve the fitness of RIB3 mutant strains, to learn about the mechanisms by which genes in the glycerol pathway can suppress RIB3 deletion lethality.

D23

Unlocking the genetic potential of sterile industrial polyploid yeasts by return to growth

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Breeding and domestication have generated widely exploited crops, animals and microbes.
Domesticated *Saccharomyces cerevisiae* yeast strains are largely sterile, preventing genetic improvement strategies based on selective breeding. Here, we present a novel strain improvement approach based on the budding yeasts' property to promote genetic recombination when meiosis is interrupted and cells return-to-mitotic-growth (RTG). We applied the RTG framework to two unrelated industrial polyploid *S. cerevisiae* strains, which suffer from nearly complete gamete inviability. We demonstrated that both strains were RTG-competent by engineering several deletion mutants and genetic systems through CRISPR-Cas9, and we developed a visual screening for easy identification of recombined RTG clones based on natural colony phenotypes. Whole genome sequencing of the evolved clones revealed unprecedented levels of RTG-induced recombination in both polyploids. Then, we extensively phenotyped the RTG library for industrial traits, and identified improved RTG clones matching with industrial standards. Thus, we propose the RTG-framework as a complete workflow to rapidly improve industrial yeasts that can be easily brought to the market.

D24

Identification of mechanisms regulating *Candida albicans* stress-induced genome instability

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Candida albicans is a commensal harmless organism that colonizes the human population. However, this benign organism can become a pathogen causing infections that are fatal in ~50% of cases. During colonization and infection, *C. albicans* encounters many different host environments to which it must adapt rapidly. This lifestyle gives rise to a key question: "How does *C. albicans* respond rapidly to the continuous changes in the environment it encounters in the host?". In recent years, the importance of stress-induced genome instability has emerged as an adaptive mechanism of fungal pathogens. Although excessive genome instability is harmful, moderate genome instability facilitates rapid adaptation to environmental insults. Genomic instability can increase genetic diversity, thereby allowing selection of genotype(s) better adapted in a new environment. The drivers and regulatory mechanisms underlying *C. albicans* stress-induced genome instability are largely unknown. Our hypothesis is that replicative stress, controlled by specific DNA damage pathways, drives genome instability in stress environments. The goal of this project is to identify novel factors regulating DNA damage and stress-induced genome instability in *C. albicans*. We performed different parallel genetic screens using a homozygous *C. albicans* gene deletion library1. This approach has led to the identification of ULP2 C. albicans gene. This gene is known to be involved in SUMO deconjugation, however during this genetic screening it has shown to be important for sensing different types of DNA lesions, including UV irradiation, drugs exposure and temperature stress. Moreover, when we look at genome instability, ulp2Δ/Δ cells exhibit defect in chromosome segregation under normal conditions and good tolerance to high fluconazole exposure. In addition, this fluconazole exposure seems to lead to chromosome rearrangements. These results show that ULP2 gene might been involved in stress-induced genome instability and adaptation.
1.Noble, S. M., French, S., Kohn, L. A., Chen, V. & Alexander, D. HHS 42, 590–598, 2011

D25

Species-wide exploration of phenotypic expressivity in a yeast natural population

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Understanding the genetic basis of traits, the underlying genetic complexity and how it varies depending on the genetic background is of prime interest to gain better insight into the genetic architecture of traits. The dichotomy between monogenic and complex traits is overly simplistic as the genetic complexity of a trait can change depending on the genetic background of the individual. Such variations in complexity can often lead to phenotypic expressivity. Until now, no systematic and species-wide assessment of this phenotypic expressivity has been performed.

To assess the prevalence of phenotypic expressivity at a population scale, we constructed a half-diallel panel by pairwise crossing genetically diverse *Saccharomyces cerevisiae* natural isolates producing 190 unique hybrids. A haploid progeny of a total of 30,400 individuals (160 individuals for each hybrid) was generated and its growth fitness was measured in 50 conditions containing various chemical compounds or carbon sources. As the phenotypic distributions of the progeny allow to infer the inheritance patterns and complexity, we evaluated these patterns for a total of 9,450 cross/trait combinations. We found that 86% displayed a complex inheritance, 4.4% a monogenic inheritance and 9.6% an oligogenic inheritance. In addition, we identified 26 major effect loci for various traits. Interestingly, a specific case of phenotypic expressivity revealed a dual effect of the GAL3 gene on growth in galactose media. Some GAL3 alleles have a high effect and cause large phenotypic shifts, whereas other GAL3 alleles act as modifier genes and influence the phenotype only under certain given genetic backgrounds.

Altogether, these results lay the foundation for a more comprehensive exploration of genetic variants causing variable complexity and therefore phenotypic expressivity. The dissection of the genetic basis of the different cases observed gives us a better insight into how phenotypic expressivity can occur in natural populations.

D26

Chromosome-level genome assembly and annotation of the oleaginous yeasts with high biotechnological potential *Rhodotorula toruloides* and *R. babjevae*

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Rhodotorula toruloides and *R. babjevae* are oleaginous yeasts with high biotechnological potential to obtain lipids and carotenoids for the production of biofuels, food and feed additives. In order to better understand the molecular physiology of lipid synthesis and to advance metabolic engineering, high-resolution genomes are required. We present here a draft of *R. toruloides* CBS 14 genome and for the first time genome assemblies of strains of *R. babjevae*. The de novo assemblies were constructed using a hybrid approach consisting of short and long reads generated by Illumina and Nanopore sequencing, respectively. The *R. toruloides* CBS 14 genome consists of 23 contigs and 3 scaffolds with a length N50 of 1,529,952 bp, 20,534,857 bp total size and 61.83% GC content. The mitochondrial genome was recovered in one contig. Transcriptomic data from different growth conditions was used to aid species-specific gene annotation. In total we annotated 9,464 genes and identified 11,691 transcripts. Furthermore, we demonstrated the presence of a potential plasmid, an extrachromosomal circular structure of about 11 kb with a copy number about three times as high as the other chromosomes. *R. babjevae* CBS 7808 draft genome has a total size of 21,862,387 bp and 68.23% GC content. It consists of 24 contigs and 3 scaffolds with a length N50 of 1,067,634 bp. A final number of 7,591 protein-coding genes and 7,607 transcripts were annotated. *R. babjevae* DBVPG 8058 draft genome has a total size of 21,522,072 bp and 68.24% GC content. It was assembled in 33 contigs and one scaffold with a length N50 of 789,767 bp. 7,481 protein-coding genes and 7,516 transcripts were annotated. The high genetic divergence between *R. babjevae* strains suggests that they belong to different species. The three generated genome assemblies have high quality and are largely representing chromosomal organization.

D27

Emergence of the new opportunistic pathogenic yeast *Candida vulturna*

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Candida haemulonii complex in the *Saccharomycetale*s order, contains three species and varieties: *C. haemulonii, C. haemulonii var. vulnera* and *C. duobushaemulonii*. The species *C. pseudohaemulonii* and the recently described *C. vulturna* are closely related to this complex. All are close to the emerging species *C. auris*, and have been described as responsible for human invasive infection. However, since the description of the species, *C. vultarna* has rarely been reported as responsible for human infection.

Among the 98 clinical isolates received at the National Reference Center for invasive Infections & Antifungals since 2004 and identified as *Candida cf. haemulonii*, 8 were retrospectively identified as *C. vulturna*. They were recovered from blood (7/8) from 7 patients (median age of 39 years, range 0.06-77, male ratio 1.33) hospitalized in 4 hospitals. We compared sequence of ITS regions, MALDI-ToF profile and in vitro antifungal susceptibility of those isolates with the type strains of *C. haemulonii* complex and related species.

For the 8 isolates, using the Maldi-Tof (Bruker Biotyper), *C. pseudohaemulonii* was the closest identification. All ITS sequences had 100% similarity with those of CBS 14366, type strain of *C. vulturna*. They were all able to grow at the maximum temperature of 37°C, confirming their potential pathogenicity. Their antifungal susceptibility profiles were very similar to those of the *C. haemulonii* complex with high minimum inhibitory concentrations (MICs) of azoles and amphotericin B but low MICs of echinocandins and flucytosin.

To our knowledge, this is the first time since the description of the species that several isolates of *C. vulturna* are reported as responsible for invasive infections. Interestingly, these isolates were mainly recovered from tropical areas (6/8), and had specific MALDI-Tof profiles. More investigations are needed to understand whether this peculiar geographic distribution is correlated with a specific environmental niche or metabolic properties.

D28

Disruption and characterization of the *Candida glabrata* ERG6 gene encoding sterol methyltransferase

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The rise in the frequency of fungal infections and the increased resistance noted to the widely employed azole antifungals make the development of new antifungals imperative for human health. The sterol biosynthetic pathway has been exploited for the development of several antifungal agents (allylamines, morpholines, azoles), but additional potential sites for antifungal agent development are yet to be fully investigated. The sterol methyltransferase gene (ERG6) catalyzes a biosynthetic step not found in humans. The *Candida glabrata* ERG6 gene was disrupted by transforming with the TRP1 distruption cassette. The resulting Cgerg6Δ strain was shown to be susceptible to a number of metabolic inhibitors, including terbinafine, fluphenazine, hygromycin B, caspofungin, micafungin, aureobasidin A and myriocin. No increase in susceptibility to azoles and polyenes was noted. The CgERG6 deletion leads to plasma membrane hyperpolarization, increased susceptibility of mutant cells to alkali metal cations and changes in mutant cells susceptibility to ROS inducing agents. These results point to the fact that defects in sterol transmethylation appear to cause a multitude of physiological effects in *C. glabrata* cells.The availability of CgERG6 would allow for its use as a screen for new antifungals targeted specifically to the sterol methyltransferase.

D29

Stress effect of new antifungal agents on pathogenic *Candida* species

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Photodynamic therapy is a minimally invasive approach, in which a photosensitizer compound is activated by exposure to visible light, resulting in generation of reactive oxygen species that kill pathogens via an oxidative stress. In contrast to conventional drug therapy, photodynamic therapy arises as a promising strategy without the risk of inducing resistance, as the mechanism of action involves multiple targets. Singlet oxygen ($^1O_2$) is a highly reactive molecule that can oxidize lipids, proteins, and nucleic acids, thus causing death of yeast cells. One photosensitizer molecule can generate thousands of molecules of singlet oxygen before being destroyed. In our study, we used sulfonated polystyrene nanoparticles with an encapsulated tetraphenylporphyrin (TPP) photosensitizer. The encapsulated photosensitizer is well protected against external quenchers and aggregation by the shell of the polystyrene nanoparticles. We showed that antifungal photodynamic inactivation mediated by TPP can be potentiated by KI. The proposed mechanism for this potentiating effect of iodide is likely due to reaction of $^1O_2$ with iodide and formation of iodine radicals, hydrogen peroxide, and molecular iodine. We also evaluated the positive effect of higher physiological temperature (37°C) on the antifungal effect of nanoparticles in comparison with a room temperature. The antifungal effect of TPP nanoparticles was species-dependent, the nanoparticles exhibited stronger killing efficiency against *C. glabrata* than *C. albicans*.

The treatment of fungal infections with photodynamic therapy is suitable especially for recurrent superficial infections of skin and mucosa, since oxidative damage takes place in close vicinity to the photosensitizer molecule. The treatment might be an alternative to conventional antifungal agents.

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D30

Kti12 enables wobble uridine modifications by the Elongator complex through direct tRNA binding.

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In eukaryotes, Kti12 enables carboxymethylation (cm^5) of transfer ribonucleic acids (tRNA) carried out by the Elongator complex. The modification is located inside the anticodon of tRNA and supports correct translation via improved base pairing during decoding of messenger RNA (mRNA). Hence, the Elongator proved to be essential in higher eukaryotes and dysfunctionalities have been associated with distinct diseases such as amyotrophic lateral sclerosis (ALS). Surprisingly, activity of the tRNA modifier has been found to be dependent on the accessory protein Kti12 for yet unclarified reasons. Here, we identify several tRNA binding residues in the C-terminal domain of Kti12 from yeast. Cumulative alanine substitutions of tRNA binding amino acid residues in Kti12 lead to a progressive decline in Elongator activity and a simultaneous decrease in Elongator interaction. Our experiments further indicate a differentiation between Elongator bound and unbound Kti12, which seems to be modulated by tRNA. Taken together, our findings propose Kti12 as a mediator in tRNA flux towards and from the Elongator in order to support proper wobble uridine modifications within the cell.

D31

A novel yeast model for tyrosine accumulation and self-assembly

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The formation of amyloid assemblies has been associated with several major human maladies, mostly neurodegenerative disorders. For over a decade, the self-assembly process was attributed only to proteins and polypeptides. Yet, by utilizing a reductionist approach, using shorter amyloidogenic building block, it was demonstrated in our lab that metabolites, such as amino acids and nucleobases, can also from typical amyloidal assemblies. As a result of inborn mutations in single genes encoding for metabolic enzymes these metabolites can accumulate and might be associated with the pathology of several inborn error of metabolism (IEM) disorders. Most of the studies regarding metabolite amyloid-like structures were conducted so far only in vitro. Recently, a yeast model for adenine accumulation and self-assembly that recapitulates adenine IEM disorders was established. Furthermore, the intracellular assembly of adenine amyloidlike structures has been demonstrated using this yeast model. Herein, we established an additional model for tyrosine accumulation and self-assembly in yeast. By using strain partially blocked in the shikimate pathway, we observed non-linear dose-dependent growth inhibition upon tyrosine feeding which was shown to be associated with increased intracellular accumulation of tyrosine. Staining with amyloid-specific dye demonstrated the presence of tyrosine amyloid-like assemblies. Moreover, treatment with epigallocatechin gallate (EGCG) resulted in a significant reduction in the formation of the toxic tyrosine amyloid-like assemblies which further supports the notion that toxicity is triggered by tyrosine assemblies. This model can provide insights into the mechanism of metabolite self-assembly in tyrosinemia and a platform to discover disease-modifying therapeutic avenues.

D32

The absence of Las17, or nucleolar retention of this protein, both lead to nucleolus division defects or fragmentation

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Wiskott-Aldrich syndrome is a rare genetic disease caused by mutations in a WAS gene, which encodes WASP. This protein is produced exclusively in hematopoietic cells. Mutations in WAS gene are connected with symptoms affecting blood cells, such as immunodeficiency. In yeast cells, Las17 plays similar role as WASP in human cells, acting as an actin assembly factor, stimulating the nucleation of branched actin filaments. Cells lacking functional Las17 protein show defects in endocytosis, bud site selection, and cytokinesis.
In previous work, we showed that besides its cytoplasmic localization in the cortical actin patches, Las17 is localized in the nucleolus. We also showed that cells lacking Las17 display a high nucleolus fragmentation score. Here, we analyzed whether Las17 regulators (Vrp1, Sla1, Bbc1) influence its localization in the nucleolus as well. In the strains *vrp1*Δ and *sla1*Δ, we observed an increase in the number of cells showing nucleolar localization of Las17. Additionally, the more cells showed nucleolar localization of Las17, the higher was the nucleolus fragmentation. Cells lacking Vrp1, Sla1, or Bbc1 proteins had a greater number of nucleolus division defects compared to WT cells. We concluded that the absence of Las17, or nucleolar retention of this protein, both lead to dysfunction of this organelle. Using PFGE, we also showed that chromosome XII migrates aberrantly in *las17*Δ and *bbc1*Δ-derived samples. The Southern blot analysis, using radiolabeled RDN25-1 probe, confirmed that the aberrantly migrating chromosomal band contained rDNA, suggesting atypical structures acquired by chromosome XII, likely involving the rDNA array, which affects chromosome XII migration in the gel. Indeed, the chromosome comet assay showed atypical chromosome structures in *las17*Δ and *bbc1*Δ -derived samples. These data suggest involvement of Las17, likely via its actin nucleation function, in the rDNA region maintenance.

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D33

Towards molecular characterization of the newly identified GDT1 protein family

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Nowadays, hundreds of protein families remain uncharacterized. Last year, the Uncharacterized Protein Family 0016 (UPF0016) has been removed from this list and renamed the GDT1 family in reference to the *S. cerevisiae* member known as Gdt1p. The first member from this family has been identified in 2012 due to mutations in the gene encoding the transmembrane protein 165 (TMEM165) in patients suffering from a Congenital Disorder of Glycosylation (CDG). Over the past ten years, studies highlighted the role of this family in manganese, calcium and pH homeostasis. It was proposed to be a family of secondary cation transporters. Although the characterization increased over the past decade, no one has studied the structural characteristics and molecular mechanism of these transporters yet. For this purpose, we are currently optimizing production, solubilization and purification of both human and yeast members in order to perform cryo-EM analysis and in vitro transport assays using proteoliposomes. In an attempt to yield functional proteins after the critical solubilization step, we assessed two different approaches. The first consisted in the use of classical “head-and-tail” detergents and the second aimed to directly extract complexes containing both membrane proteins and native lipids from the membranes of the producing host, thus forming native nanodiscs. Today, heterologous overexpression of TMEM165 in different systems is ongoing and so far insect cells showed the best yield. Solubilization and purification trials of this protein are still in the early stages but already gave promising results. Regarding the yeast member, several trials revealed that Gdt1p was efficiently overexpressed in L. lactis bacteria and solubilized by both aforementioned techniques. Detergent-solubilized proteins are well incorporated in liposomes, therefore allowing further in vitro transport experiments. These transport assays are now in development. These early successes may lead to better insights into the GDT1 family.

D34

A yeast-based repurposing approach revealed modulation of dNTP pool as a therapeutic target to treat Mitochondrial DNA Depletion Syndromes

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Mitochondrial DNA Depletion Syndromes (MDS) are a group of clinically heterogenous and often severe diseases characterized by a reduction of the mitochondrial DNA (mtDNA) copy number in affected tissues. There are still no satisfactory therapies and, since mitochondrial diseases, taken individually, are rare, therapeutic strategies with potential general applicability to several mitochondrial diseases would be desirable. Yeast has proved to be an excellent model for the study of the mechanisms underlying mitochondrial pathologies and also for the discovery of new therapies thanks to the development of a high–throughput yeast-based assay. We identified ten drugs active against MPV17 disorder modelled in yeast, whose homologous gene is SYM1. MPV17/SYM1 encodes a non-selective channel in the inner mitochondrial membrane whose physiological role and nature of the cargo remains elusive. Recessive mutations in this gene cause a hepatocerebral form of MDS and Navajo neurohepatopathy. All the ten molecules identified determine a concomitant increase of both mitochondrial dNTP pool and mtDNA stability strongly suggesting that the reduced availability of DNA synthesis precursors is the cause of the mtDNA deletion/depletion in Sym1 deficiency. We also assessed the effect of these molecules on mtDNA stability of two additional MDS yeast models characterized by mutations in MIP1 and RNR2, orthologs of the human genes POLG and RRM2B, respectively, extending the potential use of these drugs to other MDS patients. As a drug repurposing approach of FDA-approved drugs was used, this could speed up a possible clinical use of the drugs.

D35

Acetic acid as a trigger to activate human Bax heterologously expressed in yeast

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Proteins of the Bcl-2 family, including pro-apoptotic Bax and anti-apoptotic Bcl-xL, are critical for mitochondrial-mediated apoptosis regulation. Since yeast lacks obvious orthologs of Bcl-2 family members, their heterologous expression has been extensively used to investigate their molecular and functional aspects. The main advantage is to avoid misinterpretations that may arise from the interference of redundant mammalian Bcl-2 members. Active Bax is involved in the formation of mitochondrial outer membrane pores, through which apoptogenic factors such as cytochrome c are released, triggering a cascade of downstream apoptotic events. However, when in its inactive form, Bax is generally largely cytosolic or weakly bound to mitochondria. Given the central role of Bax in apoptosis, studies aiming to understand its regulation are of paramount importance towards its exploitation as a therapeutic target. So far, studies taking advantage of heterologous expression of human Bax in yeast have relied on the use of artificial mutated or tagged Bax for its activation, rather than the wild-type protein. Here, we propose acetic acid as a trigger to activate wild-type Bax that more closely resembles the natural Bax activation in the cellular context. Indeed, we found that concentrations of acetic acid that are not lethal to wild-type yeast cells trigger death in cells expressing Bax, which was associated with Bax mitochondrial translocation and cytochrome c release. This active Bax-mediated cell death was reverted either by co-expression with Bcl-xL, but not with its truncated form, and in the absence of Rim11p, the yeast ortholog of mammalian GSK3β. This novel system can therefore function as a platform to uncover novel Bax regulators and explore its therapeutic modulation.

D36

Full humanization of the glycolytic pathway in *Saccharomyces cerevisiae*

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The yeast *Saccharomyces cerevisiae* is a popular model organism to study human cellular processes. Humanization of genes in yeast is a widely used strategy to improve yeast as metazoan model, allowing exploration of gene functionality and drug testing. Hindered by the high genetic redundancy of eukaryotic genomes and limitations of molecular tools, to date humanization studies have mostly focused on single gene complementation. Recent synthetic biology advances can overcome these challenges and allow humanization of full pathways or processes. As proof of principle, we demonstrate the full humanization of the yeast glycolytic pathway. Combining single gene complementation, full pathway humanization and laboratory evolution, the functionality of 25 human enzymes in yeast was explored. All enzymes besides hexokinases 1 to 3 directly complemented the catalytic function of their yeast ortholog. Full pathway transplantation of the muscle glycolytic pathway was possible, leading to humanized yeast strains with altered physiology. Interestingly, the hexokinases HsHK1 and HsHK2 required mutations alleviating inhibition by glucose-6-phosphate to complement yeast growth on glucose. Besides catalytic functions, human aldolase and enolase enzymes also had conserved moonlighting functions. Laboratory evolution suggested a remarkable variety of cellular mechanisms deployed to optimize the growth of strains with fully humanized glycolysis. Comparison with skeletal muscle cells showed that, for most tested human enzymes, transplantation in yeast did not affect their turnover number (kcat). This demonstration of transplantation of an entire, essential pathway paves the way to the establishment of species, tissue and disease-specific metazoan models.

D37

Proton irradiation of probiotic yeast strains

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Lyophilized preparations of the yeast *Saccharomyces boulardii* have been used for the treatment of antibiotic-induced gastrointestinal disorders and acute enteritis. Also, probiotics have the potential to improve stress conditions that manifest during space travel, such as gastrointestinal disorders, dermatitis, and respiratory infections. The aim of this research is to select probiotic strains with additional radioprotective properties.
We examined two isolates from probiotic preparations ("Enterol", Biocodex, France; "Cosm-o-tentic", Putramos, Belgium) and compared these strains with well-studied laboratory strains of *Saccharomyces cerevisiae*. Biochemical and genetic characteristics were evaluated. PCR-RT analysis showed that the probiotic strains differed in nucleotide sequence among themselves and from *Saccharomyces cerevisiae*. Proton irradiation of yeast cells had more pronounced lethal effect on the laboratory strains. Moreover, the probiotic strains were more genetically stable than the laboratory strains. They had a low frequency of spontaneous and induced mutations in nuclear and mitochondrial DNA. These data allow us to suggest that probiotic preparations may have radioprotective properties. Further, we are going to study antioxidant characteristics of probiotic yeast strains and test probiotic and radioprotective properties in experiments with mice.

D38

Conserved proline 246 is an important residue determining substrate specificity and transport activity of Homo sapiens Na+/H+ antiporter NHA2

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Human Na+/H+ antiporter NHA2 (SLC9B2) transports Na+ or Li+ across the plasma membrane in exchange for protons. In mammals, it is predominantly expressed in the kidney (distal tubule) and has been found to be implicated e.g., in essential hypertension or in insulin secretion. Thus, HsNHA2 seems to be an important clinical marker. Nevertheless, information about its structure and mechanism of transport remains limited. Topology prediction algorithms suggest that this 537 amino acids long protein may consists of 14 transmembrane helices. In this work, we functionally expressed HsNHA2 in a salt sensitive *Saccharomyces cerevisiae* strain and conducted rationally designed mutagenesis analysis to: (i) identify new structural and functional elements involved in ion selectivity, and (ii) validate the current 3D model of HsNHA2. Mutated HsNHA2 versions were characterized in terms of their localization, transport activity and substrate specificity in yeast cells.

Our data show that a highly conserved P246, localized in the core of the protein, plays a crucial role in ion selectivity. The substitution of this residue with polar amino acids (serine, threonine) altered the substrate specificity of the antiporter and changed its pH profile. The mutants became highly active not only at acidic pH 4.0 (as the native antiporter), but also at pH 7.0. Our experiments also suggest that the two titratable residues (E215 and R432) may be spatially close to each other and engaged in a salt bridge, predicted in silico by the model structure, since mutual exchange of both residues resulted in an active antiporter that transports Li+, while the respective single mutants, i.e., E215R and R432E, were inactive. Overall, our data bring new knowledge into the structure of human Na+/H+ antiporter NHA2 and confirm *S. cerevisiae* as an ideal organism for the structural analysis of mammalian membrane transporters.

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D39

Yeast-based screening to discover therapies for Complex III assembly defects

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Mitochondrial dysfunctions, including mitochondrial complex III deficiency due to assembly defects, are responsible for a number of heterogeneous clinical manifestations. Mutations in the assembly factor LYRM7 were identified, and yeast, thanks to the presence of the orthologue gene MZM1, was used to support the pathogenicity of the mutations and to study their molecular consequences. Since there are no effective therapies to treat these patients, we have exploited a yeast MZM1 model available in our laboratory, carrying mutation D25N, to perform a high-throughput screening on a drug library composed of about 1000 FDA approved molecules, and we have identified ten beneficial compounds. To establish whether the drugs can be potentially beneficial for patients affected by different mutations in LYRM7, we have tested the identified molecules on other mzm1 mutant strains, including the null mutant, characterized by a more severe phenotype. The obtained data indicates that the majority of the drugs are active even on the mzm1Δ mutant suggesting their applicability to all patients, regardless of the specific mutation. In order to understand if a specific molecule could be potentially used to treat a wider range of patients, we have tested the ten drugs on another model of mitochondrial complex III assembly defect, characterized by mutations in the BCS1 gene, coding for a fundamental assembly factor. In particular, we have used two mutants with phenotype ranging from leaky to severe. More than half of the drugs proved to be effective also on these models, suggesting a potential use to treat both patients with mutations in LYRM1 and BCS1L. In conclusion, considering the fact that an FDA-approved drug library and a drug repurposing approach were used to identify the potentially therapeutic drugs, this could allow direct use in patients, speeding up a possible clinical use of the drug.

D40

Exogenous iron causes precipitation that precedes iron mediated cell death in yeast

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The essential micronutrient iron is associated with different forms of human pathophysiologies; anemia because of inadequate iron and toxicity because of excess iron. Its ability to gain and lose electrons as it interconverts between ferrous and ferric forms makes it indispensable as an electron carrier in many cellular processes. Additionally, it makes iron toxic to cells given that free electrons react with cellular constituents to produce highly toxic Reactive Oxygen Species (ROS). The prevailing model of iron toxicity is that excess iron enters the cell and induces cell death by increasing ROS. We use *Saccharomyces cerevisiae* as a simplified model to study cellular responses to excess iron. Our previous results show that excess iron does not enter cells and is associated with the formation of a precipitate. Here we are testing the hypothesis that exogenous iron causes cell death by precipitating with an essential component of yeast growth media. Using spot assays, we show that iron is a dose-dependent inhibitor of yeast growth. No growth inhibition was observed at concentrations below 5mM while complete growth inhibition occurred at 8mM $FeCl_3$. The iron mediated precipitation in growth media was also found to increase in a dose-dependent manner. Pronounced precipitation was observed at 0.4mM while maximum precipitation required 20mM $FeCl_3$. These observations demonstrate that iron mediated precipitation in growth media precedes the negative effects of iron on yeast cell growth, suggesting that precipitation serves to mediate the toxic effects of excess iron. Analysis of the components of yeast growth media suggests that phosphate is responsible for the observed precipitate. Phosphate is present at sufficiently high concentrations to account for the precipitate and is highly insoluble with iron. Our results showing that iron mediated precipitation precedes iron mediated growth inhibition supports our hypothesis that iron toxicity is due to the depletion of phosphate.

D41

Fmp40 - the only known ampylase in *S. cerevisiae* - role in redox homeostasis

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AMPylation (adenylation) is one of the post-translational protein modifications (PTM) leading to diversification of protein functions and activity, in which the AMP is attached through its α phosphate to the serine, threonine or tyrosine residues via phosphodiester linkage. Recently with our collaborators we discovered that the yeast Fmp40 protein of unknown function, the homologue of human SelO, has the ampylase activity, conserved in SelO proteins of human, yeast and bacteria. We have shown that yeast SelO protein is involved in response to oxidative stress: cells lacking Fmp40 ampylase died faster than the wild type cells upon H_2O_2 and menadione treatment. *E. coli* SelO ampylates glutaredoxin Grx, and the glutathionylation level of proteins is reduced in bacterial and yeast cells lacking SelO.
The decreased survival of *fmp40*Δ cells upon H_2O_2 stress prompted us to verify if increased cell death of *fmp40*Δ cells is dependent on Ca2+-dependent cysteine protease Yca1 or oxidation of redoxin Trx3, shown to favor PCD in yeast when accumulated in the oxidized state. We have found that *fmp40*Δ survival is restored when Yca1 or Trx3 (and other redoxins Grx2 –the yeast homologue of bacterial Grx– or Prx1) are missing. The acetic acid - another routinely used inducer of PCD in yeast, also led to decreased survival of *fmp40*Δ cells. Therefore, we hypothesize that the Fmp40 ampylase regulates the ROS signaling through redoxins, in consequence of its deficiency, cells undergo to death. We have found that mitochondrial redoxins are the substrate of Fmp40 in vitro. The in vivo experiments are ongoing.

D42

Iron depletion induces bulk autophagy and life extension in *Saccharomyces cerevisiae*

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We have demonstrated that in exponential cultures of *Saccharomyces cerevisiae* iron limitation provokes one primary response inducing bulk autophagy mediated by TORC1. Accordingly, Atg13 became dephosphorylated meanwhile Atg1 appeared phosphorylated. Iron starvation promotes accumulation of trehalose and the increase in stress resistance leading to a quiescent state in cells. We have proved that the signal of iron deprivation requires Tor2/Ypk1 activity and TORC1 inactivation leading to Atg13 dephosphorylation, consequently triggering the autophagy process. Iron replenishment, reduces autophagyc flux by the AMPK Snf1 and the activity of the iron-responsive transcription factor, Aft1. All these effects confer chronological life span extension, in a manner totally dependent on autophagy activation.

D43

Telomerase-independent telomere maintenance by Alternative Lengthening of Telomeres is mediated by RAD52 in *Naumovozyma castellii*

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Telomeres are functional nucleoprotein structures located at the ends of linear eukaryotic chromosomes. While essential, due to limitations of the canonical DNA replication machinery, the telomeric DNA structure shortens progressively after every replication cycle. Most eukaryotes rely on the activity of the enzyme telomerase to prevent the progressive shortening of telomeres. In budding yeast, telomerase activity requires two main structural components: The catalytic subunit (Est2p) that acts as a reverse transcriptase, and a non-coding telomeric RNA (TLC1) which functions as the template for DNA synthesis. Only few *Saccharomyces cerevisiae* cells lacking telomerase activity manage to survive the lethal consequences of telomere attrition. Survivors activate alternative lengthening of telomeres (ALT) mechanisms that rely on recombination between either subtelomeric elements or telomeric sequences.

The budding yeast *Naumovozyma castellii* has a processive telomerase that incorporates its characteristic octamer telomeric repeat onto chromosomal ends. In the absence of telomerase, *N. castellii* telomeres are maintained through a novel ALT mechanism which is effectively activated as the telomeres of the yeast shorten, allowing for sustained long-term growth of the cells. Here, we demonstrate that *N. castellii* ALT mechanism is dependent on homologous DNA recombination mediated by RAD52, the main recombination gene in yeast. In this work, we identified and deleted the RAD52 gene in *N. castellii* to develop diploid mutant strains heterozygous for the TLC1 and RAD52 genes. Through the sporulation of these diploid strains and microdissection of tetrads we discovered that telomerase negative cells lacking RAD52 are unable survive beyond ~72 generations. Analysis of the telomere structure of these mutant strains showed that the cells are incapable of maintaining telomeres by ALT. Thus, our results show that establishment of the N. castellii ALT mechanism requires RAD52 gene function to allow for the sustained growth of cells in the absence of telomerase.

D44

Adaptation to life with human chromatin in budding yeast

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Faithful segregation of chromosomes is critical during cellular division, ensuring the equal transmission of genetic material. Histones play a central role in chromosome segregation with Cse4 (CENP-A), a specialized histone H3 variant, defining the centromere region and serving as the anchoring point of kinetochore-microtubule attachments to the chromosomes. Through an unbiased genetic screen to identify suppressors of human histones in budding yeasts, we isolated various mutants of the DASH/Dam1c, a component of the outer kinetochore in fungi. These mutant proteins strongly suppressed human histones, displayed limited phenotypic effect in WT yeasts, and phenotypically suppressed various known kinetochore mutants. Further, analysis of ploidy in humanized yeast showed that these mutants stabilized ploidy across the population, leading to euploidization by selection despite persistently unattached centromeres. Lastly, using molecular modeling we predict these mutants may perturb the oligomerized state of the Dam1c through an evolutionary conserved interaction surface. Our data provide insight into how kinetochore attachments are modulated independently of the centromere.

D45

Exploring the roles of genes that provide resistance to cold temperatures in the *Saccharomyces* genus.

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The molecular mechanisms that affect the adaption of microbial species at different temperatures are important to understand how biodiversity originates and can be maintained in a constantly changing environment.

In this study, we investigated the role of a group of genes identified by genome screening studies (1) that provide resistance to cold temperatures. We deleted both copies of five non-essential genes, ADH3, GUT2, NMA1, YND1 and FAA1, in eight *Saccharomyces* species. Upon deletion of these genes, *S. kudriavzevii* and *S. arboricola* were more compromised in term of fitness when compared with the other species. We also determine the expression of these genes in their native species and observed a significantly higher expression at cold for *S. paradoxus*, *S. kudriavzevii* and *S. jurei* compared to *S. cerevisiae*.

We created different combinations of double mutants and scored the fitness in the different species. The double mutant data showed a greater defect at cold for ΔADH3/ΔADH3 ΔYND1/ΔYND1 strains in *S. paradoxus*, *S. uvarum* and *S. kudriavzevii*. Interestingly, the majority of the interactions showed positive and negative epistasis in *S. cerevisiae* and in *S. kudriavzevii*, respectively.

To tease apart the role of promoters (i.e., gene expression) from the allele type (i.e., genetic) in cold-adaptation, we replaced independently either the ADH3 and YND1 promoters or their alleles in *S. cerevisiae*, *S. paradoxus, S. jurei* and *S. eubayanus* with the promoters and alelles belonging to *S. kudriavzevii*. A fitness improvement was detected at cold in the strains carrying *S. kudriavzevii* promoter for both ADH3 and YND1, while yeast fitness was lower upon alleles swapping, suggesting a crucial role of the strength of expression over the allele type for growth at cold temperature.

Ref:

1. Paget et al., (2014) Environmental systems biology of cold-tolerant phenotype Saccharomyces species adapted to grow at different temperatures. Molecular Ecology.

D46

Genome plasticity through circularization and re-integration of endogenous DNA biosensors in *Saccharomyces cerevisiae*

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In eukaryotes, circular DNA of chromosomic origin is responsible for rapid gene amplifications in mitotically dividing cells. Studies of cancer cell lines have shown that circular DNA can reintegrate into chromosomes and thereby fix gene amplifications. However, little is known about how frequently circular DNA integrates in other cell types and what impact they might have on the evolution of eukaryotic genomes. Here, we use *Saccharomyces cerevisiae* to create endogenous circular DNAs of molecular biosensors via the Cre-LoxP recombination system. This system allows us to follow their replication and segregation during population cell growth and measure their re-integration rate back into the linear genome. We show that 1) cells relying on circular DNA under selective pressure grow slower due to asymmetric segregation of the circular DNA and 2) we find that re-integration frequencies are higher than mutation rates for other structural changes, such as translocations. We also follow circular DNA through meiosis and find that it is 3) inherited from diploid cells to spores in a non-Mendelian manner at high frequencies, which is contrary to what is found for ribosomal circular DNAs (ERCs). Overall, we show that circular DNA can have lasting effects across generations by re-integration in the genome and inherence through meiosis.

D47

Evidences of horizontal gene transfer and coevolution with lactic acid bacteria in genomes of *S.cerevisiae* strains isolated from Kefir.

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This study addresses the genomic structure of 10 *Saccharomyces cerevisiae* and 2 *Kluyveromyces marxianus* isolates from Kefir from the Yaghnob valley. Strain DNA was sequenced using Illumina chemistry on a Hi-Seq 2000, the genome assembly was then confirmed using a Sequel 2.0 from pac Bio. The K. marxianus strains showed two significantly different genotypes, one of which extremely different from those sequenced so far. The *S.cerevisiae* strains differed significantly in several phenotypic characteristics, such as tolerance toward high temperatures, low pH, and acidity. Comparing these strains to 350 previously described strains, showed increased similarity of the Kefir *S. cerevisiae* to two different ancestry origins, both distinct from the wine and beer strains, and similar to strains isolated from human and insects' feces, suggesting a peculiar origin of these strains. Additionally, the Kefir strains were able to utilize lactose as sole carbon source. The sequence confirmed the presence of lactose permeases and genes involved in galactose metabolic pathways, as potential result of horizontal gene transfer from other yeasts and lactobacilli. Additionally, the strains showed increase in copy number of several genes involved in BCA metabolism. We then studied the ability of a *S.cerevisiae* strain from Yaghnob, CL4, to improve the growth of *Lactobacillus delbrukei*, demonstrating the potential of this strain to improve the growth of lactobacilli.

D48

Is apoptosis the cost for domesticating mitochondria?

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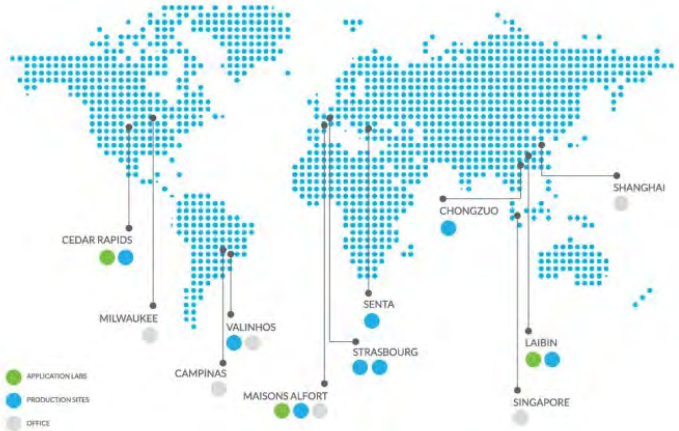
Apoptosis, a common type of programmed cell death, is a mechanism that allows cells to self-destruct when stimulated by the appropriate trigger. In addition to higher eukaryotes, it's also reported in unicellular eukaryotes as well as prokaryotes. We aim to experimentally verify that the apoptotic machinery is a primeval adaptation acquired during mitochondrial domestication. *Saccharomyces* Genome Database analysis revealed the role of 31 known proteins, deletion of which decreased apoptotic activity and 13 proteins whose overexpression induced apoptosis. Four apoptotic proteins overlapping these two sets i.e. protease metacaspase Mca1, nuclease Nuc1, and apoptosis-inducing factors Ndi1 and Aif1 were selected for the study. Core HTRA/Omi protease (Nma111) was also included based on literature studies. We plan to test the retention of ancient functions of apoptotic gene homologs across the various kingdoms i.e. bacteria, protists, plants, and animals etc. in yeast by their ability to replace their yeast orthologs. The strategy aims at making a simultaneous deletion and gene replacement of these yeast apoptotic genes in wild-type yeast diploid strain with homologous ORFs from other organisms. A common vector containing an external terminator i.e. CPS1, as well as KanMX4 cassette as well as 3' homology region of the each selected native yeast gene terminator was constructed. The codon-optimized orthologs were commercially synthesized and were cloned in front of CPS1 terminator. The yeast transformants were created and further are being tested to check functional complementation. Furthermore, GFP tagged version of these homologs are under construction and will be used to verify the expression and localisation of foreign genes in the yeast. If the homologs of the genes forming the core machinery of apoptosis can rescue their respective functions, this further strengthens the proof of apoptotic pathway conservation in the complex course of evolution as well as its pre-genesis before eukaryotic apoptosis.

LET'S GROW TOGETHER

Procelys by Lesaffre



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