YEAST BIOTECH 2.0

NEW YEAST, KNOWLEDGE & APPLICATIONS

INTERNATIONAL SPECIALISED SYMPOSIUM ON YEASTS

ISSY 2023

27 NOVEMBER - 01 DECEMBER

NATIONAL WINE CENTRE
ADELAIDE, AUSTRALIA

PROGRAM & ABSTRACTS BOOK

THE UNIVERSITY of ADELAIDE

CONGRESS MANAGEMENT: EASTERN SUN EVENTS
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WELCOME

On behalf of the International Commission for Yeasts and the Conference Organising Committees, we are excited to invite your organisation to partner with us in the 37th International Specialised Symposium on Yeasts (ISSY37).

Co-chaired by Professors Vladimir Jiranek (University of Adelaide) and Sakkie Pretorius (Macquarie University), the theme for ISSY37 is **Yeast Biotech 2.0: New Yeast, Knowledge and Applications**. The meeting will bring together leading researchers, biotech executives, suppliers, government and early career researchers, whose activities span the spectrum of fundamental to applied investigations of yeast.

Planned themes will likely include:

- Biodiversity and new yeast
- Yeast-derived food and beverages
- Yeast cell factories
- Yeast in plant-based foods and meat/dairy alternatives
- Synthetic genomes, organelles and communities
- Designer yeast
- Bioenergy
- Biomedicine
- Biosafety, amongst others.

Australia and New Zealand have vibrant yeast research communities, brought to together by the Australasian Yeast Group, and one which is well networked with researchers and businesses across the globe.

ISSY37 provides an opportunity for the world's yeast communities to get together in the iconic travel destination that is Australia. Adelaide, capital of South Australia, is at the heart of the majority of Australia's wine production and supporting research activities.

To punctuate that fact, ISSY37 will be held at the National Wine Centre in the downtown area. Adelaide is a vibrant, safe, attractive and affordable destination in close proximity to pristine beaches, world-renowned wine regions, natural beauty and unique flora and fauna. Adelaide's central location places is less than 4 hrs flight from other major attractions in Australia.

We look forward to working with you and welcoming the world to ISSY37 in Adelaide.

Vladimir Jiranek **Sakkie Pretorius**
Conference Co-convenors

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NEW YEAST, KNOWLEDGE & APPLICATIONS
INTERNATIONAL SPECIALISED SYMPOSIUM ON YEASTS

ISSY 2023

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SYMPOSIUM VENUE

National Wine Centre of Australia

THE UNIVERSITY of ADELAIDE

The National Wine Centre of Australia, located in Adelaide is a world-renowned attraction that offers a unique and memorable experience for wine enthusiasts, industry professionals, and tourists alike. Offering an extensive selection of food and wine experiences that cater to all levels of wine enthusiasts. With its state-of-the-art Conference Centre in Adelaide, it's the perfect venue for hosting corporate events, conferences, and social gatherings.

THANK YOU TO OUR SPONSORS

SYMPOSIUM PARTNER

The University of Adelaide—a member of Australia's prestigious Group of Eight research-intensive universities—stands tall among the world's leading institutions of learning and innovation. The vast majority of our research is rated 'above or well above world standard' by the Australian Research Council's Excellence in Research Australia program. Our distinguished alumni include five Nobel Laureates; and Australia's first female prime minister and Supreme Court judge. Many of our staff and teachers are internationally recognised leaders in their fields. Our extensive international network of students, academics, and commercial collaborators provides a stimulating and unique environment for research and student training.

GOLD SPONSOR

Lallemand is a privately held Canadian company that specialises in the development, production, and marketing of yeasts, bacteria, and their derivatives. Lallemand provides microbiological solutions for different industries, from human and animal health and nutrition to baking, oenology, brewing, food ingredients, probiotics, and biofuels. Our bio-inspired approach has led to some of the most exciting innovations in fermentation.

Lallemand was one of the first companies to understand the importance of collaborating with universities and research institutes to be at the forefront of worldwide technological advances. Moreover, our Research & Development team has contributed to enriching our knowledge and expertise in microbiology. Our in-depth production processes, exhaustive characterisation studies, full process of validation of new solutions, and rigorous quality policy allow us to offer a reliable and complete range of natural and visionary solutions to fit the unique needs of our customers, with the support, knowledge, and expertise that comes with over a hundred years of continuous research in this field.

www.lallemand.com

SILVER SPONSOR

A key global player in fermentation for more than a century, Lesaffre, with a 2,7 billion euro turnover, and established on all continents, counts 11,000 employees and more than 90 nationalities. We work with customers, partners and researchers to find answers to the needs of food, nutrition, and health while respecting our environment. Thus, every day, we explore the infinite potential of microorganisms. To nourish 9 billion people in 2050 by making the most of our planet's resources is a major and unprecedented task, we believe that fermentation is one of the most promising answers to this challenge.

SYMPOSIUM BEER SPONSOR

Coopers Brewery is Australia's largest family-owned brewery. Established in 1862 by Thomas Cooper, the company is now in the hands of the fifth and sixth-generation descendants. Coopers moved to its present state-of-the-art brewery at Regency Park, South Australia, in 2001. It now accounts for approximately 5% of the national beer market and is renowned for its unique premium quality ales, stout and lagers. Coopers' portfolio of international partners includes Carlsberg, Kronenbourg, Sapporo, Molson Coors and Thatchers Cider. Coopers is also the world's largest producer and exporter of DIY brewing concentrates, and the Australia's leading supplier of malt extract to the food industry.

SYMPOSIUM DINNER SPONSOR

MicroBioGen is an Australian based biotech company with global success in improving the industrial capabilities of *Saccharomyces cerevisiae*. This yeast underpins ~US$2 trillion in products worldwide, from biofuels and pharmaceuticals to food and feed. With a proprietary platform technology and extensive library of elite yeast genetics developed over 20 years, MicroBioGen delivers 'yeast innovation as a service' to industry leaders globally. Demonstrated through MicroBioGen's partnership with Novozymes on the Innova ethanol yeasts, MicroBioGen is the collaborator of choice for yeast biotechnology, with opportunities in existing and emerging industries, ranging from optimizing yeast for biofuels and baking to sustainable feed.

NETWORKING WELCOME RECEPTION SPONSOR

Main Sequence, co-founded by Australia's science agency CSIRO, invests in visionary inventors tackling global challenges with science-driven innovations. Precision fermentation is a critical part of the firm's platform and is attending ISSY with two portfolio companies - Eden Brew and Nourish Ingredients. Eden Brew is developing the world-first casein micelle for animal free dairy. Nourish Ingredients are producing lipids that deliver the experience of animal-based meat and dairy to plant-based products.

SYMPOSIUM WINE SPONSORS

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LANYARD SPONSOR

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Yeast and fermentation solutions - Fermentis is an expert in the art of fermentation.

Our active dry yeasts and yeast derivatives cover almost all professional requirements: from safeguarding production to expressing sensory characteristics in beer, wine, spirits and more. Take a look at the various types of products in our range. Fermentis works with everyone in the world of beer, wine, spirits and other fermented beverages. Our Research & Development team is always looking for ways to improve or develop new products and services for the beverage industry. Our products are available wherever you are. We have chosen the right partners on a global scale. To be as close as possible to our customers, we have opened offices right where our new markets are, joining forces with trusted and reliable partners. Today, you will find Fermentis experts and distributors in the United States, China, Australia, Mexico, South Africa, as well as anywhere in the heart of Europe.

EXHIBITORS

fermentation an Open Access Journal by MDPI

METABOLOMICS AUSTRALIA

Pathtech First Choice In Science

ROWE SCIENTIFIC

Fermentation is an international, scientific, peer-reviewed, and open access journal published monthly online by MDPI. The scope of Fermentation covers almost all research areas of fermentation, from the development of new and emerging products through to fermentation technology, fermentation processes, and designs of various instruments for fermentation.

The scope includes the following:

- Fermentation processes and product development;
- Strain improvement;
- Bioprocess and metabolic engineering;
- Fermentation of food and beverages;
- Scale up of fermentation processes;
- Downstream processing of fermentation products;
- Microbial physiology and metabolism;
- Applied genetics and molecular biotechnology;
- Bioreactor design, monitoring, biosensors, and instrumentation;
- Biosafety and biosecurity;
- Biopharmaceuticals and biotech drugs.

Metabolomics SA is a specialised R&D facility that houses cutting-edge technology and expertise to support both academia and industry. It provides analysis of small molecules including trace metabolites, secondary metabolites, volatiles, biomarkers, and chemical quality markers, using advanced mass spectrometry and NMR techniques.

The team provides a diverse range of specialist capabilities including:

- expert consultation
- experimental design
- building bespoke analytical techniques and methodologies
- targeted analysis of known compounds
- non-targeted profiling of complex samples
- data analysis using univariate and multivariate statistic and visualisation tools
- training on the use of analytical equipment
- contributions to research collaborations.

Metabolomics SA can help solve fundamental biological research questions or support the commercialisation and performance testing of new products for the food, agriculture, beverage, environmental and livestock sectors.

Metabolomics SA is a NCRIS-enabled facility supported by Bioplatforms Australia (BPA), the Australian Wine Research Institute (AWRI) and the South Australian Government.

For over 37 years **Pathtech** have operated as an innovative, ethical and successful distributor within the Australian Scientific, Forensic and Business communities. We are partners with leading manufacturers around the world and our range includes over 4,000 high-quality products. This globally comprehensive product list combined with exceptional customer service, provides cutting-edge solutions tailored to help meet our customers' needs.

With a sales team in all major Australian markets, we are committed to ensuring our clients get the most from our products. Our expert staff work with you to gain a complete understanding of your business needs. Where relevant, we can provide product training and ongoing guidance to ensure your investment performs to its full capacity.

SUPPORTERS

FEMS AMBASSADOR PROGRAM

Federation of European Microbiological Societies

STUDENT ATTENDANCE SUPPORT

SBMB

AYG

SYMPOSIUM PROGRAM

Monday 27th November

TIME	PROGRAM	LOCATION
15:30-17:30	**Registration and Exhibitor Bump in**	National Wine Centre
17:15-18:30	**Welcome Reception**	
	Welcome to Country	
	Own arrangements for dinner	

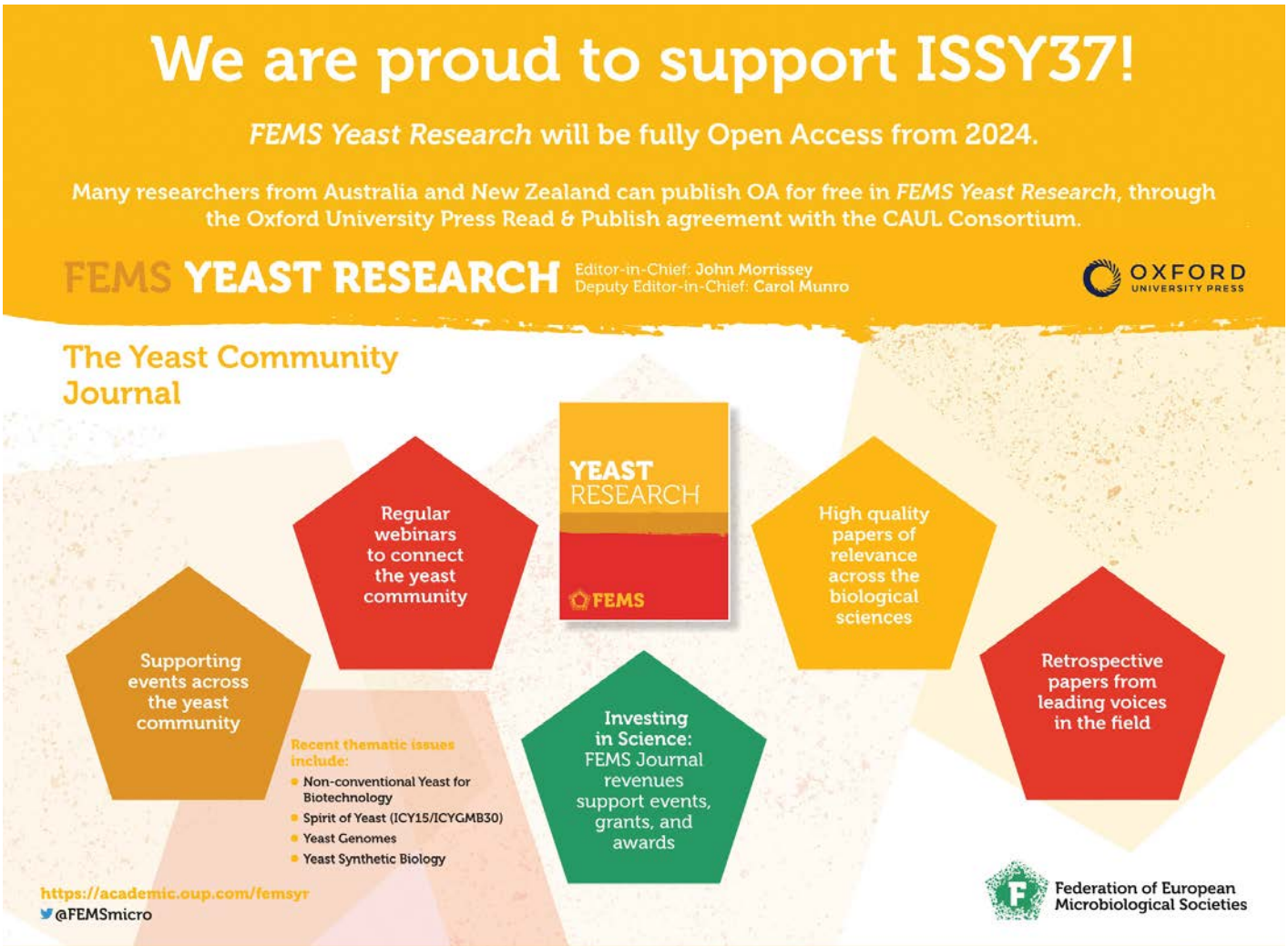
Tuesday 28th November

TIME	PROGRAM		DURATION (min)
07:30	Registrations Open		
08:30-08:45	**Official Opening**	**Conference Chair Welcome and Official Opening**	15
08:45-09:30	**Keynote Address**	**Brenda Andrews** – Using budding yeast to map the spectrum of possible morphological phenotypes in a model eukaryotic cell	45
09:30-10:35	**Session 1** Yeast in Medicine **Prof Dee Carter + Prof Johan Thevelein**	**Dee Carter** – Repurposing bisphosphonate drugs to kill yeast pathogen	18
		Johan Thevelein – Novel Warbicin® family of phosphorylation-dependent glucose-uptake inhibitors in yeast and human cells as potential anti-cancer drugs	18
		Uwe Himmelreich – Do the different molecular types of the *Cryptococcus neoformans/ Cryptococcus gattii* species complexes cause different clinical manifestations? A longitudinal *in vivo* assessment study of host-pathogen interactions and pathogen dissemination routes in murine cerebral cryptococcosis models	18
		Danfeng Long – Yeast extract exhibits great potential in mitigating alcohol-induced liver injury	8
10:35-11:00	MORNING TEA		25
11:00-12:30	**Session 2** Bioprospecting and Collections **Dr Kyria Boundy-Mills + Dr Krista Sumby**	**Kyria Boundy-Mills** – Myriad bioprospecting opportunities using the Phaff Yeast Culture Collection	18
		Benjamin Schulz – Capturing and characterising wild yeast for beer brewing	18
		Cristian Varela – A special drop: Characterising yeast isolates associated with fermented beverages produced by Australia's Indigenous peoples	18
		Vivien Measday – Whole genome sequencing of North American *Saccharomyces cerevisiae* strains isolated from spontaneous wine fermentations reveals a new Pacific West Coast Wine Clade	18
		Jiao Jiang – Indigenous wine yeast from China: biodiversity, oenological properties, and potential application in winemaking	8
		Tess Snyder – Digging deeper into Microbial Terroir: Biogeography of *Hanseniaspora* in Oregon's Willamette Valley Wine Region	8
12:30-13:30	LUNCH		60
13:30-15:05	**Session 3** New Knowledge via Yeast **Dr Jen Gardner + Prof John Morrissey**	**Sonja Billerbeck** – The yeast toxicome: A potentially rich source of antifungals for food, agriculture, health, and biotech	18
		Adrianna Skoneczna – Live while the DNA lasts. The autophagy-dependent DNA loss in diploid yeast during chronological aging	18
		Nilima Walunjkar – Widespread divergence in protein stability between two *Saccharomyces* species	8
		Marek Skoneczny – *Saccharomyces cerevisiae* Hsp31p as a tool in the studies of Unconventional Protein Secretion	8
		Víctor Garrigós – Cytosolic peroxiredoxin Tsa1 modulates acetic acid levels in wine yeasts under fermentative and respiratory conditions	8
		Julius Battjes – Ethanol-lactate transition of *Lachancea thermotolerans* is linked to nitrogen metabolism	8
		Zhenni Zhu – Anoxia-induced rearrangements of cellular dynamics	8
		Shulei Liu – Yeast secretome response to the endoplasmic reticulum stress with revealing of stress-induced Bip secretion	8
		Marina Jecmenica – Exploring the genetics governing quantitative phenotypes in the yeast *Komagataella phaffii*	8
15:05-15:30	AFTERNOON TEA		25

SYMPOSIUM PROGRAM

Tuesday 28th November continued

TIME	PROGRAM		DURATION (min)
15:30-17:05	**Session 4** Yeast Interactions and Microbiomes **Prof Florian Bauer + Dr Jenny Bellon**	**Florian F. Bauer** – Characterisation of molecular targets of the co-evolution of the microalga *Chlorella sorokiniana* and the yeast *Saccharomyces cerevisiae*	18
		Sarah Knight – Effects of fungicide applications on non-target yeast communities during wine production	15
		Pilar Morales – Extracellular vesicles shuttle information between different wine yeast strains	15
		Benjamin Binder – Modelling spatial growth pattern formation in yeast colonies	15
		I Nyoman Sumerta – The interplay of yeasts in a complex environment of palm wine fermentation	8
		Maria J. Valera – Friendly yeasts for winemaking: flavours involved in yeast interactions mediated by cooperation or competition events	8
		Nguyen (Kevin) Hu – Cross-feeding promotes heterogeneity within cell populations, and the strategies to harness it for bioengineering	8
		Anna Julien-Ortiz – Metabolic interactions of *Saccharomyces cerevisiae* cocultures: a way to extend the aroma and chemical diversity of Chardonnay wine	8
17:05-18:30	**Posters – Odd numbers**	Exhibition Hall	
18:30-21:30	**ICY Dinner** (Commissioners only – The Vines Room)	Own arrangements for dinner	



SYMPOSIUM PROGRAM

Wednesday 29th November

TIME	PROGRAM		DURATION (min)
07:30	Registrations Open		
08:30-09:15	**Keynote Address**	**Anton Glieder** – Next generation strains, vectors and methods for gene expression employing *Komagataella phaffi* (*P. pastoris*)	45
09:15-10:35	**Session 5** The Yeast Bioeconomy **Prof Andriy Sybirny + Assoc Prof Victoria Haritos**	**Andriy Sybirnyy** – Non-conventional yeasts as promising producers of biofuel and high-value chemicals	18
		Weerawat Runguphan – Metabolic engineering of *Pichia pastoris* for sustainable production of biofuels and chemicals	18
		Riaan Den Haan – Engineering natural isolates of *Saccharomyces cerevisiae* for applications in lignocellulosic biorefineries	8
		Manja Mølgaard Severinsen – Conversion of methanol to itaconic acid with engineered *Komagataella phaffii*	8
		Roksolana Vasylyshyn – Engineering of *Ogataea polymorpha* strains with ability for high-temperature alcohol fermentation of cellobiose	8
		Kelly Boyd – Improving *Saccharomyces cerevisiae* phenotypes for enhanced first-generation ethanol production	8
		Johan Quezada – Overexpression of dihydroxyacetone synthase 1 in Mut+ *Komagataella phaffii* (*Pichia pastoris*) improves the tolerance to methanol and the productivity of recombinant proteins	8
10:35-11:00	MORNING TEA		25
11:00-12:40	**Session 6** Yeast Cell Factories **Dr Paul Attfield + Prof Milan Čertík**	**Paul Attfield** – Yeast applications in decarbonising fuel and food production	18
		Milan Čertík – Construction of *Yarrowia lipolytica* strains for biosynthesis of conjugated trienoic fatty acids	18
		Patrick Fickers – Molecular toolbox for gene expression from erythritol regulated promoters	18
		Inge Van Bogaert – Turning oleaginous yeasts into free fatty acid producers: the mystery of fatty acid secretion	18
		Justyna Ruchala – Yeasts as cell factories for the production of bacterial compounds - aminoriboflavin and roseoflavin	8
		Ewelina Celinska – 'Mother (Nature) knows best' - hijacking nature-designed transcriptional programs for enhancing stress resistance and protein production in *Yarrowia lipolytica*; presentation of YaliFunTome database	8
		Helena Godon – Unfolded protein response biosensors for recombinant protein expression	8
12:40-13:40	LUNCH		60
12:40-13:40	**FEMS Yeast Research Lunch Meeting – Exhibition Hall**		
13:40-15:10	**Session 7** Yeast in Food + Beverages (1) **Prof Hiroshi Takagi + Dr Cristian Varela**	**John Morrissey** – Domestication of hybrid yeasts in fermentation environments	18
		Hiroshi Takagi – Functional amino acids engineering: A new breeding technology for brewing yeasts	18
		Graeme Walker – Fission yeast fermentations	18
		Jennifer Bellon – *Saccharomyces* interspecific hybridisation delivers evidence of hybrid heterosis in winemaking	18
		Natalia Caliani – Could fungicide treatment lead to changes in the makeup of native grape yeast communities?	8
		Nitnipa Soontorngun – Enhanced nutritional value of fermented drink by novel yeast strains with superior fermentability for bioconversion	8
15:10-15:40	AFTERNOON TEA		30
15:40-16:55	**Session 8** Yeast in Food + Beverages (2) **Prof Francisco Carrau + Assoc Prof Kate Howell**	**Kate Howell** – Diversity and dynamics of fungi during spontaneous fermentations and association with unique aroma profiles in wine	18
		Francisco Carrau – *Hanseniaspora vineae* application for low input strategies in fermented foods. Searching for sustainability and flavor complexity	18
		Anna Wittwer – *Kazachstania* yeasts may lower bread fructan content in extended dough fermentations	8
		Rosalba Lanciotti – *Yarrowia lipolytica* strain potential to valorize cheese whey into sustainable adjunct for cheese sector	8
		Francesca Patrignani – *Yarrowia lipolytica* potential to valorize cricket powder into a versatile and safe food ingredient	8
		Jin-Chen Li – Getting rid of greenness in wines - A yeast strategy	8
		Jason Amos – Introduction to "Celebration of Yeast" Workshop	7
17:00-18:30	**Posters – Even numbers**	Exhibition Hall	
17:00-18:30	**Workshop**	"Celebration of Yeast" sponsored by LALLEMAND	
	Own arrangements for dinner		

SYMPOSIUM PROGRAM

Thursday 30th November

TIME	PROGRAM		DURATION (min)
08:00	Registrations Open		
09:00-09:45	**Keynote Address**	**Tom Williams** – Competing with complexity: unlocking nature's potential using synthetic biology	45
09:45-10:30	**Session 9** Posters Alive! 1-3 Slides in 5 mins **Jason Amos**	**Teresa Zoladek** – Genetic and physical interaction of Vps13 lipid transporter with Rsp5 E3 ubiquitin ligase: coordination of lipid synthesis with lipid transport?	5
		Ryoya Tanahashi – Isolation of *Saccharomyces cerevisiae* strains with higher proline uptake and their applications to beer and wine fermentation	5
		Sinead Robinson-Cast – *Saccharomyces cerevisiae* that grow without addition of vitamins	5
		Masako Takashima – Investigation of characteristic orthologs of Ascomycota and Basidiomycota: insights from principal coordinates analysis	5
		Lethukuthula Ngobese – Antagonist wine yeast *Saccharomyces cerevisiae* T206 challenges laboratory strains over-expressing dominant FLO genes	5
		Chris Curtin – Impacts of environmental conditions and initial yeast community composition upon Kombucha SCOBY community assembly and fermentation outcomes	5
		Shailja Mishra – Volatile chemistry of yeast and Drosophila interactions	5
		Simon Schmidt – Uncovering the interplay between copper and SO_2 tolerance in *Saccharomyces cerevisiae*	5
		Eun Jung Thak – Extension of O-Mannosylation Is critical for cell wall integrity signaling and interaction with host cells in *Cryptococcus neoformans*	5
10:30-11:00	MORNING TEA		30
11:00-12:20	**Session 10** **Panel Workshop** Yeast as a Source of Ingredients in Plant-based Foods	Panel Chair: **Phil Morle** (Partner, Main Sequence)	10
		Panelists: **Maciej Holowko** (Head of Biofoundry, Nourish) **Laura Navone** (R&D Director, Eden Brew) **Robert Speight** (Director, CSIRO Advanced Engineering Biology Future Science Platform) **Stella Child** (Research and Grants Manager, Good Food Institute)	40
		Discussion	30
12:20-13:20	LUNCH		60
13:20-14:40	**Session 11** Yeast in Food + Beverages (3) **Dr Jin Zhang** **+ Dr Jean-Luc Legras**	**Jean-Luc Legras** – QTL mapping reveals novel mechanisms underlying variations in H_2S production during alcoholic fermentation in *Saccharomyces cerevisiae*	18
		Peter Costello – SO_2-production potential and early, transient acetaldehyde formation by *Saccharomyces cerevisiae*: implications for *Oenococcus oeni* co-fermentation	8
		Irina Borodina – Metabolic engineering of yeasts for the production of betalain-type natural colors	18
		Hidde Yaël Berg – Unlocking the secrets of peptide transport in wine yeast: Insights into oligopeptide transporter functions and nitrogen source preferences	8
		Claudia Vickers – Metabolic engineering in yeast for isoprenoid flavour, fragrance, and pigment molecules	12
		Ramon Gonzalez – Softer wine with an improved combination of aeration and yeast strains	12
14:40-15:10	AFTERNOON TEA		30
15:10-16:20	**Session 12** Genomics and Evolution **Prof Vladimir Jiranek +** **Dr Philip Bell**	**Philip Bell** – Exploring the phenotypic plasticity of *Saccharomyces cerevisiae* using long term evolutionary programs	18
		Heide-Marie Daniel – New *Starmerella* genomes: genomic characteristics, molecular evolution, species divergence, and horizontal gene transfers	18
		Johnathan Crandall – An adaptive interaction between cell type and metabolism drives ploidy evolution in *Saccharomyces eubayanus*	18
		Estéfani García-Ríos – Deciphering subtelomeric variation in low temperature fermentation	8
		Yuuki Kobayashi – Intrageneric genomic relationship and evolution of non-model *Basidiomycota* yeasts based on chromosome-level genome assemblies	8
19.00-23.00	**Symposium Dinner**	Playford Hotel, North Terrace, Adelaide	

SYMPOSIUM PROGRAM

Friday 1st December

TIME	PROGRAM		DURATION (min)
08:00	Registrations Open		
09:00-09:45	**Keynote Address**	**Verena Siewers** – *In vivo* directed evolution for metabolic engineering	45
09:45-10:50	**Session 13** Designer Yeast - Systems/Synthetic Biology **Prof Sakkie Pretorius + Dr Tom Williams**	**Tom Collier** – A lipid neochromosome for the over-expression of *Yarrowia lipolytica* lipid metabolism enzymes in *Saccharomyces cerevisiae*	18
		Charles Moritz – Improving the efficiency of one carbon metabolism in *K. phaffii* through enzyme and metabolic engineering	18
		Golnaz Memari – Pathway design for mixotrophic production of chemicals from CO_2 and methanol in yeasts	18
		Charlotte Cautereels – Pathway engineering using recombination-based expression optimization and Cas3-guided base editing	8
10:50-11:15	MORNING TEA		25
11:15-12:15	**Session 14** Yeast in Space and other extreme environments **Prof Vladimir Jiranek**	**Kate Poole** – Can we use fission yeast as a model to understand the impact of microgravity at the cellular level?	30
		Corey Nislow – The Yeast Deletion Collection at 25: A snapshot of progress and a view towards the future	30
12:15-13:00	**Final announcements and Symposium close**	**Jiranek & Pretorius** – Poster Prizes	10
		Mattanovich – ICY updates	10
		Bauer – ICY 2024	10
		TBA – ISSY 38	5
		Jiranek & Pretorius – Final thanks and Symposium close	10

12th International Mycological Congress, Maastricht, Netherlands,11-15 August 2024

Theme 3: Evolution, biodiversity and systematics

Symposium 13 (13 August) session name

THE AMAZING WORLD OF YEASTS: THEIR EVOLUTION, BIODIVERSITY AND BIOTECHNOLOGICAL POTENTIAL

Chairs: Marizeth Groenewald, Heide-Marie Daniel

Invited speakers:

Antonis Rokas, Fantastic yeasts and how they evolved to be; the evolution of the *Saccharomycotina* subphylum

Jean Luc Legras, Evolution and adaptation of yeast strains in changing environments; *S. cerevisiae* as example

Neža Čadež, Genomic adaptation of *Hanseniaspora* to fermenting environments

Benedetta Turchetti, *Mrakia*: an emerging cold adapted yeast genus for innovative brewing

Abstract submission open	6 November 2023
Abstract submission deadline	20 March 2024
Early registration deadline	21 May 2024

Four offered talks (10 min each) will be selected from abstracts.

Please submit abstracts on the IMC website https://imc12.org/abstract-submission/

ABSTRACTS

Metabolic engineering in yeast for isoprenoid flavour, fragrance, and pigment molecules

Claudia Vickers

ARC Centre of Excellence in Synthetic Biology, Centre for Agriculture and the Bioeconomy, and School of Biology and Environmental Science, Queensland University of Technology; Griffith Institute for Drug Discovery, Griffith University; and BioBuilt Solutions, Brisbane, QLD, Australia

The terpenes/terpenoids/isoprenoids group of natural products includes many flavours, fragrances, and pigments that are important in the food industry. Examples include the fragrance/flavour molecules limonene and citral (citrus/lemon), menthol (mint), nerolidol (strawberry), farnesene (green apple), eucalyptos (eucalyptus), nootkatone (gapefruit); and the orange, red, yellow, purple, and blue cartenoid pigments. Many of these are rare and/or difficult to produce economically from their natural sources, so metabolic engineers have turned to producing them in heterologous systems such as yeast. This requires a delicated ability to redirect carbon through the metabolic network. To control carbon flux at specific metabolic nodes for delivery of different classes of isoprenoids, we have developed a variety of molecular tools. Here I will present protein degradation as a metabolic engineering tool as well as our most recent tool, the HapAmp system. This system uses haploinsufficiency as evolutionary force to drive *in vivo* gene amplification, de-bottlenecking metabolic flux to massively increase titres of target isoprenoids. Limonene titre was improved by 20-fold in a single engineering step, delivering ~1 g L^{-1} in flask cultivation. HapAmp is an efficient approach to unlock metabolic bottlenecks rapidly for development of microbial cell factories.

'Mother(Nature) knows best' – hijacking nature-designed transcriptional programs for enhancing stress resistance and protein production in Yarrowia lipolytica; presentation of YaliFunTome database

M. Gorczyca[1], W. Białas[1], J.M. Nicaud[2], E. Celinska[1]

[1]Department of Biotechnology and Food Microbiology, Poznan University of Life Sciences, Poland
[2]INRAE, UMR1319, MICALIS, Biologie intégrative du Métabolisme Lipidique, France

In the era of rationally designed synthetic biology, heterologous metabolites production, and other counter-nature engineering of cellular metabolism, we took a step back and recalled that 'Mother(Nature) knows best'. While still aiming at synthetic, non-natural outcomes of generating an 'over-production phenotype' we dug into the pre-designed transcriptional programs evolved in our host organism – *Yarrowia lipolytica*, hoping that some of these fine-tuned orchestrated programs could be hijacked and used. Having an interest in the practical outcomes of the research, we targeted industrially-relevant functionalities – stress resistance and enhanced synthesis of proteins, and gauged them over extensive experimental design's completion.

Technically, the problem was addressed by screening a broad library of over 120 Y. lipolytica strains under 72 combinations of variables through a carefully pre-optimized high-throughput cultivation protocol, which enabled actual phenotype development. The abundance of the transcription program elicitors – transcription factors (TFs), was secured by their overexpression, while challenging the strains with the multitude of conditions was inflicted to impact their activation stratus. The data were subjected to mathematical modelling to increase their informativeness. The amount of the gathered data prompted us to present them in the form of a searchable catalog – the YaliFunTome database (http://109.173.226.32/projectup_v4/) - to facilitate the withdrawal of biological sense from numerical data.

A Dive into Hybrid Technology and *Saccharomyces cerevisiae* Maltose Negative Yeast

Molly Browning[1], Eric Abbott[1], Andrew Paterson[1], Avi Shayevitz[1], Gianmaria Ricciardi[1], Richard Chamberlin[1]

[1]Lallemand Brewing, LALLEMAND Inc, Montreal, Canada

The production of non-alcoholic or low-alcohol beer can be accomplished using physical or biological methods, each with its own advantages and disadvantages. Physical methods typically have high start-up costs and require moderate to high infrastructure investment, while biological methods may incorporate a combination of physical methods, such as immobilized yeast fermentation, or rely on non-*Saccharomyces cerevisiae* maltose-negative yeasts to perform partial fermentations.

This poster aims to showcase the application of cutting-edge hybridization techniques to develop tailored strains of *Saccharomyces cerevisiae* that demonstrate maltose-negative characteristics.

A Lipid Neochromosome for the Over-Expression of *Yarrowia Lipolytica* Lipid Metabolism Enzymes in *Saccharomyces cerevisiae*

Thomas R V. Collier[1], Fergus S M. Harrison[1], Felix. Meier[1], Xue-Rong. Zhou[3], Hugh D. Goold[2], Bei. Dong[3], Surinder P. Singh[3], Thomas Vanhercke[3], Roy S. K. Walker[1], Isak S. Pretorius[1], Thomas C. Williams[1], Ian T. Paulsen[1]*

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Yarrowia lipolytica is an oleaginous yeast species that is known for its ability to produce high levels of lipids. However, *Y. lipolytica* still has a limited molecular toolbox and is difficult to engineer relative to better-characterised host species such as *Saccharomyces cerevisiae*. In this study, we investigated the effects of over-expressing the lipid pathway of *Y. lipolytica* within the model organism *S. cerevisiae* by designing a custom lipid neochromosome. We selected 33 *Y. lipolytica* genes flanked by loxPsym sites for future randomised Cre-mediated recombination to be assembled into a 115,000bp synthetic lipidthemed neochromosome. Assembly attempts resulted in an over-expression of *Y. lipolytica* lipid-related genes in *S. cerevisiae* (BY4741) capable of accumulating 65% total lipids with the majority consisting of triglycerides (590 mg/gCDW). Here we report the highest TAG and total lipid yields in a lab-based *S. cerevisiae* strain to the best of our knowledge. Finally, these results were achieved in a single transformation and without prior strain manipulation suggesting further enhancements to *S. cerevisiae* lipid production capabilities could be achieved before a significant metabolic burden is encountered. This research represents the first example of a lipid-neochromosome in the dominant biotechnological host and model eukaryote *S. cerevisiae* and suggests it as a candidate for industrial lipid applications.

A special drop: Characterising yeast isolates associated with fermented beverages produced by Australia's Indigenous peoples

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Over the course of human history and in most societies, fermented beverages have played a unique role due to their economic and cultural importance. In Australia, *way-a-linah*, an alcoholic beverage produced from the fermented sap of *Eucalyptus gunnii*, and *tuba*, a fermented drink made from the syrup of *Cocos nucifera* fructifying bud, are two of several fermented beverages produced by Australian Aboriginal and Torres Strait people. Here we describe the characterisation of yeast isolates associated with the fermentation of *way-a-linah* and *tuba*. Microbial isolates were obtained from two different geographical locations in Australia - the Central Plateau in Tasmania, and Erub Island in the Torres Strait. Amplicon-based phylotyping enabled the identification of 1029 isolates from Tasmania and 71 isolates from Erub Island. A selected number of isolates were screened for their tolerance to common stress conditions found during the production of fermented beverages; for enzyme activities relevant to the appearance, aroma and flavour of these beverages; and for their ability to ferment media containing different sugars. Based on screening results, eight isolates were evaluated for their volatile profile during the fermentation of wort, apple juice and grape juice. Diverse volatile profiles were observed for beers, ciders and wines fermented with different isolates. These findings reveal the potential of these isolates to produce fermented beverages with unique aroma and flavour profiles and highlight the vast microbial diversity associated with fermented beverages produced by Australia's Indigenous peoples. One of the species found in Tasmania, *Lachancea cidri*, is also found in Patagonia mainly associated with *Nothofagus* and *Araucaria* forests. Phylogenomics was used to investigate the genetic variation of *L. cidri* isolates from Australia and South America, revealing the presence of two main lineages according to their geographic distribution. Estimation of the divergence time suggests that both lineages diverged near the last glacial maximum event during the Pleistocene (64-8 KYA).

An adaptive interaction between cell type and metabolism drives ploidy evolution in *Saccharomyces eubayanus*

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Ploidy is an evolutionarily labile trait, and its variation across the tree of life—particularly among yeasts—has profound impacts on evolutionary trajectories and life histories. Extreme mating type skews in some fungi hint at links between cell type and adaptive traits, but the immediate consequences of ploidy variation on organismal fitness—and the underlying molecular causes—have remained elusive, even in the model eukaryote *S. cerevisiae*. We previously subjected a strain of *Saccharomyces eubayanus*, the wild ancestor of hybrid lager-brewing yeasts, to adaptive laboratory evolution under selection for improved growth on the disaccharide maltose. We discovered an unusual ploidy reduction from the ancestral diploid state to haploidy in replicate populations of this experiment. We find that haploids in this background have a substantial, but conditional, fitness advantage in the absence of other genetic variation. Using engineered genotypes that decouple the effects of ploidy and cell type, we show that increased fitness is primarily due to the distinct transcriptional program deployed by haploid-like cell types. The link between cell-type specification and the carbon metabolism adaptation can be traced to the noncanonical regulation of a maltose transporter by a haploid-specific gene also involved in invasive growth. Our results thus provide novel mechanistic insight into the molecular basis of an environment-cell type fitness interaction and illustrate how selection on traits unexpectedly linked to ploidy states or cell types can drive karyotypic evolution in fungi.

ABSTRACTS

An Investigation into Low and Non Alcohol Sensory using a Hybrid *Saccharomyces cerevisiae* Maltose Negative Yeast

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One of the most noticeable issues with low or non-alcoholic beer is that flavor often does not often match or compare with traditional beer flavor. Low or non alcohol beer can be made either through physical or biological methods, which the latter relying on maltose and maltotriose negative yeast. These yeast tend to produce unique flavors (phenolic, worty) and most are non-*Saccharomyces cerevisiae* strains.

This poster seeks to explore how a novel hybrid *Saccharomyces cerevisiae* maltose negative yeast ("Yeast LN") offers new flavor advantages in the production of these beers.

Anoxia-induced Rearrangements of Cellular Dynamics

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Oxygen is crucial in cellular energy production because it acts as the terminal electron acceptor in mitochondrial oxidative phosphorylation. Acquiring and utilizing oxygen is essential for protozoans and metazoans on earth. In many organisms, including budding yeast, worms, and zebrafish, cells can enter a reversible suspended animation state under anoxia (0-100 ppm O_2), an extreme form of hypoxia. Still, the specific molecular mechanisms involved are unclear, and the responses appear different from hypoxia. Our current research is centred on elucidating molecular mechanisms and cellular pathways in entering anoxia-induced suspended animation, in which many organelles rearrange their organization to facilitate the arrest. We have performed a genome-wide deletion gene mutant screening in budding yeast and identified 34 AIS (anoxia-induced suspended animation-required) candidate genes that can be grouped into different functional categories, such as cell cycle regulation, nucleus regulation, and chromosome organizations. We have observed immobile mitotic spindle and nucleolus, rearranged nuclear envelope, and condensed chromosomes by time-lapse live-cell imaging. Specifically, we identified a conserved cell division cycle gene CDC50 that is potentially involved in polarized proliferation during anoxic recovery. In conclusion, anoxia-induced rearrangements of the cellular dynamics are essential for cell survival through anoxia. Such understanding will provide insights into therapeutic approaches that may prevent the proliferation and metastasis of dormant cancer cells upon oxygen availability.

Antagonist wine yeast *Saccharomyces cerevisiae* T206 challenges laboratory strains over-expressing dominant *FLO* genes

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The *FLO* gene family of *Saccharomyces cerevisiae* encodes for GPI-anchored glycoproteins that are attached to the β-1,6-glucan network of the yeast cell wall. The functions of these glycoproteins is yet to be fully elucidated. However, research has suggested that they play a role in the protection or survival of the yeast strains under harsh environmental conditions. Some yeast strains of *Saccharomyces cerevisiae* are known to produce one of the five documented killer toxins (K1, K2, K28, Klus and Kx) which offer them a competitive advantage against sensitive strains in the growth medium. The toxin, which is a protein by nature, is released extracellularly into the media and binds to and kills the sensitive strains, while not harming the releasing strain. The K2 toxin is known to bind the β-1,6-glucan of the cell wall of the susceptible strain, thereby creating pores and affecting the structural integrity of the plasma membrane. In this study we genetically modified a non-flocculent, non-invasive and K2 toxin susceptible laboratory strain BY4741 via a promoter replacement strategy so that it constitutively expresses *FLO1, FLO5* and *FLO11*. The three transgenic strains displayed two distinct phenotypes, viz., flocculation (BY4741-F1P and BY4741-F5P) and invasive growth (BY4741-F11P). The transgenic strains were tested for resilience against a well-known K2-toxin producing strain *S. cerevisiae* T206 via the methylene blue agar plate technique. However, no major resistance to the toxin by the transgenic strains over-expressing *FLO1*, *FLO5* and *FLO11* was observed on the MBA plates.

Capturing and Characterising Wild Yeast for Beer Brewing

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Beer is typically made using fermentation with *Saccharomyces cerevisiae* or *Saccharomyces pastorianus*, domesticated brewing yeasts. Historically, wild, non-*Saccharomyces* yeasts have also been frequently used in mixed culture fermentations to provide interesting and unique flavours to beer. However, brewing using mixed cultures or by spontaneous fermentation makes reproducing flavours and beer styles extremely difficult. Here, we describe a pipeline from collection of native wild yeast from plant material to the characterisation and commercial scale production of beer using wild yeast. We isolated 23 wild yeast strains, performed fermentation assays and measured ethanol production. We used growth assays, proteomics, metabolomics, and genomics to understand the sugar and amino acid utilisation profiles of two candidate production strains of wild *Torulaspora* and a poorly performing wild strain of the same genus compared to commonly used craft beer brewing yeast US05. We then investigated media composition and fermentation parameters that could affect growth, identified the underlying mechanisms that limited growth of wild yeast at industrial scale, and implemented process modifications that allowed effective commercial fermentation.

Characterisation of molecular targets of the co-evolution of the microalga *Chlorella sorokiniana* and the yeast *Saccharomyces cerevisiae*

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Evolutionary experiments play a pivotal role in research, as they provide invaluable insights into the dynamic processes of adaptation and selection in the natural environment. Here, we present a new synthetic ecology-based method for evolving cooperative behaviour between yeast *Saccharomyces cerevisiae* and microalga *Chlorella sorokiniana*.

These two species evolved through an obligatory mutualism in a continuous co-evolution. After 100 generations in co-evolution, isolated strains exhibited enhanced biomass production in co-culture conditions. RNAseq data revealed significant differences in the expression of genes involved in transmembrane transport.

Co-evolved yeast strains were genome sequenced to understand the genetic changes responsible for improved cooperative behaviour. Genetic alterations in evolved *S. cerevisiae* isolates were identified, and several predicted high-impact SNPs were further investigated in using deletion mutants. Specific SNPs in genes leading to altered cooperativity with the deletion mutants were re-introduced into the original parental strain. Mutations in two genes involved in ethanol tolerance and carbon catabolite repression and in nitrogen catabolite repression, *ETP1* and *GAT1*, both individually improved cooperativity. Combining the two mutations further improved the phenotype, suggesting synergistic roles in cooperative behaviour. These findings highlight the importance of considering biotic selection pressures in strain development projects and offer valuable insights into the molecular adaptations that promote cooperation between yeast and algae.

Characterization of *Yarrowia lipolytica* Ceramidase, YlYcd1p, for Functional Application to the Secretory Production of Sphingoid Bases in Yeast

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Ceramidase plays vital role in regulating ceramide levels by hydrolyzing ceramide, a central molecule in the pathway of sphingolipid metabolism. Here, we performed functional analysis of *Yarrowia lipolytica YDC1*, encoding a predicted protein with 36% and 35% identity to *Saccharomyces cerevisiae* ceramidases Ypc1p and Ydc1p, respectively. *Y. lipolytica* Ydc1 protein (YlYdc1p), composed of 320 amino acids, has a ceramidase domain and seven transmembrane segments. Despite the absence of an ER retention sequence in YlYdc1p, the cellular localization analysis using GFP fusion confirmed the presence of YlYdc1p is localized to at the ER membrane. The level of Yl*YDC1* mRNA increased in cultivation at GB media, sphingolipid overproduction condition. We overexpressed Yl*YDC1* in the *S. cerevisiae* wild-type (WT) and *ypc1ydc1* double null mutant strains and investigated the profile change of sphingoid long-chain bases, the breakdown products of ceramides, by TLC analysis. Notably, the *S. cerevisiae* overexpressing Yl*YDC1* showed the profiles, both in WT and *ypc1ydc1* background, which were more like those of the *S. cerevisiae* overexpressing Sc*YPC1* than those of the *S. cerevisiae* overexpressing Sc*YDC1*. The increase of both dihydrosphigosine and phytosphingosine at comparable levels by Yl*YDC1* overexpression strongly indicated that YlYdc1p can cleavage both dihydroceramide and phytoceramide without apparent substrate preference. The overexpression of Yl*YDC1* enhanced the secretory production of LCBs in the recombinant acetylated *S. cerevisiae* and *Y. lipolytica* strains, engineered by expressing *Wickerhamomyces ciferrii SLI1* encoding a sphingoid base *N*-/*O*-acetyltransferase. Moreover, the improved production of LCBs was also observed in the Yl*sur2sld1* double mutant, which secretes LCBs without acetylation, by the Yl*YDC1* overexpression. Our results present YlYdc1p as a manipulation target in engineering yeast cells to increase the secretory production of sphingoid bases with high industrial potential as pharmaceutical and cosmeceutical ingredients.

Competing with complexity: unlocking nature's potential using synthetic biology

Dr Tom Williams

Synthetic biology has enormous potential to enable sustainable biomanufacturing, novel therapeutics and diagnostics, and greater insights into fundamental aspects of biology. However, this potential is limited by the complexity of living systems and our incomplete knowledge of them. I will present recent research that highlights strategies we are employing to engineer complex biological systems more effectively and uncover biology's 'unknown unknowns'. As illustrative examples, I will cover the use of Adaptive Laboratory Evolution for altered carbon source utilization in *Saccharomyces cerevisiae*, the creation and use of biosensors to screen for metabolite production phenotypes, and work towards the engineering of simplified minimal yeast genomes.

ABSTRACTS

Construction of a barcoded collection of wild and domestic *Saccharomyces cerevisiae* strains for competitive fitness assays using CRISPR-Cas9

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The budding yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) is a powerful model system for eukaryotic biology. Experimental designs are often restricted however to a limited set of domesticated laboratory strains, typically derived from S288c. Here we describe a genetically diverse collection of barcoded *S. cerevisiae* strains, representing multiple phylogenetic clades, that enable pooled competitive fitness assays in non-laboratory strains. Marker-less genetic barcodes were introduced into the genomes of each strain by targeting the *HO* locus via CRISPR-Cas9 and replacing the gene with single-stranded oligonucleotide donor DNA (ssDNA) consisting of a 20-nucleotide barcode and two short 40-nucleotide homology arms. High-throughput transformation of CRISPR-Cas9 machinery and ssDNA into each strain was achieved in 96-well format using an optimized protocol, and barcode insertion was confirmed via Sanger sequencing. The barcoded collection will be utilized for high-throughput drug screening via pooled liquid-phenotyping bar-seq assays in response to antineoplastic and antifungal compounds targeting various biological pathways. Examples of compounds include methotrexate, an anticancer agent that inhibits DNA synthesis, and benomyl, an antifungal that disrupts microtubule dynamics and is functionally similar to the chemotherapeutic taxol. A preliminary benomyl screen has identified one strain with 10.5-fold higher resistance than S288c, which has been partially mapped to a missense mutation in the beta-tubulin Tub2. After drug screening, genotype-phenotype relationships will be investigated via genome-wide association studies, allele-swap experiments, and bulk-segregant analysis. This study will provide comprehensive gene-trait mapping in *S. cerevisiae* and will increase the relevance of this model system when studying complex trait variation.

Construction of *Yarrowia lipolytica* strains for biosynthesis of conjugated trienoic fatty acids

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Oleaginous yeast *Yarrowia lipolytica* appears as one of the most attractive microbes for wide biotechnological applications. Rational re-design of lipid metabolic pathways by gene engineering leads to production of unusual lipid structures in this yeast. Conjugated trienoic fatty acids (CTFAs) are interesting compounds which are mainly biosynthesized only in some plants. Genes coding specific conjugases for formation of punicic acid (*PgFADX* from *Punica granatum*), α-eleostearic acid (*VfFADX* from *Vernicia fordii*), calendic acid (*CoFADX* from *Calendula officinalis*) and jacaric acid (*JmFADX* from *Jacaranda mimosifolia*) have been optimized for *Y. lipolytica* and transformed by insertion cassettes equipped with special promoters to the yeast. All conjugases showed bifunctional feature with both conjugation and desaturation activities. However, accumulation of CTFAs in yeast lipids was low. Therefore, further strain engineering was needed to improve CTFAs biosynthesis and storage in *Y. lipolytica*. The first attempt was focused on punicic acid (PuA). Because *PgFADX* transforms linoleic acid bound to phosphatidylcholine to PuA, the aim was to enhance PuA removal from phosphatidylcholine to acyl-CoA pool and its subsequent incorporation into triacylglycerols. So various lysophosphatidylcholine acyltransferases from *P. granatum* (*PgLPCAT*), *Lichi chinensis* (*LchLPCAT*) and *Physaria fendleri* (*PfLPCAT*) were co-expressed with *PgFADX*. Moreover, four acyltransferase genes from *P. granatum* (*PgDGAT1, PgDGAT2, PgDGAT3, PgPDAT*) were also co-expressed with *PgFADX*. This strategy proved successful PuA accumulation in the yeast lipids. Thus, *Y. lipolytica* with rational metabolic engineering strategies showed a great potential for the production of various CTFAs.

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Conversion of methanol to itaconic acid with engineered *Komagataella phaffii*

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Itaconic acid is a value-added organic acid with multiple applications within many different industries. Today, itaconic acid is primarily produced industrially through microbial fermentation of sugars or starch, which rely on agricultural cultivation and compete with food and feed production. Methanol, a C1 carbon source offers a more sustainable possibility to produce itaconic acid, as technology allows electrochemical reduction of CO_2 to methanol. As part of the VIVALDI project, we strive for a sustainable production of itaconic acid from green methanol with engineered *Komagataella phaffii*.

Integration of the heterologous pathway for itaconic acid from *Aspergillus terreus* enabled *K. phaffii* to produce itaconic acid from methanol, and first experiments in lab scale bioreactors demonstrated titers of up to 28 g/L, however at the cost of a high biomass accumulation. Further optimization strategies, including genetic engineering and process optimization were employed and improved the production efficiency significantly. For instance, higher fermentation temperatures have proven to be beneficial for itaconic acid production and improved the $Y_{P/X}$ of the methanol fermentation by 91%. Multicopy integration of the heterologous genes *cadA*, *mttA* and *mfsA* enabled final titers of 50 g/L and further enhanced the $Y_{P/X}$ by 92% and the $Y_{P/S}$ by 68%.

In the light of our results we show that it is possible to produce itaconic acid from methanol using the methylotrophic yeast *K. phaffii*. We believe with further optimization *K. phaffii* can become a competing production host for itaconic acid in industry, thereby enabling a shift towards a more sustainable bio-based economy.

Could fungicide treatment lead to changes in the makeup of native grape yeast communities?

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Consumers are increasingly interested in trying new and unique wine experiences, seeking wines that reflect a sense of place (regional typicity). One tool with which to achieve such wines is uninoculated fermentations, performed by the diverse native yeast communities to boost wine sensory attributes. Many of these native yeasts can ferment grape juice even without the addition of commercial yeast. This is how wines are produced with special sensory attributes (aroma, texture, and length) that are part of what some winemakers call the 'terroir'.

The diversity of yeast in fermentations starts in the vineyard and can be modified by various factors including climate (rain and temperature) and viticultural practices (fungicide applications). Applications of fungicides are necessary to control the multiple grapevine diseases caused by fungi and oomycetes every season which cause multi-million-dollar losses to the wine industry. However, whilst this keeps the developing bunches healthy, native yeast populations become off-target organisms whose populations are affected by the sprays.

Our hypothesis is that organic vineyard practices might drive less diverse yeast populations as they rely on broad spectrum fungicides; whilst conventional vineyards might contain more diverse populations as the products used have specific sites of action. Therefore, in this study, we characterised the yeast communities identified from biodynamic and conventionally grown Riesling grape washes, juice, and fermentation. We aimed to elucidate the link between these treatments and the observed yeast diversity.

Both biodynamic and conventional grapes were manually harvested and split into separate treatments. Grape samples were collected and washed to harvest fungal species and both treatments were also processed by crushing in separate batches. The resulting juice was split into 6 replicates which were incubated at 23 °C and sampled over three weeks (uninoculated fermentations). ITS1 Illumina sequencing was used to identify the different yeast species in each sample and their relative abundance.

We found that the conventional grape washes and juice had the highest diversity. However, we observed a shift in the biodynamic treatment during fermentation, with *Saccharomyces cerevisiae* being the dominant species from mid-fermentation. This information could help tailor fungicide spraying schemes to optimise their effectiveness, whilst maintaining vineyard ecological balance.

Cross-feeding promotes heterogeneity within cell populations, and the strategies to harness it for bioengineering

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Currently, one of the most common methods of producing recombinant proteins is through the use of high-copy plasmid-based yeast expression platforms, wherein plasmids are maintained under auxotrophic selection pressure. Using fluorescent proteins as models for intracellular recombinant protein production, our study revealed there is a high degree of cell-to-cell heterogeneity in recombinant protein expression within a clonal-originated *ura3*-deficient *Saccharomyces cerevisiae* population complemented with *URA3*-containing episomal plasmids. In particular, even when cultivated in a supposedly pyrimidine-free selective medium, a subpopulation of plasmid-free cells was observed to be proliferative alongside plasmid-carrying cells. We experimentally proved the growth of these plasmid-free auxotrophs was supported by the complementary metabolites excreted from plasmidcarrying prototrophs into the shared exo-metabolomic space, thereby compromised the auxotrophic selection pressure. We then demonstrated that through targeted genetic engineering it is possible to effectively repress the growth and thus the proportion of plasmid-free cells within the recombinant

population when cultivated in either selective or non-selective media, which in turn improved both single-cell and population-ensemble recombinant protein productivity. The mechanism of complementary metabolite cross-feeding was also exploited to design yeastyeast and yeastbacteria coculture systems with selfregulation capability, which can be a useful feature that facilitates the efficient division of labour within synthetic microbial communities.

Cytosolic peroxiredoxin Tsa1 modulates acetic acid levels in wine yeasts under fermentative and respiratory conditions

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During its industrial use in winemaking, the yeast *Saccharomyces cerevisiae* must withstand several metabolic transitions and stress conditions. Among these stresses is oxidative stress, caused by reactive oxygen species (ROS). Peroxiredoxins are a family of peroxide-degrading enzymes that challenge oxidative stress. The main cytosolic peroxiredoxin is Tsa1, which is involved in many other processes as a redox sensor, and we have proved that it has an impact at different stages of the industrial use of *S. cerevisiae*.

TSA1 deletion in industrial wine yeasts decreases their fermentative capacity in grape juice fermentation and alters acetic acid production. Regulation of acetic acid metabolism during winemaking is important for wine's quality, as high levels worsen its organoleptic profile. In synthetic grape juice fermentation, *TSA1* deletion causes a drop in acetic acid production on the first day of vinification, but this difference is lost at later stages. That profile correlates with a lower cytosolic aldehyde dehydrogenase Ald6 activity, and not mitochondrial Ald4, in the *tsa1*Δ mutant in early fermentation. To demonstrate that the role of Tsa1 on acetate metabolism is dependent on the metabolic status of the cell, we also study it under respiratory conditions in the post-diauxic phase of growth where *tsa1*Δ mutant decreases acetic acid. We have shown that Tsa1 regulates pH homeostasis in both liquid and plate assays. By labelling the main aldehyde dehydrogenases, we will study whether they have cysteine residues that can be oxidised and undergo transient Tsa1-dependent redox regulation during the early stages of winemaking.

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Deciphering subtelomeric variation in low temperature fermentation

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Temperature is one of the most important parameters to affect the duration and rate of alcoholic fermentation and final wine quality. Wine produced at low temperature is often considered to have improved sensory qualities. However, there are certain drawbacks to low-temperature fermentations, such as slow growth rate, long lag phase, and sluggish or stuck fermentations. In this study, a multiomic approach was performed in strains with divergent phenotype at low temperatures and the analysis of the subtelomeric variation through PacBio technology was also carried out. Sulfur assimilation, nitrogen uptake, maintenance of the membrane asymmetry and oxidative stress response proved to be crucial mechanisms in cold and they have been deeply analyzed by using different biochemical approaches.

Diversity and dynamics of fungi during spontaneous fermentations and association with unique aroma profiles in wine

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Microbial ecology is an integral part of an agricultural ecosystem and influences the quality of agricultural commodities. Microbial activity influences grapevine health and crop production, conversion of sugar to ethanol during fermentation, thus forming wine aroma and flavour. There are regionally differentiated microbial patterns in grapevines and must but how microbial patterns contribute to wine regional distinctiveness (terroir) at small scale (<100 km) is not well defined. Here we characterise fungal communities, yeast populations, and *Saccharomyces cerevisiae* populations during spontaneous fermentation using metagenomics and population genetics to investigate microbial distribution and fungal contributions to the resultant wine. We found differentiation of fungi, yeasts, and *S. cerevisiae* between geographic origins (estate/vineyard), with influences from the grape variety. Growth and dominance of *S. cerevisiae* during fermentation reshaped the fungal community and showed geographic structure at the strain level. Associations between fungal microbiota diversity and wine chemicals suggest that *S. cerevisiae* plays a primary role in determining wine aroma profiles at a sub-regional scale. The geographic distribution at scales of less than 12 km supports that differential microbial communities, including the dominant fermentative yeast *S. cerevisiae* can be distinct in a local setting. These findings provide further evidence for microbial contributions to wine terroir, and perspectives for sustainable agricultural practices to maintain microbial diversity and optimise fermentation function to craft beverage quality.

Do the different molecular types of the *Cryptococcus neoformans/ Cryptococcus gattii* cause different clinical manifestations? A longitudinal *in vivo* assessment study of host-pathogen interactions and pathogen dissemination routes in murine cerebral cryptococcosis models

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Yeast isolates of the *Cryptococcus neoformans (CN)/ Cryptococcus gattii (CG)* species complexes are opportunistic pathogens, considered the most high-risk fungal pathogen worldwide. During disease dissemination, cryptococci are able to cross the blood-brain barrier, subsequently spreading through the central nervous system. Patients with cryptococcosis can display a wide variety of symptoms. As the species complexes consist of at least eight molecular types with partially different manifestation in human patients, it was our aim to further characterize these eight molecular types in terms of phenotypical and clinical parameters in a standardized murine model by using repeated *in vivo* molecular imaging and immunohistochemistry.

BALB/c mice were infected with one of eight isolates representing one of the eight major molecular types of CN (VNI - IV) and CG (VGI - VG IV) as previously identified by *Meyer et al*. *In vivo* radiological exams (MRI/ NMR spectroscopy) were performed on day 3 and 6 post infection to assess dissemination to the brain. *In vivo* imaging was correlated with histopathology for lesion formation and host-pathogen interactions (H/E, PAS, CD68, CD206, CD301, CD11b, CD4, CD8, MHCI, MHCII, CD31).

We found notable differences between major molecular types within the same species complex, ranging from manifestation, lesion formation, affected anatomical brain regions and immune response. Our results indicate a provocation of an autoimmune-like reaction during a cryptococcal infection based upon an immunosuppressed phenotype in the CNS, an upregulated M2 macrophage activity within the brain parenchyma, which differs between the various molecular types. These results indicate the need to communicate on molecular types that provoke different pathologies.

Reference: Meyer, W. *et al.* Consensus multi-locus sequence typing scheme for *Cryptococcus neoformans* and *Cryptococcus gattii*. *Med Mycol* **47**, 561–570 (2009).

Domestication of hybrid yeasts in fermentation environments

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Yeasts have been used by humans for thousands of years for the production of fermented foods and beverages. When considering this, one immediately thinks of bread, beer and wine, but there is also a long history of fermented dairy, meat and vegetable products involving both yeast and bacteria. Our work on *Kluyveromyces* yeasts uncovered a complex history of domestication. One of the unusual features we observed was that all dairy isolates of *K. marxianus* are either diploid intra-species hybrids or triploid strains. The hybrid genomes all showed features typical of domesticated species. Further work uncovered extensive evidence of domestication in the genus. Of course, the most famous hybrid is the lager yeast, *Saccharomyces pastorianus*, born around the 16th century when parental *S. cerevisiae* and *Saccharomyces eubayanus* strains mated. The details of when and how this happened have been the subject of much debate but new studies are offering some insights. The isolation of *S. eubayanus* in Ireland confirms that European populations exist, and an investigation of old Central European brewing records led us to propose that the actual hybridisation happened in the Munich äuhaus between a Bohemian wheat strain of *S. cerevisiae* and a native strain of *S. eubayanus* that was part of a yeast mixture that had been used in Bavaria for hundreds of years. The integration of historical research with modern phylogenomics shows how an interdisciplinary approach can help unlock long-held secrets and answer key questions. The links between human activity and evolutionary events will be discussed.

Effects of fungicide applications on non-target yeast communities during wine production

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Fungal infections of grapes can result in major economic losses for wine producers and thus fungicides are routinely applied throughout the grape growing season to control for unwanted pathogens and maintain fruit quality. However, the mode of action of these fungicides may not be pathogen-specific and could affect desirable fungi such as yeast. Yeast are essential for the fermentation of grapes to wine where traditionally a diverse succession of species and *Saccharomyces* strains drive the process. Traditionally derived from the vineyard where they reside on fruit prior to harvest, these yeast communities are regionally differentiated both in their species composition and strain genetic diversity. Given different species and strains of yeast contribute unique attributes to wine, lending themselves as important components of wine quality and style, these regional differences in yeast communities may contribute to a wines sense of place, or terroir. In fact, experimental trials have demonstrated this with geographically distinct populations of *Saccharomyces cerevisiae* contributing to regionally distinct Sauvignon blanc chemical

properties, and more recently, regionally distinct mixed communities of yeast contributing to regionally distinct Pinot noir chemical properties in New Zealand. The potential effects of fungicide applications on the composition of these yeast communities may thus have consequences for regional wine styles. Evidence from commercial vineyard microbiomes is assessed alongside laboratory microcosm and growth rate experiments to further our understanding of the impacts of fungicide applications on yeast communities.

Effects of nutrient levels and mixed fermentation on wine flavor

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Yeast plays a crucial role in wine fermentation, with specific nutritional requirements resulting in diverse fermentation outcomes including flavor profiles. Among all factors, grape-derived carbon and nitrogen impose significant influences on yeast growth and metabolism. *Saccharomyces cerevisiae*, dominant in winemaking process, has been extensively investigated on such aspects. Non-*Saccharomyces* yeasts, on the other hand, attracted research attention in recent decades due to their versatile fermentative activities. Mixed fermentation of both *S. cerevisiae* and non-*Saccharomyces* yeasts become popular practices, with scientific regulatory mechanisms therein to be fully elucidated.

In this study, mixed fermentation with diverse initial carbon/nitrogen levels were conducted in chemically-defined grape juice media to investigate the effects of nutrient levels on yeast fermentation performances, especially volatile production. GC-O-MS and GC-MS were conducted at the end of fermentation for aroma analysis. Results showed that mixed fermentation of *Aureobasidium pullulans* and *S. cerevisiae* was better at producing volatile compounds than *Papiliotrema laurentii* RY1 and *Meyerozyma guilliermondii,* under all nutrient conditions tested. Most abundant volatile productions were noted at nutrient combinations of 270 g/L sugar, 90 mg N/L nitrogen and 200 g/L sugar, 380 mg N/L nitrogen. In addition, the most aldehydes were detected under low nitrogen level, regardless of sugar contents. And similarly, the major volatiles identified at high sugar content were esters irrespective of nitrogen concentrations. In exploring the interactions between nutrient levels and aroma-oriented yeast fermentation performances, this study can be of referential values for winery practices aiming at better aroma production under diverse nutrient conditions.

Efficient CRISPR-Cas9 Production of Non-GMO Yeast with Desirable Winemaking Characteristics

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During alcoholic fermentation of grape sugars, wine yeast produce a range of secondary metabolites that play an important role in the aroma profile of wines. However, not all yeast-derived compounds are desirable, such as excessive production of volatile acidity; while other characteristics can be difficult to achieve, such as the production of 'fruity' aroma compounds. At present, the ability to alter yeast characteristics in a way that does not result in the yeast produced being labelled as a genetically modified organism (GMO) is limited to a few methodologies such as hybridisation, or mutagenesis and selection. While these methodologies have been successful in the past for the generation of commercially available industrial strains with improved winemaking properties, these techniques are also regarded as time-consuming and unpredictable. To overcome these limitations we have developed a more efficient and targeted non-GMO method for acquiring desirable traits in wine yeast based on the CRISPR-Cas9 system. Through this system we were able to successfully reduce the production of volatile acidity and to improve yeast's ability to release 'fruity' aroma compounds during laboratory-scale fermentation conditions

This work is an important step to efficiently develop non-GM wine yeast with a wide range of optimised, improved or oenological properties that can add value to the wine.

Engineering natural isolates of *Saccharomyces cerevisiae* for applications in lignocellulosic biorefineries

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Utilizing a significant portion of the sugars available in renewable biomass is crucial for the development of sustainable strategies in biofuel and green chemical production. *Saccharomyces cerevisiae* has emerged as a promising candidate in this industry using a consolidated bioprocessing approach, due to its high ethanol production, genetic malleability, and tolerance to industry relevant stresses. However, this yeast lacks the ability to produce cellulolytic enzymes and utilize the pentose sugar xylose, which can constitute 35% of plant dry weight. Therefore, significant strain engineering is necessary to adapt this organism for use in biorefineries. Domesticated strains of *S. cerevisiae* often exhibit low levels of protein secretion and a lack of process robustness, making the diverse genetic background of natural isolates appealing. We have employed CRISPR/Cas9 tools to develop strains capable of producing a core set of cellulases. Among the natural isolate derivatives, YI13_BECC and YI59_BECC demonstrated superior secretion capacity for heterologous cellulases under high incubation temperatures and in the presence of acetic acid. These strains achieved ethanol concentrations of up to 4.5 g/L when cultivated on crystalline cellulose, without the need for exogenous enzymes. Similarly, we developed natural isolate strains to utilize xylose, xylo-oligosaccharides, and xylan, allowing direct conversion of these to ethanol. Recognizing the importance of adding value to the cellulosic ethanol industry through higher-priced co-products, we also engineered the production of xylitol directly from polymeric substrates. The development of a diverse set of strains would contribute to the overall economic feasibility of utilizing lignocellulosic biomass in biorefineries.

Engineering of *Ogataea polymorpha* strains with ability for high-temperature alcohol fermentation of cellobiose

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Plant cell walls are a promising source of sugars for biofuel production. The main complex sugars included in their composition are cellulose (a polymer of glucose) and hemicellulose (a heterogeneous polymer of pentoses, hexoses, and sugar acids). Successful conversion of cellulosic biomass into biofuel requires organisms that can efficiently utilize xylose, cellodextrins, and glucose. *gataea polymorpha* is a thermotolerant yeast that naturally metabolizes xylose, making it a good candidate for biofuel production. However, *O. polymorpha* cannot naturally ferment cellodextrins like cellobiose, which is a disadvantage for producing cellulosic biofuels.

Two cellobiases were selected for the metabolism of cellobiose in the yeast *O. polymorpha*: β-glucosidase (gh1-1) from *Neurospora crassa*, which hydrolyzes cellobiose into two glucose molecules, and cellobiose phosphorylase (CBP) from *Saccharophagus degradans*, which cleaves the disaccharide into glucose and glucose-1-phosphate. Modified cellobiose transporter proteins, CDT-1m and CDT-2m from *N. crassa*, were selected due to their high affinity for cellobiose. Overexpression of *gh1-1/CDT-1m*, *CBP/CDT-1m*, *gh1-1/CDT-2m*, and *CBP/CDT-2m* gene combinations in the best ethanol producer (*BEP*/cat8Δ) of the yeast *O. polymorpha*, did not have a significant positive effect on the fermentation of 10% cellobiose. Laboratory evolution and UV-mutagenesis of the transformants characterized by the best growth on cellobiose allowed the obtaining of mutant strains with a 1,5 and 4-fold increase in ethanol production from cellobiose, reaching a maximum of 4.2 g ethanol/L during cellobiose alcoholic fermentation at 45°C. Moreover, co-fermentation of a sugar mixture by a mutant with overexpression of *CBP/CDT-2m* demonstrated co-consumption of sugars despite a significant initial amount of glucose.

Enhanced nutritional value of fermented drink by novel yeast strains with superior fermentability for bioconversion

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We presented the development of fermented beverages, using the evolutionally engineered probiotic yeast *Saccharomyces boulardii* GT-EVE- and/or *Torulaspora delbrueckii* GT-ROSE1-assisted fermentation process of local honey, tea and rice variety. The fermented drink showed altered chemical profiles of raw agricultural materials as shown by nontargeted HS-SPME-GC-MS and HPC/LC-MS methods. Fermentation improved sensory quality and offered value-added characteristics of healthy products. These included augmentation of aroma and flavor of 2-phenlyethanol, amino acids and several fresh fruity compounds with low volatile acids and alcohols. Combination of health-promoting molecules prebiotics and probiotics enhanced nutritional value of symbiotic beverage including the antioxidant, anti-inflammatory and anti-ageing effect. This research study promotes the delivery of fresh and premium quality of yeast-derived products from local farms to global functional food markets.

Ethanol-lactate transition of *Lachancea thermotolerans* is linked to nitrogen metabolism

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Climate change increases sugar content in grapes, resulting in unwanted increase in ethanol content of wine. *Lachancea thermotolerans* ferments glucose and fructose into both ethanol and lactate, decreasing final ethanol content and positively affecting wine acidity. Reported *Lachancea thermotolerans* strains show big variation in lactate production during fermentation. However, a mechanistic understanding of this lactate producing phenotype is currently lacking. Through a combination of metabolomics, transcriptomics, genomics and computational methods we show that the lactate production is induced by amino acid limitation in a high lactate producing strain. We found in fermentations in synthetic grape juice media that lactate production starts in the last stages of growth, marked by decreased growth rate and increased expression levels of stress related genes. This onset of lactate production is specific for the high lactate producing strain and independent of oxygen availability. The onset of lactate production was changed by increased amino

acid content of the media, and it is shown by both computational methods and amino acid measurements that at the onset of lactate production amino acids become limiting for growth. This study shows that lactate production of *Lachancea thermotolerans* is directly linked to nitrogen availability in the media, an insight that can further aid in the improvement of wine quality.

Evaluating Diverse British Columbian Yeast Strains for New-Make Spirit Applications

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Whisky is a distilled alcoholic beverage (spirit) made from a malt liquid extract called wort, which then undergoes alcoholic fermentation commonly by *Saccharomyces cerevisiae* (*S. cerevisiae*) yeast. Whisky that has not undergone maturation in oak barrels is called new-make spirit. Most whisky distilleries utilize one of a handful of commercial distilling strains. Recently, global interest has increased in diverse non-traditional strains for spirits production, including *S. cerevisiae* and non-*S. cerevisiae* species, which offer novel organoleptic properties compared to commercial strains, and this may lead to interesting new brands of whisky. We screened 88 strains isolated from spontaneous wine fermentations from the Okanagan Valley, British Columbia, representing four species, for use in whisky production. Maltose utilization and malt-extract fermentation efficiency phenotypes for some strains suggested their potential application for whisky production. Of 88 strains, four *S. cerevisiae* strains belonging to three lineages, as well as a *Saccharomyces uvarum*, *Torulaspora delbrueckii* and *Lachancia thermotolerans* strain, were selected for triplicate 19L pilot scale wort fermentations and subsequent new-make spirit distillation. Non-*S. cerevisiae* strains had a commercial whisky *S. cerevisiae* strain sequentially inoculated after 72 hours to aid fermentation progression. Ethanol, glycerol, sugars, and organic acids from fermentations were measured by high-performance liquid chromatography. Quantitative and semi-quantitative analysis for volatile compounds from new-make spirit were measured by headspace solid-phase microextraction gas chromatography. This project aims to demonstrate diverse Okanagan Valley yeast strain potential for creating new interesting and exciting brands of Canadian whisky.

Evaluation of rain-shelter cultivation mode effects on microbial diversity during Cabernet Sauvignon (*Vitis vinifera* L.) maturation in Jingyang, Shaanxi, China

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Rainfall particularly under continental climates with monsoonal tendency impacts the vineyard microbial niches during grapevine growth. With microbial community shifts, vine traits (grape flavor and yield) cultivated/ protected under rain-shelter may ultimately be altered. Such cultivation may influence microflora dynamics via meteorological parameter variations, however this is unclear yet. Here, we used Cabernet Sauvignon, a prevalent red cultivar among wine growing regions, to evaluate the effects of the rain-shelter cultivation on the microorganism diversity. We found that average air temperature under rain-shelter conditions was 2–3℃ higher than the non-covered group, while air humidity the maximum reduction was 5.79% (p<0.05). After grape setting stage, similar trends were observed on soil temperature (increased) and humidity (lowered) under the treatments (p<0.05). UV and precipitation of rain-shelter treatment were less by a total of 72% and 96%, respectively (p<0.05). The rain-shelter management presented lower fungal and bacterial OTUs. The fungal alpha diversity on leaves and branches under rain-shelter was lower (p<0.05) than the control as the grape ripeness, with *Ascomycota*, *Mycosphaerella* and *Cladosporium* as the principal fungi. Our results revealed that the fungal microbiota patterns were differentiated by the cultivations from setting stage to the entire veraison and then tended to be similar at harvesting. Only branch fungal patterns were observed asymmetrically at all stages. Meanwhile, bacterial diversity and distribution varied on colonization locations where *Proteobacteria* and *Actinobacteria* were the primary bacteria phyla. Bacterial community structures overlapped at harvest, while the differences were observed between two cultivations at other stages, excluding grape berry. The rain-shelter cultivation reduced the abundance of *Alternaria* and *Colletotrichum* that may adversely affect grapevine health. Multivariate statistical analysis suggested that the effect of vineyard microclimate on microbiota distribution and succession were influenced by cultivation modes and grapevine developmental stages. This research provides evidence to address the dynamics of microbial ecology from vineyard to grape under rain-shelter cultivation, and its benefits as a sustainable vineyard management.

Exploring the genetics governing quantitative phenotypes in the yeast *Komagataella phaffii*

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Komagataella phaffii (formerly known *Pichia pastoris*) is a methylotrophic yeast widely used in industry for recombinant protein production. Particularly derivatives of the isolate CBS2612 have been used for manufacturing a wide variety of products and were studied extensively. While lots of efforts have been made for the thorough investigation of these industrially-relevant strains, the majority of natural isolates within this species remains unexplored. Yet, as demonstrated in other yeast species, particularly isolates of *Saccharomyces spp.*, exploiting intra-species variation can benefit strain development for industrially-relevant phenotypic traits. Using quantitative trait loci (QTL) mapping, we aimed at resolving the recombinant protein production potential, as well as temperature tolerance between a cross of a natural isolate and a close descendant of CBS2612. Both parental strains show highly contrasting phenotypes with regard to product yields in small-scale cultivations and also growth at non-optimum temperatures of 39°C. We generated F14 recombinant inbred lines, of which single hybrids were screened and ranked for their ability to secrete a fluorescence protein and for their growth performance at high (39°C) and at low temperatures (12°C). Hybrids exhibiting extreme values of respective phenotypes were pooled, genomic DNA was extracted for whole-genome re-sequencing and finally, allele frequencies were compared among contrasting pools. Single nucleotide polymorphisms (SNP) within QTL regions were analysed for their effect and indeed, meaningful candidate alleles were identified for each phenotype. While final allele swaps are currently still being conducted, this study already highlights the great potential of QTL mapping for the identification of beneficial gene variants.

Exploring the influence of non-*Saccharomyces* yeast on fermented beverage sensory qualities

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Fermented beverages have been consumed in one form or another by nearly all cultures around the world for millennia, and today fermented beverages such as beer, wine, and cider represent some of the most economically important beverages in the industry. In recent years, there has been a considerable increase in interest in the use of non-*Saccharomyces* yeast in fermentations to meet consumer demands for new beverages with diverse and interesting flavour and aroma profiles. While beer, wine, and cider, are made from relatively few and simple ingredients, the variety of flavours and aromas found in these fermented beverages can vary greatly, with much of this owed to the enzymes and metabolites produced by the yeast strain used in the fermentation process. The increasing use of non-*Saccharomyces* yeasts in fermentations requires a deeper understanding of the metabolic and enzymatic diversity of these yeasts to provide a better knowledge of how their use can impact the sensory properties of the finished ferments. Here we have used a combination of genomics, liquid chromatography-mass spectrometry-based metabolomics, proteomics, and lipidomics approaches to characterize the metabolomic and enzymatic diversity present in a library of native wild non-*Saccharomyces* yeasts. We have identified significant metabolite differences between these native wild yeasts when compared with a commercial *S. cerevisiae* brewing strain, US05, as well as significant differences between genera.

Exploring the phenotypic plasticity of *Saccharomyces cerevisiae* using long term evolutionary programs

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Evidence suggests that the yeast *Saccharomyces cerevisiae* was originally just one of the many varieties of yeast species that inhabit the temperate forests of Asia. Intriguingly, members of this single species have shown sufficient phenotypic plasticity to adapt to and dominate many human derived niches. These domesticated niches include traditional applications as diverse as the manufacture of beer, bread, wine and sake, each of which requires different specialised phenotypes. For over 20 years our research group has been exploiting this plasticity to evolve multiple novel phenotypes in *S. cerevisiae* that expand its industrial utility. Our longest established program has been running for >20 years and has successfully increased the substrate range of *S. cerevisiae* to include the 5-carbon sugar xylose. We also have evolved strains of *S. cerevisiae* that can thrive at pH 2, tolerate the presence of 15% lactic acid, and even vigorously grow in the complete absence of any vitamins or other complex organic nutrients such as inositol. These evolutionary programs demonstrate the remarkable phenotypic plasticity of *S. cerevisiae* and potentially provide a deep insight into the genetic mechanisms underlying the evolution of new phenotypes. However, the programs themselves were inspired by the desire to address the crucial global challenges of providing sustainable renewable fuels to displace fossil carbon and address the challenge of providing sufficient high protein feeds in a future with increasing climate disruption.

Exploring the properties of the lager yeast *Saccharomyces pastorianus* and its ancestral species *Saccharomyces eubayanus* by investigating differences in drug sensitivity

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Saccharomyces pastorianus, a yeast used to brew lager beer, is believed to have resulted from interspecific hybridization between *Saccharomyces cerevisiae* and *Saccharomyces eubayanus*. *S. pastorianus* has some properties derived from *S. eubayanus*, such as low temperature tolerance. However, as *S. eubayanus* was discovered relatively recently (Lidkind *et al. PNAS.* 2011;108(35):14539-1454), many of its supposedly derived properties and their mechanisms are unknown. Therefore, we aimed to explore the properties of *S. pastorianus* and *S. eubayanus* that differ from those of *S. cerevisiae* and to investigate the mechanisms responsible for these properties.

To consider the effects of ploidy and hybridization on drug sensitivity, we first created triploids and tetraploids of *S. cerevisiae*, *S. eubayanus*, and their hybrid strains. We then examined whether the sensitivity of *S. pastorianus*, *S. eubayanus*, *S. cerevisiae*, and the hybrid strains to various drugs was different. We found differences in sensitivity among species to some drugs. We also found that the ploidy of yeasts did not significantly affect their drug sensitivity. In this presentation, we focus on the higher sensitivity of *S. eubayanus* to the ergosterol synthesis inhibitor fluconazole and present some data on the possible mechanisms responsible for these differences.

Exploring the Terroir: Bioprospecting Indigenous Vineyard Yeast for Sustainable Winemaking

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Vineyards are an essential part of the 'terroir', which encompasses the soil, climate, and viticultural practices, which are intimately linked to the regional typicity of wine. The 'terroir' also includes the unique microbiological makeup which defines distinctive wine attributes. Within this microbial terroir, we find non-*Saccharomyces* yeasts, which have gained the interest of winemakers worldwide, as they could be used as a microbiological tool to enhance wine quality (aroma, texture, and length). However, winemakers currently only have access to a handful of non-*Saccharomyces* strains, and this project aims to diversify their choices. Some of the main characteristics of a new yeast candidate are linked to stress tolerance (ethanol, sulfur dioxide, pH, and deficient nitrogen availability), fermentation performance, desirable secondary metabolite production, and the ability to survive freeze-drying. In this study, we created an indigenous yeast library containing 600+ single colonies isolated from the Chalmers Merbein vineyard (Victoria, Australia) and Yalumba Pewsey Vale vineyard (SA, Australia). They were identified using internal transcribed spacer (ITS)sequencing. Nineteen of the Chalmers isolates were pre-screened to assess their ethanol and SO2 tolerance, using YPD agar spiked with increasing concentrations of both stressors, based on species identification. Of these, only thirteen isolates showed some degree of tolerance. Their sugar consumption was evaluated in a chemically defined grape juice (CDGJM) medium, adding twelve Pewsey Vale isolates to the screening. Lastly, SO2 tolerance was evaluated using CDGJM spiked with 20, 35, 50, and 65 ppm using an automated fermentation platform. The most promising candidates (*Hanseniaspora, Kazachstania, Metschnikowia, Saccharomyces,* and *Torulaspora*) withstood 65 ppm SO2, similar to the *Saccharomyces cerevisiae* control. They will be tested further for their volatile production in white and red grape juice, with a view towards broadening the portfolio of winemaking starter cultures.

Extension of *O*-Mannosylation Is Critical for Cell Wall Integrity Signaling and Interaction with Host Cells in *Cryptococcus neoformans*

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C. neoformans assembles two types of *O*-linked glycans on its surface proteins, the more abundant major *O*-glycans that do not contain xylose residues and minor *O*-glycans containing xylose. Two cell surface sensor proteins, Wml1 (WSC/Mid2-like) and Wml2, were found to be independent substrates of Cap6-mediated minor or Ktr3-mediated major *O*-mannosylation, respectively. The double deletion of *KTR3* and *CAP6* (*ktr3*Δ *cap6*Δ) completely blocked the mannose addition at the second position of *O*-glycans, resulting in the accumulation of proteins with *O*-glycans carrying only a single mannose. Tunicamycin (TM)-induced phosphorylation of the Mpk1 mitogen-activated protein kinase (MAPK) was greatly decreased in both *ktr3*Δ *cap6*Δ and *wml1*Δ *wml2*Δ strains. Transcriptome profiling of the *ktr3*Δ *cap6*Δ strain upon TM treatment revealed decreased expression of genes involved in the Mpk1-dependent cell wall integrity (CWI) pathway. Consistent with its defective growth under several stress conditions, the *ktr3*Δ *cap6*Δ strain was avirulent in a mouse model of cryptococcosis. Associated with this virulence defect, the *ktr3*Δ *cap6*Δ strain showed decreased adhesion to lung epithelial cells, decreased proliferation within macrophages, and reduced transcytosis of the blood-brain barrier (BBB). In conclusion, *O*-glycan extension in the Golgi apparatus plays critical roles in various pathobiological processes, such as CWI signaling and stress resistance and interaction with host cells in *C. neoformans*. As there are no human homologs for Cap6 or Ktr3, these fungus-specific mannosyltransferases are novel targets for antifungal therapy.

Extracellular vesicles shuttle information between different wine yeast strains

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Since the beginning of this century, non-*Saccharomyces* yeast starters are gaining market share in the winemaking industry. They are commonly combined with *Saccharomyces cerevisiae* to warrant complete sugar consumption of the grape juice. Researchers in this field are currently interested in the possible interactions between yeast starters, as these can influence the outcome of wine fermentation. This work explores possible communication mechanisms between different wine yeast species. Our group had previously shown that a wine strain of *S. cerevisiae* responds to the presence of competitors by changing its transcription program as soon as 2-3 hours after co-inoculation. It was also shown that *Metschnikowia pulcherrima* extracellular vesicles (EVs) induce in *S. cerevisiae* transcriptional responses akin to those induced by whole living cells, suggesting stimulation of sugar consumption and growth. We have now explored the time course of this transcriptional response, as well as the capacity of EVs produced by other wine yeast species to induce transcriptional changes in *S. cerevisiae*. All yeast species analysed had an impact on the transcription program of *S. cerevisiae* in synthetic grape must. Both common and divergent features arose in the transcription profile. Observed variations might be species or strain-specific; but they can also be influenced by differences in the amount of EVs produced or recovered, or in the response timing. This work was funded by the Spanish Government through grants PID2019-105159RB-I00 and PRE2020-093420 funded by MCIN/AEI/10.13039/501100011033 (and "ESF Investing in your future" training contract for MM).

Fifty Shades Of Red – Production of natural food dyes with yeast cell factories

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Synthetic food dyes are extensively used in the food industry. Recently, however, multiple studies have shown that they can cause hyperactivity, trigger hypersensitivity and be carcinogenic. This, together with an increasing consumer awareness towards sustainable and healthy food ingredients has led to a growing demand for natural alternatives. Betalains are a group of natural pigments that cover a colour spectrum from yellow to red-violet. The bright red betanin (E162) is widely used as red food dye and is the only betalain on the market so far. Betalains come from a renewable resource and are strong antioxidants, but their production is expensive, seasonal and competes with crop plants for resources, making their extraction from plants a non-sustainable process with limited scalability. The production of nature-identical betalains by microbial fermentation is a promising alternative, offering the benefits of natural colours without the disadvantages. We've developed a fermentation-based process for the production of betanin by engineering *Saccharomyces cerevisiae* and *Yarrowia lipolytica* using state-of-the-art genome editing methods.

A downside of betalains is their limited stability. When subjected to light, oxygen, or heat, betanin forms undesired yellow-brown degradation products which prevents a more extensive use of the pigment in food applications. Therefore, there is a need for more stable betalain variants. Furthermore, there is a commercial interest in other shades of red food dye such as a pink or purple colour to broaden the colour portfolio. To address these issues, we exploited the diversity of betalains in nature. Betalains are present not only in beetroots but in many other plants of the Caryophyllales order. By mining the transcriptomes of these plants, we identified candidate genes for "decorating enzymes" that can modify the betanin scaffold by adding sugars or acyl groups and thereby influence both stability and hue of the pigment. The selected genes were screened *in-vivo* in betanin-producing platform strains and led to the production of three new betalain variants in *S. cerevisiae* and *Y. lipolytica* with improved stability properties and a colour shift. These compounds are a starting point for the synthesis of a portfolio of decorated betalains to be used as food colourants.

Fission yeast fermentations

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Schizosaccharomyces pombe is a quite remarkable yeast. From biology to biomedicine and to biotechnology is has contributed greatly to enhancing both fundamental scientific knowledge and development of bioproducts. For example, studies with this yeast species have unravelled the molecular genetic mechanisms of eukaryotic cell cycle control pertinent to human cancer. In the fermentation industries, this fission yeast has not been exploited to the

same extent as the major ethanologenic yeast, *Saccharomyces cerevisiae*. Nevertheless, it does exhibit physiological and metabolic characteristics of interest to winemakers, brewers and distillers such that the flavour of wine, beer and spirits can be favourably influenced by the presence of *Schiz. pombe* in fruit, cereal and sugar fermentations. In oenology, *Schiz. pombe* is able to conduct malo-ethanolic fermentations to deacidify wine, but its role in malt wort fermentations is less well known. Work will be presented on recent research pertinent to beer and whisky fission yeast fermentations. Regarding Scotch Whisky, there is scope to employ more flavoursome non-*Saccharomyces* yeasts and *Schiz. pombe* has been evaluated in fermentations designed to mimic industrial-scale malt whisky processes. By monitoring CO_2 evolution kinetics it was found that *Schiz. pombe* fermented malt whisky wort well compared with a commercial distilling strain of *S. cerevisiae*. This presentation will review some of the fermentative properties of *Schiz. pombe* that distinguish it from *S. cerevisiae* in alcoholic beverage production processes and will highlight several of the biotechnological attributes of fission yeast that make it a prime candidate for further exploitation in industrial fermentations.

Friendly yeasts for winemaking: flavours involved in yeast interactions mediated by cooperation or competition events

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In the last decade yeasts diversity in wine fermentation begun to be considered an alternative to traditional inoculation using pure *Saccharomyces cerevisiae* strains. Particularly, the non-*Saccharomyces* yeast *Hanseniaspora vineae* has been characterized by allowing the growth of other native yeasts compared to the competitive behaviour of most selected *Saccharomyces* strains. Therefore *H. vineae* has been considered a friendly yeast for vinification1, being successfully used in winemaking through mixed cultures, giving desirable aromas to wine. In fact, some flavour molecules have been described as potential mediators in yeast interaction. Among them, some positive flavour compounds such as benzenoids and acetate esters of higher alcohols are produced at significantly higher levels in *H. vineae* compared to *S. cerevisiae*. By contrast, the production of higher alcohols and short chain fatty acids have been related with more competitive strains that reduce the natural biodiversity in fermentations. The present study focuses on evaluating the impact of higher alcohols derived from aromatic amino acids and their corresponding acetates on the growth of different yeast strains. Three *H. vineae* and four *S. cerevisiae* strains applied in oenology were grown in microplates, their optical density was monitored at 620nm for 48 hours in presence of different higher alcohols and their acetylated derivatives in order to analyse their effect. The results obtained suggest that high levels of alcohols and mixed alcohols and acetates caused a decrease in yeast growth for some strains compared with pure acetates. Highly acetylation capacity could be related with the friendly character of *H. vineae*

Functional amino acids engineering: A new breeding technology for brewing yeasts

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Amino acids are important not only as protein components of living organisms, but also as nutrients and energy sources. In recent years, many amino acids exist in free form and play important roles in cells, and thus, their physiological functions have been attracting attention. Various foods, beverages, nutritional supplements, and cosmetics, containing these amino acids have been commercialized worldwide. In yeast, amino acid metabolism vary under different growth environments and metabolic modes by regulating anabolic and catabolic processes, including uptake and export. Controlling the amino acid content is expected to contribute to improved productivity and value-added fermented foods and alcoholic beverages. The development of industrial yeast strains that overproduce "functional amino acids" could lead to improvement of fermentation ability, diversity of product taste and flavour, addition of healthy images, or increase of nutritional value. To emphasize these advantages, I named this breeding technology 'functional amino acids engineering'. The yeast *Saccharomyces cerevisiae*, which is widely used in the fermentation industry, has been certified as 'Generally Recognized as Safe (GRAS)' by the US Food and Drug Administration (FDA), demonstrating its high safety. In this lecture, I will introduce two topics of "functional amino acids engineering" with application to brewing of alcoholic beverages, focused on the new metabolic regulatory mechanisms of 'valine' and 'proline' in *S. cerevisiae*, e.g., 1) improvement of valine and isobutanol production in sake yeast by the acetohydroxy acid synthase variant and 2) isolation of *S. cerevisiae* strains with higher utilization of proline abundant in grape must and wort.

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Genetic and physical interaction of Vps13 lipid transporter with Rsp5 E3 ubiquitin ligase: coordination of lipid synthesis with lipid transport?

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Several rare neurodegenerative diseases depend on mutations in *VPS13A-D* genes, including chorea-acanthocytosis and early-onset Parkinsonism. *VPS13* genes are conserved from yeast to humans and yeast with unique *VPS13* is a good model system to study function of Vps13 proteins. Recent findings show that Vps13 protein transports bulk lipids at membrane contact sites. Localization of Vps13 is dependent on interactions with specific proteins and lipids which are characteristic to different subcellular compartments. The lipids determining the localization of Vps13 are quite well defined, but the proteins are not. To investigate Vps13 protein partners we purified Vps13 from yeast cells by pull down and identified interacting proteins using mass spectrometry. Among proteins recognized, we found Rsp5 ubiquitin ligase. The Vps13-Rsp5 interaction was confirmed by immunoprecipitation and Western Blot. Rsp5 is important for the lipid homeostasis. It ubiquitinates and is involved in proteasomal maturation of the transcriptional activators, Spt23 and Mga2, which regulate the expression of genes encoding lipid biosynthetic enzymes. Increased levels of activated Spt23 or Mga2 result in the formation of gigantic lipid droplets. This prompted us to analyse genetic interaction between *VPS13* and *RSP5* genes and we found that additional copies of the *RSP5* gene introduced into *vps13*Δ mutant cells increase their sensitivity to sodium dodecyl sulphate. We also observed the lower level of Rsp5 E3 ubiquitin ligase in *vps13*Δ compared to the wild type cells. These results suggest that the Vps13-Rsp5 interaction may be important for coordination of the lipid synthesis with lipid transport capacity of the cell.

Getting Rid of Greenness in Wines - A Yeast Strategy

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3-Isobutyl-2-methoxypyrazine (IBMP) is an aroma compound with potent smells reminiscent of green capsicum. It naturally occurs in many wine grape cultivars, such as Cabernet Sauvignon and Sauvignon Blanc. High levels of such compound in grapes may result in excessive greenness in wine products, thus reducing quality. Non-*Saccharomyces* yeasts, identified with versatile metabolic activities, were applied in mixed fermentation as a potential postharvest remediation strategy. In this study, 11 strains of non-*Saccharomyces* were investigated on their impacts on the concentration and perception of IBMP in wines through fermentation of IBMP-spiked juices. Wines were sequentially inoculated with these strains and *Saccharomyces cerevisiae* yeast strain EC1118, and several non-*Saccharomyces* yeast strains were noted to differentially mask the organoleptic perception of IBMP. Specifically, strains of *Kazachstania servazzii*, *Metschnikowia pulcherrima*, *K. aerobia*, *Hanseniaspora uvarum*, *Meyerozyma guillermondii* and *Candida krusei* were identified as promising candidates since the resulting wines were evaluated with stronger fruitiness and less greenness in sensory study, even though no significant difference on IBMP concentrations was observed amongst yeast treatments. For grapes containing excessive levels of IBMP, application of specific non-*Saccharomyces* yeasts during fermentation may well be a valid strategy for greenness modulation from sensory perspectives.

Hanseniaspora vineae application for low input strategies in fermented foods. Searching for sustainability and flavor complexity.

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Environmental conditions, such as climate, soil composition, water management, winds and air quality, altitude, fauna and flora, and microbes, are considered part of "terroir" and contribute to differentiate fermented food styles. If "low input production" strategies are applied, the terroir or regional effect can be expected to be more authentic in terms of quality differentiation. Curiously, role of microbial flora associated with grapes, apples or cereal flours has been little studied until a few years ago, when new genetic technologies were developed for the mass identification of species. These biotechnologies together with native yeast strains selection and pure yeast fermentations for sensory screenings allowed us to identify *Hanseniaspora vineae* as a superior flavor yeast producer. Some strains of this species are consistent producers of excelent flavors and there were defined as "friendly" yeast because share fermentation niches with native yeast from grapes, including diverse strains of *Saccharomyces*[2].In this work we explain the interesting positive effects on

yeast and flavor diversity and quality of wine, cider and bread, obtained using *H. vineae* strain HV205, the current commercial strain available of this species. Sensory data are correlated with chemical GC-MS analysis that showed the flavor footprinting characteristics of *H. vineae* under fermentation. Briefly, in the three food substrates studied significant increases of acetoin, isoprenoids, phenylpropanoids and their acetate esters; and decreases of higher alcohols and medium chain fatty acids were obtained in the final products. Furthermore, increased yeast strains diversity was measured during fermentations with *H. vineae* compared to conventional *Saccharomyces* strains inoculated processes.

Hyphal growth in *Trichosporon asahii* is induced by the addition of magnesium

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Fungal dimorphism involves two morphologies: a unicellular yeast cell and a multicellular hyphal form. Invasion of hyphae into human cells causes severe opportunistic infections. The transition between yeast and hyphal forms is associated with the virulence of fungi; however, the mechanism is poorly understood. Therefore, we aimed to identify factors that induce hyphal growth of *Trichosporon asahii*, a dimorphic basidiomycetous yeast known as a human fungal pathogen that causes trichosporonosis. Here, we demonstrated that magnesium is a key factor for hyphal growth, and the addition of magnesium causes changes in gene expression pattern in *T. asahii*. We found that *T. asahii* showed poor growth and formed small cells containing large lipid droplets and fragmented mitochondria when cultivated in a nutrient-deficient medium. These phenotypes were suppressed via the addition of yeast nitrogen base. When *T. asahii* cells were cultivated in the presence of different compounds present in the yeast nitrogen base, we found that magnesium sulfate was a key factor for inducing cell elongation, and its addition dramatically restored hyphal growth in *T. asahii*. When magnesium sulfate was added, vacuoles were enlarged, the size of lipid droplets was decreased, mitochondria were distributed throughout the cell cytoplasm and adjacent to the cell walls; expression of some genes was increased. Collectively, our results suggest that an increase in magnesium levels triggers the transition from yeast to hypha in *T. asahii*, which accompanied by changes in dynamics of cell organelles and expression of several genes; and will support studies on the pathogenesis of fungi.

Impacts of Environmental Conditions and Initial Yeast Community Composition upon Kombucha SCOBY Community Assembly and Fermentation Outcomes

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Kombucha is sweetened tea fermented with a cellulosic biofilm known as the SCOBY (Symbiotic Culture of Bacteria and Yeast), that is re-pitched into successive batches. Most commercially used SCOBY are dominated by two core species: the yeast *Brettanomyces bruxellensis* and acetic acid bacterium *Komagaetibacter rhaeticus* (BK archetype). However, we previously observed three minor archetypes across Northern America. What drives assembly of the BK archetype, and under what conditions are these core species interchangeable? As a first step towards addressing this question we varied kombucha fermentation environmental conditions and microbial inoculum complexity, then evaluated yeast (ITS) and bacterial (16S) community composition in the biofilm and fermenting liquid by metabarcoding.

Environmental conditions (sugar type/concentration, surface-area-to-volume (SA:V) ratio, and temperature) had significant effects on tea acidification, whereas biofilm formation was most affected by SA:V ratio. PERMANOVA on dissimilarity matrices for ITS and 16S amplified sequence variants showed there were significant differences in yeast and bacterial communities between biofilm and liquid, and that community structures were affected by environmental conditions. Surprisingly, we did not observe the dominant BK SCOBY archetype under any conditions. Instead, *Picha occidentalis* and *K. rhaeticus* were most abundant in biofilm while *Lachancea fermentati* and *Gluconobacter oxydans* dominated liquid phase. Interestingly, when *L. fermentati* was excluded from the inoculum no biofilm formation was observed. When *P. occidentalis* was excluded, *L. fermentati* was abundant in both phases. Our results highlight the impact of yeast upon kombucha fermentation outcomes and show that biotic interactions influenced spatial heterogeneity in kombucha microbial community assemblies.

Improving *Saccharomyces cerevisiae* phenotypes for enhanced first-generation ethanol production

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First-generation (1G) ethanol is produced by fermentation of six-carbon sugars (glucose and fructose) using starch-rich or sugar crops. Over 130 billion litres of 1G ethanol are produced per year with the USA (corn) and Brazil (sugarcane) being the major producers. Strains of *Saccharomyces cerevisiae* are the primary yeasts used for 1G ethanol production, taking advantage of the strong anaerobic fermentation capabilities of this species. Speed of fermentation, ethanol tolerance, organic acid resistance, thermotolerance, and other phenotypes are critical to the yields and productivities of ethanol. Until recently, the corn ethanol industry was dominated by one incumbent yeast strain known as Ethanol Red. This yeast is an immensely proficient industrial biofuels strain, but it has limitations particularly in terms of ethanol tolerance and thermotolerance. There is therefore a need for yeast strains with stronger performance features. We have used classical genetics to develop yeast strains with improved phenotypes for 1G ethanol applications. These improvements have included enhanced speeds of fermentation, abilities to reach higher titres of ethanol, reduced production of unwanted byproducts, improved thermotolerance enabling fermentations to run at higher temperatures without damaging yeast performance, and greater osmotic resistance allowing for higher concentrations of substrates to be used. The classical approach to strain development has successfully pushed the phenotypic boundaries of 1G yeast, transcending the previously recognised limits of its capabilities. In this presentation we will highlight the advancements in yeast phenotypes raising the intriguing question of "what is the true potential of yeast"?

Improving the efficiency of one carbon metabolism in *K. phaffii* through enzyme and metabolic engineering

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Komagataella phaffii (Pichia pastoris) is a methylotrophic yeast species used widely in the biotechnology industry primarily as a host for heterologous protein production. However, due to the ongoing development of methanol as a green industrial feedstock, there is also growing interest in the potential of *K. phaffii* as a whole-cell biocatalyst for the conversion of methanol into biomass and/or industrial chemicals. The first step in the yeast methanol utilization pathway, catalyzed by methanol oxidizing enzymes Aox1 and Aox2, is a limitation to the efficiency of this conversion as a large part of the energy is lost as heat instead being transferred to a cellular energy carrier. It has been demonstrated that in the absence of Aox1 and Aox2 *K. phaffii* can instead utilize methanol through the native alcohol dehydrogenase 2 (Adh2), significantly increasing the energetic efficiency of the pathway through the production of NADH (1). We have applied metabolic and process engineering to develop such Adh-based *K. phaffii* strains as hosts to produce biomass and organic acids from both methanol and CO_2 (2). In parallel we aimed to use enzyme engineering to improve the activity of Adh2 towards methanol using both a targeted semi-rational approach and a directed evolution approach coupled with high-throughput screening. Here we present our recent progress from the combined strain and enzyme engineering efforts towards generating a robust, carbon-efficient *K. phaffii* host for the production of industrial chemicals from methanol and CO_2.

Increased production of riboflavin on lignocellulose hydrolysates by the flavinogenic yeast *Candida famata* as the effect of adaptive laboratory mutagenesis

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Riboflavin, also known as vitamin B_2 is a precursor of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which play a key role as cofactors in energy metabolism and are required for co-enzyme function in numerous oxidation and reduction reactions in all aerobic forms of life. Thanks to many genetic modifications and the use of modern methods of metabolic engineering, *Candida famata* yeast can still compete with other species used in industry for the production of riboflavin. Taking into account the fact that one day natural energy sources will run out, the search for alternative energy sources has already begun and measures have been taken to reuse industrial waste, that can be reused to produce useful compounds such as vitamin B_2. The general hunger prevailing in the world makes it unethical to use sugar-rich raw materials such as cereals or sugar cane in industry, but lignocellulosic waste obtained from energy crops, wood chips and sawdust, as well as agricultural residues are the world's largest source of renewable biomass, which are treated as non-edibility waste. As we have shown, the use of waste, which is lignocellulosic hydrolysate (HL) from wheat straw, can be used as an excellent alternative for the production of riboflavin. The most efficient strains in minimal medium with 20% HL were able to achieve a productivity of 320 mg RF/g biomass. Inhibitors created during lignocellulose hydrolysis (furfural, HMF, acetic acid) have a negative effect on cells, which is why we decided to use evolutionary mutagenesis to select cells better adapted to difficult conditions. In addition, the strains were exposed to UV radiation, which further increased the mutation rate. The obtained mutants were characterized by better growth and/or better production of riboflavin. The best of them achieved a productivity of 350 mg RF/g of biomass. The maximum concentration of riboflavin in the medium during fermentation for the mutant was about 660 mg/L, which turned out to be a better result by 10% compared to the initial strain. The use of this method, especially long-term (> one month), may determine the growth of mutants showing better growth and production of vitamin B2.

ABSTRACTS

Increasing the carbon efficiency of citric acid production

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Citric acid is one of the most important organic acids produced by fermentation with the filamentous fungus *Aspergillus niger* using glucose as substrate. This organic acid is extensively used in food, cosmetic and pharmaceutical industries with a production capacity of nearly 2 million tons per year (Steiger et al., 2017). Citric acid is produced in the tricarboxylic acid cycle (TCA) from oxaloacetate and acetyl-CoA. In glycolysis, 1 mol glucose is oxidized and converted into 2 mol of pyruvate. 1 mol of pyruvate is carboxylated to oxaloacetate, the other is decarboxylated to acetyl-CoA, resulting in the net reaction: Glucose + 3 NAD+ + H_2O ⇌ Citrate + 3 NADH While this pathway leads to a theoretically balanced carbon yield it is not redox balanced and re-oxidizing the NADH leads to high oxygen consumption and heat release (Karaffa & Kubicek, 2003). Mixed-substrate conversion allows to incorporate CO_2, a cheap carbon source, into products (e.g. organic acids) with higher oxidation states than the co-substrate (e.g. glucose). This is a promising strategy to fix CO_2 in an industrial process and increase the total carbon yield of the process without requiring oxygen as an electron acceptor, hence reducing the need for extensive cooling (Steiger et al., 2017). The aim of this research is to increase the carbon efficiency of citric acid production by developing a synthetic pathway that avoids decarboxylation, hence leading to a net CO_2 assimilation during the mixed-substrate production of citric acid. The pathway, expressing the respective genes under control of methanol regulated promoters, is being incorporated into the yeast *Komagataella phaffii (Pichia pastoris)* to create an orthogonal test system. Preliminary results of this research have shown that the citric acid transporter genes of Yarrowia lipolytica and *Aspergillus niger* were successfully expressed in *K. phaffii*. After evaluation, the best pathway variants will be transferred into *A. niger*.

Indigenous Wine Yeast from China: Biodiversity, Oenological Properties, and Potential Application in Winemaking

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To comprehensively explore the indigenous wine yeast resources from China, over 20,000 yeast isolates were collected from spontaneous fermentations over multiple vintages in typical regions. Results show that yeast species and dynamics exhibit distinct distribution patterns at national, regional and vineyard scales. They were also largely influenced by grape varieties, fermentation stage and anthropogenic practices. Various genotypes of *Saccharomyces cerevisiae* and strains of non-*Saccharomyces* yeast were evaluated for oenological properties, including tolerance to typical winemaking related stressors, H_2S production, fermentation duration and flavour formation. Yeast strains with desirable traits, e.g. near-freezing temperature tolerance, high sugar tolerance, low H_2S and higher alcohol production, and ability to induce flavour modulation were obtained. This further allowed investigation of the related underlying mechanism. In specific, for *S. cerevisiae*, QTL mapping revealed that *NAT1* played a role in the near-freezing temperature tolerance whilst RNA-seq showed that *MET2*, *MET3*, *SER33* were associated with H_2S production. We also demonstrated that the amount of higher alcohols produced by *S. cerevisiae* can be ameliorated by both chaptalisation and supplement of nitrogen during fermentation. The best performing yeast strains were then evaluated by pilot and winery-scale trials across multiple vintages in various wine regions. Strain NX11424 and LFE1225 continuously displayed high sugar tolerance and greater production of esters, and were successfully commercialised by Angel Yeast, known as CECA and CEC01, respectively. Our study showed the high biodiversity of yeast with varying phenotypes harboured in Chinese wine regions, and highlighted the use of indigenous yeast to enhance Chinese wine quality.

Intrageneric genomic relationship and evolution of non-model Basidiomycota yeasts based on chromosome-level genome assemblies

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Genomic information is a powerful tool for exploring fungal characteristics and phylogeny. To elucidate the fungal diversity in the environment and taxonomic relationships of non-model yeast strains, we sequenced the multiple genomes from two groups of Basidiomycota yeasts, *Cutaneotrichosporon* and Sporidiobolaceae. These taxa include both industrially useful species, viz oleaginous yeasts, and medically important species, with *C. oleagenosum* and *Rhodotorula toruloides* being the representatives in the former and *C. cutaneum* and *R. musilaginosa* in the latter.

In *Cutaneotrichosporon*, we sequenced six strains including *C. cavernicola* and related species. Chromosome-level assemblies indicated that the chromosomal synteny was little conserved between *C. cavernicola* and a related species, *C. spelumceum*. As their ITS barcode sequences are well conserved (98.7% identity) and the Average Nucleotide Identity (ANI) was 81%, chromosomal synteny data were highly divergent from those shown in the model yeast genus *Saccharomyces* (98.9% ITS identity, in *S. cerevisiae* and *S. paradoxus*). The data suggested that the rate of evolutionary speed among barcode sequence, whole-genome sequence and chromosome synteny differ among fungal taxa.

In Sporidiobolaceae, we sequenced a total of 24 strains including *R. toruloides*, *R. kratochivirovae*, *R. sphaeocarpa* and several type strains of this and related genera. ANI indicated that *R. toruloides* had higher intraspecific diversity than other species examined, *R. kratochvilovae* and *R. sphaerocarpa*. In addition, the genomes of *R. toruloides* JCM 10020 and JCM 10021 had significant chromosome rearrangement levels. We present the genomic relationship of these yeast strains at the sequence and structural levels and discuss phylogenetic relationships and evolution.

Investigating mitotic stability of a synthetic pan-genome neo-chromosome

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A synthetic pan-genome neo-chromosome (PGNC) was built using unique pan-genomic sequences from over 200 industrial and environmental isolates of *Saccharomyces cerevisiae*. These unique sequences were incorporated into a single DNA molecule and expressed in the laboratory strain S288c. To assess the mitotic stability of the PGNC, its circular and linear variants were serially passaged under non-selective conditions (media without ClonNAT) for 50 generations. The results showed that a substantial number of isolates of the circular, as well as the linear variants, lost the PGNC element during growth in a non-selective environment. The losses ranged from nearly 40% in the most stable circular variant, to almost complete loss in the least stable linear variant. In this study, we evaluated the impact of an efficient, early-firing autonomously replicating sequence from laboratory *S. cerevisiae* S288c, ARS305, on the mitotic stability of PGNC.

Investigation of characteristic orthologs of Ascomycota and Basidiomycota: insights from principal coordinates analysis

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Genomic data are useful for constructing a reliable molecular phylogenetic tree as well as for identifying phenotypic or genomic information that distinguishes a clade from others. In the previous ISSY meeting, we reported that the characteristic orthologous groups (OGs) of Ascomycota (Saccharomycotina and Pezizomycotina) fall into two categories: those that were present in the ancestors of Dikarya and commonly lost in Basidiomycota, and those that were not present in the ancestors of Dikarya but were obtained by Ascomycota after it diverged from Basidiomycota. As the characteristic OGs of Basidiomycota showed a similar pattern, we assumed that an important genomic change occurred when Ascomycota and Basidiomycota diverged from the ancestors of Dikarya.

As the morphology of the sexual state is widely considered the most important taxonomic criterion in fungi, we investigated the OGs potentially involved in sexual state development based on ortholog group annotation data. The results showed that OGs that included genes annotated as "sporocarp" were distributed across most fungal species, whereas the OG containing "basidium/basidia" was not found in the dataset. OGs containing "ascospore" showed a distinctive distribution pattern; most were present in numerous fungal species, but 2 OGs were present only in Ascomycota. The distribution of OGs containing "prospore membranes" also showed relatedness with taxa; for example, OG0008357 (YML066C, in *Saccharomyces cerevisiae*) was present in Saccharomycetes and Pichiomycetes, and was absent in other taxa. We consider that the presence or absence of OGs could correlate with morphology data when taxon-characteristic OGs are investigated.

In vivo directed evolution for metabolic engineering

Verena Siewers

Saccharomyces cerevisiae has been engineered to produce a plethora of industrially relevant compounds including biofuels, chemicals, nutraceuticals and pharmaceuticals. Introduced heterologous pathways can however suffer from low enzyme activity or specificity. In such cases, directed evolution is often employed to improve enzyme properties. Traditionally, this comprises iterative rounds of gene diversification *in vitro*, transformation, selection or screening and isolation of improved variants, which can be time consuming. In the past years, several methods that allow for targeted mutagenesis *in vivo*

- and thus continuous directed evolution - have been developed. Here, two systems based on Cas9 variants will be presented: yEvolvR, where a Cas9 nickase directs an error-prone DNA polymerase to its designated target site, and the employment of nucleobase deaminases coupled to a deactivated Cas9. The latter was employed to improve performance of a heterologous transporter in *S. cerevisiae*.

Isolation of *Saccharomyces cerevisiae* strains with higher proline uptake and their applications to beer and wine fermentation

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Proline is the most abundant amino acid in beer and wine because *Saccharomyces cerevisiae* cells poorly assimilate proline during fermentation processes. An excess of residual proline results in the risk of proline-polyphenol hazes, and a bitter and mild acidic taste. We recently developed a rapid method for measuring residual proline with the dye isatin (*Biosci. Biotech. Biochem.*, **87**, 358-362, 2023) and obtained here *S. cerevisiae* strains that better utilize extracellular proline. We first found that proline was not consumed in synthetic complete (SC) medium inoculated with an ale strain of *S. cerevisiae*, while no proline remained in the medium inoculated with a *Lachancea thermotolerans* strain, which had previously been known to assimilate proline. After validation of this method, additional 671 *S. cerevisiae* strains from the Phaff Yeast Culture Collection (phaffcollection.ucdavis.edu) were subjected to the screening. These strains include commercial beer and wine strains as well as strains isolated for research purposes. We finally obtained 67 strains that consumed more than 60% of the proline in the original medium. The 67 strains were also used to ferment wort and synthetic grape must (SGM). Intriguingly, 3 and 27 out of 67 strains produced ethanol as much as commercial yeasts did in the wort and SGM, respectively. These results suggest that *S. cerevisiae* strains that utilize proline contribute to improvement in beer and wine fermentation. We plan to conduct a whole genome sequencing analysis to identify what genes are responsible for proline utilization during the fermentation processes.

Kazachstania yeasts may lower bread fructan content in extended dough fermentations

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Kazachstania is a common yeast genus found in sourdough starters, and since sourdough bread tends to have a lower fructan content than non-sourdough bread, we are interested in the potential ability of *Kazachstania* yeasts to directly metabolise bread fructans. We used a Maximum Likelihood analysis to investigate the genetic similarities between *Kazachstania* yeasts derived from sourdoughs and those associated with other environments. Most sourdough-associated yeasts are found in one sub-clade of the genus. We performed several tests on a collection of *K. humilis* and *K. bulderi* yeasts, using inulin and fructo-oligosaccharides (FOS) as test carbohydrates. When grown on inulin- and FOS-supplemented media, we found that the *K. humilis* yeasts grew better than the *K. bulderi* yeasts on inulin, suggesting that the invertases of *K. humilis* may perform better on longer substrates. We then compared the ability of extracellular and cellular fractions of yeast cultures to metabolise FOS and inulin and found that FOS degradation was greater for the cellular fractions, suggesting that most of the enzymes responsible may be cell wall bound. Fermentation time in breadmaking can be a significant variable and our initial studies indicate that *Kazachstania* yeasts require longer than 12 hours to metabolise FOS, which indicates that longer fermentations may be necessary to make low-fructan bread. These data demonstrate that ability to break down fructans varies between *Kazachstania* strains and species, and that manipulation of fermentation parameters can contribute to the effectiveness of yeast metabolism in bread.

Let it stick: developing post-translational lipidations as tools for intracellular plasma membrane targeting in *Saccharomyces cerevisiae*

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Lipid binding domains and protein lipidation are essential features to recruit proteins to intracellular membranes, enabling them to function at specific sites within the cell. Membrane association can also be exploited to answer fundamental and applied research questions, from obtaining insights into the understanding of lipid metabolism to employing them for metabolic engineering to redirect fluxes.

Both lipid-binding domains of various origins and co- and post-translational lipidation signals encoded in the amino acid sequence (prenylation, palmitoylation, and myristoylation) were evaluated as tools to target the plasma membrane by fusing them to mCherry. Both native and engineered targeting sequences were tested. Qualitative and quantitative confocal microscopic image analysis were used to investigate the localization of prenylated mCherry as a reporter protein in the exponential and stationary phase. Some constructs showed cytoplasmatic or dual targeting, while others such as the combined and engineered lipidations signals were located almost exclusively at the plasma membrane.

By systematically analysing and scoring potential plasma membrane targeting signals, we developed a useful toolbox for fundamental and applied yeast research. Moreover, thanks to the conservation over species, this toolbox might find applications in other yeasts and even mammalian cells.

Live while the DNA lasts. The autophagy-dependent DNA loss in diploid yeast during chronological aging

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Aging is inevitable. Nevertheless, researchers keen to delay aging are exploring its mechanisms. Yeast cells are used as a model to study aging because it affects all cell types. There are two approaches to studying aging in yeast: replicative aging, which describes the proliferative potential of cells, and chronological aging, which is used for studying post-mitotic cells. While analyzing the chronological lifespan (CLS) of diploid yeast cells, we discovered a remarkable phenomenon: ploidy reduction during aging progression. We aimed to uncover the mechanism behind this unusual process. Using *Saccharomyces cerevisiae* strains undergoing a CLS assay, we measured various aging parameters over time, e.g., survival rate, DNA content, autophagy induction. We showed that during the CLS assay, dying cells lost their DNA, and only diploids survived. We found that autophagy was responsible for the gradual loss of DNA. The nucleophagy marker was activated at the beginning of the CLS experiment, correlating with the significant drop in cell viability. The activation of piecemeal microautophagy of nucleus (PMN) markers appeared to accompany the chronological aging process until the end. Our findings suggested that a single genomic copy was sufficient to rebuild the diploid genome and promote the survival of post-mitotic diploid cells. During chronological aging, cellular components, including DNA, are exposed to increasing stress, leading to DNA damage and fragmentation in aging cells. We propose that PMN-dependent clearance of damaged DNA from the nucleus helps prevent genome rearrangements. However, as long as one copy of the genome can be rebuilt, cells can survive.

Low temperatures reshape yeast metabolism during wine fermentation

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Low temperature fermentation is considered to contribute to aromatic enrichment and improve wine quality. The metabolism of *Saccharomyces cerevisiae* responding to low temperatures is intricate and this complexity is further enhanced by various strains and culture media. However, the effect of low-temperature fermentation on yeast metabolism is not clarified. To illustrate the formation of yeast metabolites in the low-temperature white winemaking range, fermentations were carried out at 10, 15, and 20°C using five wine yeast strains in two media. The tolerance of wine yeast to low temperatures and the composition of metabolites, including basic chemical components and volatile aromatic compounds) were analyzed, demonstrating a considerable impact of low temperatures on yeast metabolism. In the resulting wines, ethanol, ethyl acetate, and ethyl butanoate increased with decreasing temperature, while acetic acid, phenylethanol, phenylethyl acetate, ethyl decanoate, and ethyl hexadecanoate decreased with decreasing temperature. The linear relationship between fermentation temperature and the formation of ethanol, acetic acid, and phenylethanol might be fundamentally due to the temperature-induced changes in growth. The production of ethyl acetate and ethyl butyrate increased with decreasing temperature, which might be due to the stimulation of enzymes in the metabolic pathway by low temperature. These findings reveal the typical characteristics of yeast-derived metabolites during low-temperature fermentation and provide evidence for the application of low-temperature winemaking in the wine industry.

Mechanisms of stress resistance mediated by methionine metabolites

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In methionine metabolism pathway, *S*-adenosyl-L-methionine (SAM) is synthesized from methionine and ATP, and is the methyl donor utilized in the majority of biological methylation reactions. Upon demethylation, SAM is converted into *S*-adenosylhomocysteine (SAH), a potent competitive inhibitor of SAM-dependent methyltransferases. Therefore, SAH is degraded by *SAH1* which encodes SAH hydrolase. We found that *SSG1* mutant of laboratory yeast extends lifespan with a higher accumulation of SAM and SAH (Ogawa et al., 2016). The *SSG1* mutation is an allele of the *YHR032W*, whose function are unknown, and Ssg1 protein is 36 amino acids longer than Yhr032w by frameshift mutation due to *SSG1* mutation. Interestingly, industrial yeast such as *sake* yeast originally has the same sequence of *SSG1*, so *SSG1*, but not *YHR032W,* is a real wild-type gene. This suggests that *SSG1* is a mutation that can recover its original function. Accordingly, the purpose of this study is to elucidate the characteristic of brewing with *sake* yeast at the molecular level through analysis of phenotype with *SSG1*.

ABSTRACTS

We found that *SSG1* mutant shows increased stress resistance, such as oxidative stress and heat stress in addition to longevity. Interestingly, many of the phenotypes exhibited by *SSG1* mutants can be mimicked by SAH supplementation (Ogawa et al., 2022). Since yeast are exposed to various stresses during the process of fermentation, *SSG1* and SAH may play an important role in these mechanisms of stress response.

Metabolic Engineering of *Pichia pastoris* for Sustainable Production of Biofuels and Chemicals

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Metabolic engineering has become an essential tool in the quest for renewable and sustainable alternatives to traditional fuels and chemicals. In this conference, we present a collection of research achievements focusing on the metabolic engineering of the industrially relevant yeast *Pichia pastoris* for the production of biofuels and valuable bioproducts from renewable carbon sources. Our studies detail the optimization of metabolic pathways and strain improvement strategies to enhance the production of isobutanol, isopentyl acetate ester, 3-methyl-1-butanol (3M1B), and D-lactic acid in engineered *P. pastoris* strains, utilizing glucose, glycerol, and sugarcane trash hydrolysates as feedstocks. Key metabolic engineering strategies include overexpression of endogenous amino acid biosynthetic pathways, keto-acid degradation pathways, and heterologous xylose isomerase, as well as the use of CRISPR/Cas9 system for down-regulation of side-product ethanol production. Furthermore, our work demonstrates how manipulating yeast flocculation can enhance tolerance to D-lactic acid, resulting in improved bioproduct production. By integrating advanced metabolic engineering techniques with strain improvement, our findings showcase the potential of *P. pastoris* as a versatile production platform for sustainable biofuels and high-value chemicals. This work contributes to the development of economically efficient and environmentally friendly solutions to address climate change concerns and highlights the power of metabolic engineering in advancing renewable technologies.

Metabolic engineering of the sphingolipid biosynthetic pathway to enhance production of long chain bases and human-type glucosylceramides in *Yarrowia lipolytica*

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Sphingolipids are essential membrane components in mammalian cells as well as in plants and microbes. *De novo* sphingolipid biosynthesis begins with the condensation of L-serine and palmitoyl-CoA to yield 3-keto sphinganine, which is reduced to yield sphinganine (dihydrosphingosine, DHS). Fungi mostly generate two distinct species of glycosphingolipids, mannosyl-inositolphosphorylceramides (MIPCs) and glucosylceramides (GlcCers) as major products. In this study, we delineated and engineered sphingolipid biosynthesis pathway in the oleaginous yeast *Yarrowia lipolytica* with dimorphic growth. To block the fungal specific phytosphingosine-based sphingolipid biosynthesis pathway, we disrupted *Y. lipolytica SUR2*, encoding sphinganine C4-hydroxylase responsible for conversion of DHS to phytosphingosine. The resultant *Ylsur2*Δ mutant exhibited a retarded growth with increased pseudohyphal formation and displayed the increased stress sensitivity compared to the wild-type strain. Notably, the *Ylsur2*Δ mutant showed remarkably increased production of DHS, which was mostly secreted to the cell surface. The *Ylsur2*Δ mutant also showed the enhanced production of sphingosine (So) and GlcCers at the cell surface. Moreover, the altered profiles of IPCs and sterols were observed in *Ylsur2*Δ. Subsequent disruption of the *SLD1* gene, encoding fungal specific Δ8 sphingolipid desaturase, restored the filamentous growth of *Ylsur2*Δ to yeast-type growth and increased the production of human-type GlcCers. Additional introduction of mouse ceramidase into the *Ylsur2*Δ*sld1*Δ double mutants led to a significantly increased production of DHS and So. Our results present the high potential of the engineered *Y. lipolytica* strains for the secretory production of non-acetylated long chain bases and human-type GlcCers, which are useful ingredients for pharmaceutical, cosmeceutical, and nutraceutical formulations.

Metabolic engineering of yeasts for the production of betalain-type natural colors

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Betalains are natural red-purple-yellow pigments found in plants of the order Caryophyllales and fungi of the *Amanita* genus. While nearly a hundred natural betalains have been identified, only red pigment betanin is produced commercially, by extraction from red beets. The betanin content in red beets is very low, ~0.2% wet weight, and the downstream process is challenged with impurities, such as geosmin, nitrates, and sugars, co-extracted with the pigment. We developed a yeast-based fermentation process to produce betanin and several other betalain pigments. The work comprised biosynthetic pathway discovery, optimization of pathway expression and precursor supply, and prevention of product degradation. Due to the simple visual detection of betalains, parts of the strain improvement program could be carried out using high-throughput genome engineering and screening. Engineered oleaginous yeast *Yarrowia lipolytica* made ~1.2 g/L betanin in 48-h fed-batch fermentation. According to the life cycle assessment, the fermentation process would have a significantly lower impact on resources and ecosystem quality than the traditional extraction of betanin from beets.

Metabolic interactions of *Saccharomyces cerevisiae* cocultures: a way to extend the aroma and chemical diversity of Chardonnay wine

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Saccharomyces and non-*Saccharomyces* co-inoculations in winemaking have been investigated in various applications, and especially in the context of modulating the aromatic profiles of wines. Our study aimed to characterize *S. cerevisiae* interactions and their impact on wine using an comprehensive approach. We studied the fermentative capacity, chemical composition and aromatic profile of wines associated with three cocultures and their corresponding pure cultures. The various strains studied within the cocultures showed different behaviors regarding their development. The interactions induced a modulation of more than half of the 67 quantified volatile compounds including higher alcohols and their associated esters, vinylphenols and fatty acids. New aromatic expressions associated to cocultures were reported and attributed to yeast interactions. Sensory profiles of cocultures differed from the blends (50/50 v/v) of post AF wines and also from pure cultures. Complex interactions were highlighted. Indeed, cocultures appeared to be different from the simple additions of two wines represented by blend. High resolution mass spectrometry allowed to highlight thousands of interactions biomarkers. Most of these biomarkers belonged to metabolic pathways involved in nitrogen metabolism. Despite of preserved fermentative properties, the described interactions induced a modification of the chemical composition and sensory profile of the wines from the cocultures. Combining different techniques in an integrative approach seems essential to understand yeast interactions and describe the consequences on wine.

Modelling spatial growth pattern formation in yeast colonies

Benjamin Binder

Yeasts have been used for biotechnology throughout recorded history. They are important human pathogens, and major experimental models of eukaryotic cells. Although yeasts are some of the most studied organisms in biology, their modes of colony pattern formation are not fully understood. In this talk, continuum and discrete modelling approaches are implemented to investigate the mechanisms that produce spatially non-uniform colony formation. We show the continuum approach can model spatial patterning observed in floral biofilm colonies and use a discrete model to capture highly non-uniform patterning in filamentous colonies. This research challenges the ground-breaking work of Pirt (1967), who proposed that yeast growth patterns were exclusively caused by limitations due to nutrient diffusion. Thus, our ongoing aim is to provide a deeper understanding of the fundamental mechanisms for colonial morphology in the different modes of growth of *Saccharomyces cerevisiae*, with implications for this and other biofilm-forming yeasts of biotechnological or medical importance.

Molecular Mechanism of Heterosis in Yeast

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Heterosis or hybrid vigor is a common phenomenon in plants and animals, however, the molecular mechanisms underlying heterosis remain elusive, despite extensive studies on the phenomenon for more than a century. Here we constructed a large collection of F1 hybrids of *Saccharomyces cerevisiae* by spore-to-spore mating between homozygous wild strains of the species with different genetic distances and compared growth performance of the F1 hybrids with their parents. We found that heterosis was prevalent in the F1 hybrids at 40ºC. A hump-shaped relationship between heterosis and parental sequence divergence was observed. We then analyzed transcriptomes of selected heterotic and depressed F1 hybrids and their parents growing at 40ºC and found that genes associated with one-carbon metabolism and related pathways were generally upregulated in the heterotic F1 hybrids, leading to improved cellular redox homeostasis at high temperature. Consistently, genes related with DNA repair, stress responses and ion homeostasis were generally downregulated in the heterotic F1 hybrids. Furthermore, genes associated with protein quality control systems were also generally downregulated in the heterotic F1 hybrids, suggesting a lower level of protein turnover and thus higher energy-use efficiency in these strains. In contrast, the depressed F1 hybrids exhibited a largely opposite gene expression pattern to the

heterotic F1 hybrids. We provide new insights into molecular mechanisms underlying heterosis and thermotolerance of yeast and new clues for a better understanding of the molecular basis of heterosis in plants and animals.

Molecular toolbox for gene expression from erythritol regulated promoters

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Yarrowia lipolytica is an emerging cell factory to produce recombinant proteins (rProt) or specific bioproducts. Recently, we developed a set of regulated promoters for gene expression and synthetic biology that derives from the *EYK1* gene encoding erythrulose kinase. Promoter induction is repressed by glucose and glycerol and strongly induced by erythritol, a low cost 4-carbone sugar alcohol. Identification and characterization of the *EYK1* upstream activating sequences allowed the construction of tunable and hybrid promoters that significantly outperform the benchmark pTEF promoter in terms of gene expression level. The developed promoters can be also made bidirectional to allow gene coexpression. Several efficient recipient strains suitable for industrial applications have been developed together with easy to clone vectors based on chromogenic proteins. Depending on the genetic background of the recipient strain considered, the *EYK1*-derived promoters can be either constitutive, phase-dependent or regulated. For fast and efficient use, all those promoters have been made compatible with our existing *Y. lipolytica* Golden Gate assembly system. These molecular biobricks will be described in detail, and their utilization exemplified for the lipase CalB from *Candida antartica*. The developed promoters yield higher rProt productivity in *Y. lipolytica* than those obtained in the relevant industrial cell factory *P. pastoris*.

New *Starmerella* genomes: genomic characteristics, molecular evolution, species divergence, and horizontal gene transfers

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The presented research is based on the genome sequencing and assembly of mostly unique *Starmerella* strains of the BCCM/MUCL collection. The genus *Starmerella* is of interest because of its production of sophorolipids as secondary metabolites with industrial potential, the presence of large numbers of genes of bacterial origin in its genomes, the genetic diversity on species level, and a potential role of yeasts in bees.

Genomic data were obtained by Illumina technology for 21 strains of 6 species (*S. apicola*, *S. apis*, *S. bombi*, *S. bombicola*, *S. magnoliae*, *S. neotropicalis*). Four strains of species without genome in public databases were also sequenced by PacBio technology. Publicly available *Starmerella* and *Wickerhamiella* genomes were included, and the genomic data were analysed using the GEN-ERA toolbox (Cornet et al. 2022). Genomic contamination assessment was performed and phylogenomic trees constructed using bootstrap and jackknife methods based on protein and DNA sequences of 249 core genes, confirming published phylogenetic hypotheses. Different conformations of gene clusters associated with the production of sophorolipids were detected. Comparative genomics using over 7,200 bee-associated bacterial genomes indicated many lateral gene transfers from bacteria that share the ecological niche of *Starmerella* in bee guts. Pairwise average nucleotide identity was determined for 70 strains and resulted in surprisingly low values within some species, including *S. bombicola*, known for its ascospore formation. Doubling the known *Starmerella* genomes, our findings contribute to the understanding of the biology and ecology of *Starmerella* and suggest approaches for further research in this intriguing yeast genus.

Next generation strains, vectors and methods for gene expression employing *Komagataella phaffi (P. pastoris)*

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Originally generated as an alternative yeast strain to convert methanol to single cell protein for animal feed, *K. phaffii* (originally classified as *Pichia pastoris*) was converted to an efficient gene expression platform about 40 years ago. Employing next generation genome sequencing and modern tools for genetic engineering we are learning more and more about the most common and closely related specific strains, which were used for industrial biotechnology since then. However, surprisingly the platform host strains and vectors, which had been used for research, and also for industrial applications, did not change much in the past 4 decades. Therefore, the question arises, if this is due to the still unbeatable technological properties of those tools from the early days, their track record in safety and product approval, IP reasons or just the simple availability of the key strains, vectors and expression kits.

This presentation will summarize the current knowledge about the most important tools from the past, today and some new strains, vectors and technologies which might change our future for gene expression employing *K. phaffii*.

Non-conventional Yeasts as Promising Producers of Biofuel and High-Value Chemicals

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Some non-conventional yeasts possess unique metabolic or regulatory properties. Thermotolerant yeast *Ogataea polymorpha* growth at 50°C and can grow and ferment xylose and L-arabinose. Introduction of heterologous gene coding for lactate dehydrogenase converts *O. polymorpha* to lactic acid producer. Metabolic engineering and random selection led to *O. polymorpha* strains accumulating 50 times more ethanol from xylose relative to the wild-type strain (20 g/L). Genes *XYL1, XYL2, XYL3, DAS1, TAL2, CAT8, IRA1, TKL1, TAL1, ATG13* appeared to be important for efficient xylose fermentation. Engineering Hxt1 hexose transporter led to strain with simultaneous utilization of glucose and xylose. Characteristics of lactic acid synthesis also were studied on the natural yeast producer of this substance, *Lachancea thermotolerans*. Another interesting species is the flavinogenic yeast *Candida famata* (*Debaryomyces subglobosus*) which overproduces riboflavin under iron starvation. Using random selection, strain was isolated which overproduces riboflavin in iron-sufficient medium. Genome sequencing revealed many genetic changes some of them could be important for riboflavin biosynthesis like mutations in genes *SEF1, MET2* and *RIB3*. Besides, genes *RIB1, RIB6, SFU1, VMA1, RFE1* are also important for riboflavin overproduction. Metabolic engineering was used for construction of the advanced overproducers of riboflavin and flavin nucleotides also on cheap substrates like cheese whey and lignocellulosic hydrolysates. One of the best constructed strains accumulated 16 g of riboflavin/L in bioreactor during fed-batch cultivation. *C. famata* and *Komagataella phaffii* were used to construct producer of bacterial flavin antibiotics aminoriboflavin and roseoflavin. Maximal production of roseoflavin reached 130 mg/L during fed-batch cultivation in bioreactor.

Novel microbes from Australian plants: Characterisation and investigation of their industrial potential

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Yeasts are one of the most important organisms in the beverage industry, whose sales are estimated to be worth $9 billion USD by 2030. Currently, there is demand from beverage manufacturers for yeasts capable of enhancing products, for example by contributing to diverse flavour profiles. This is in direct response to consumer trends of desiring new and innovative products. The discovery of novel yeasts is therefore crucial for industry development. Australia has over 24,000 unique plant species in which lie potential niches of novel microbes. One of these, *Eucalyptus gunnii*, commonly known as the Cider Gum tree is endemic to central Tasmania. The tree sap was used by indigenous people to produce a sweet, fermented drink called *way-a-linah* and is thus an excellent place to search for useful yeasts. We have collected sap, bark, and soil samples from *E. gunnii* and begun to identify and characterise yeasts present using ITS profiling of purified and cultured isolates. Characterisation will involve analysis of growth and enzymatic activities under common industrial fermentative and stressful conditions (e.g., high ethanol, low pH, and suboptimal temperatures). We expect this project will identify novel, uniquely Australian yeast isolates suitable for wine, beer, and/or cider fermentation and also potentially for other industrial uses. We also hope to highlight the microbial diversity found in native Australian plants, in particular those used by Australian first nations peoples, and contribute and transfer useful knowledge to support efforts for cultural and heritage conservation.

Novel Warbicin® family of phosphorylation-dependent glucose-uptake inhibitors in yeast and human cells as potential anti-cancer drugs

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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 *tps1Δ* 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. They are the first inhibitors that act on both. Warbicin® compounds inhibit proliferation and trigger cell death in cancer cells in a dose-dependent manner. They also inhibit tumor growth in vivo in mice xenografts. Specific concentrations did not evoke any major toxicity in mice but increase adipose tissue levels, consistent with deviation of glucose into storage organs. 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 *tps1Δ* glucose sensitivity. We suggest that hexokinase can reversibly interact with glucose carriers and utilize the inhibitory ATP molecule bound in their cytosolic domain, converting it into non-inhibitory ADP and thereby increase the cellular influx of glucose. The yeast *tps1Δ* strain as well as cancer cells are proposed to have defective, permanent interaction resulting in persistent overactive influx of glucose. Based on their chemical structure and hydrophobicity, we suggest that Warbicin® compounds replace the inhibitory ATP in the cytosolic domain of the glucose carriers, preventing its utilization by hexokinase in transport-associated phosphorylation, and thereby reducing the overactive glucose uptake and catabolism.

Occurrence of wild yeasts in Uruguayan craft beers and its impact on physicochemical parameters

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Wild yeasts are defined as yeasts present in beer that were not intentionally introduced during the brewing process and are not fully controlled by the brewer. Detecting these contaminating wild yeasts in beer typically indicates poor hygiene and disinfection practices and can cause inconsistencies and deviations from the intended beer profile. The aim of this study was to analyze the presence of wild yeasts in Uruguayan craft beers, their evolution in bottled beer over time and their impact on physicochemical attributes. The study was conducted on the three most consumed craft beer styles in Uruguay: IPA, Blonde and Porter/Stout. The follow-up times selected were freshly bottled, and 3, 6 and 12 months after bottling. 67% of the beers exhibited contamination by non-*Saccharomyces* yeasts, while 47% showed contamination by *Saccharomyces* wild yeasts. In all instances, Saccharomyces yeast counts were significantly lower. The highest contamination of non-*Saccharomyces* yeasts observed was 2x106 cel/ml compared to 2x105 cel/ml for *Saccharomyces* wild yeasts. Wild yeast counts were highest in freshly bottled beers and began to decrease after 3 months, reaching their lowest levels at 12 months. Stouts/Porters and Blonde Ales displayed the highest contamination within the first 6 months. However, IPAs, which initially had no detectable contamination, exhibited the highest counts thereafter. A moderate correlation (R2 = 0.65) was identified between yeast contamination and density reduction. Samples with highest levels of *Saccharomyces* yeast contamination were characterized by a more pronounced pH decrease, whereas those with greater non-*Saccharomyces* counts showed a higher increase in turbidity.

Overexpression of Dihydroxyacetone Synthase 1 in Mut+ *Komagataella phaffii* (*Pichia pastoris*) Improves the Tolerance to Methanol and Formaldehyde Pulses

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Komagataella phaffii is a methanol-inducible expression system used for producing recombinant proteins (rProt). However, utilizing methanol as a carbon source results in a reduction in growth rate (μ) and the intracellular accumulation of formaldehyde, a toxic intermediate. To tackle these challenges and potentially enhance rProt productivity, the assimilative metabolism of methanol was modified by overexpressing dihydroxyacetone synthetase 1 (DAS1).

A strain of K. phaffii has been modified to overexpress DAS1 under the inducible promoter PAOX1. To assess the methanol tolerance of the +DAS strain, a kinetic characterization was conducted using varying concentrations of methanol as a carbon and energy source (5 to 18 g·L-1) through flask-batch culture. To determine formaldehyde sensitivity, flask-batch cultures with 3 g·L-1 of methanol were exposed to a formaldehyde pulse (2, 5, and 8 mM), verifying changes in growth rate.

The results indicated that the +DAS strain exhibits greater tolerance to methanol; both the specific growth rate (μmax) and the specific rate of methanol consumption (qS) were higher compared to the control. Moreover, the +DAS strain displayed significant tolerance to formaldehyde pulses, contrasting with the control strain, thus supporting this metabolic modification as a promising candidate for a recombinant protein platform.

Pathway design for mixotrophic production of chemicals from CO2 and methanol in yeasts

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Autotrophy and mixotrophy in industrial strains have the potential to contribute to the mitigation of climate change, and they are therefore of a great interest towards a more sustainable production of biochemicals.

In previous studies, functional autotrophy was engineered in yeasts by integrating the Calvin-Benson-Bessham (CBB) cycle in *Pichia pastoris* (*Komagataella phaffii)* (Gassler et al. 2020), and similarly in *Escherichia coli* (Gleizer et al. 2019), allowing growth on CO_2 as the sole carbon source. The autotrophic *P. pastoris* strain uses methanol for energy supply. In this project, we aim to design alternative pathways for assimilation of both CO_2 and methanol for the conversion of CO_2 into value-added compounds. Using methanol in both assimilatory and dissimilatory pathways lowers the overall methanol demand. Blueprints for such a mixotrophic pathway are the bacterial serine or serine-threonine cycles. Implementation of such a complex pathway in the metabolism of *K. phaffii*, requires a substantial rerouting of central metabolic fluxes to maintain growth.

By using methods for synthetic biology such as Golden Gate Assembly and CRISPR-Cas9, we are aiming to design thermodynamically feasible and energetically favorable pathways for the production of organic acids from a mixed feed of methanol and CO_2. For this purpose, additional enzymatic loops are added to the serine cycle by either introducing bacterial genes or activating native yeast pathways. Functionality of the novel cyclic pathways is assessed by the capacity to produce the target molecules and intermediates from methanol and CO_2.

Pathway engineering using recombination-based expression optimization and Cas3-guided base editing

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Metabolic engineering of microbes is essential for the sustainable production of industrially-relevant compounds. To tackle the major challenges currently faced in metabolic engineering, e.g. low enzyme activity, metabolic burden and insufficient pathway flux, we recently developed two inexpensive technologies that are fast and easy to implement.

The first technology involves a gene expression modulator for large-scale *in vivo* expression diversification. The tool uses a set of newly developed, orthogonal LoxPsym sites and Cre recombination to shuffle various regulatory elements controlling the expression of a pathway gene. The design allows multiplexed targeting of all pathway genes simultaneously and can diversify the expression of each gene over 100-fold.

The second technology (CoMuTER) combines the targetable helicase Cas3 with the cytidine deaminase rAPOBEC1 to specifically mutagenize large genomic regions. The long stretches of single-stranded DNA generated by Cas3 serve as substrate for the cytidine deaminase, enabling an activity window of up to 55 kb, with an average of 0.35 cytidine deaminations per kilobase.

As a proof-of-concept, both synthetic biology tools were applied to a heterologous pathway for the production of red carotenoids (either astaxanthin or lycopene) and profiling variants with randomized pathway mutations or expression regulations gave us new insights into optimized pathway flux and let to the identification of improved production strains, ultimately resulting in more than two-fold increase in production titre for both approaches.

Production of complex recombinant proteins in *pichia pastoris*: presence of the pro-domain and its influence on reticulum stress

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Komagataella phaffii, a methylotrophic yeast formerly known as *Pichia pastoris*, is a versatile and cost-effective system for producing recombinant proteins with pharmacological potential. Among these recombinant proteins, a subgroup known as difficult-to-express (DTE) proteins may face limitations in production due to their inherent characteristics. Previous studies have indicated that the presence of the native pro-domain could play a significant role in the folding, expression, and secretion of DTE proteins. In this study, we propose various modifications to the pro-domain of two complex proteins, namely human Bone Morphogenetic Protein 2 (BMP2) and human tissue plasminogen activator (tPA), to determine their impact on the secretion level in *K. phaffii*. We compared different structures of BMP2 and tPA, including the complete protein (native form), protein fragment (mature form), and modifications to the cleavage site. To facilitate expression in *K. phaffii*, we optimized the nucleotide sequences of the BMP2 and tPA genes, synthesized them, and cloned them into the pPICZαA vector. These constructs were then expressed under the control of the AOX promoter in K. phaffii X-33. Subsequently, all recombinant proteins were purified using a two-step ion-exchange chromatography process. The results have shown varying yields of purified culture medium for all the tested variants. This proposed strategy is expected to enhance the production of DTE proteins in *K. phaffii*, as well as

other complex proteins with pharmacological potential, within economically feasible production systems.

QTL mapping reveals novel mechanisms underlying variations in H_2S production during alcoholic fermentation in *Saccharomyces cerevisiae*

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Saccharomyces cerevisiae's requirement of reduced sulfur to synthesize methionine and cysteine during alcoholic fermentation, is mainly fulfilled through the sulfur assimilation pathway. 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 highly strain-specific and depends as well on the SO_2 concentration, often added to the grape must. Applying a Bulk Segregant Analysis to a 96 strain-progeny derived from two strains with different ability to produce H_2S we decipher new genetic bases of the H_2S production in the presence of SO_2. The comparison of the allelic frequencies along the genome of pools of segregants producing a small or a high quantity of H_2S during alcoholic fermentation pointed out to two regions (QTL) involved in the variations of H_2S production. The functional analysis of genes in the first region led to the discovery of the role of *ZWF1*, while in the second region, variants of genes previously not known for their impact on sulfur metabolism were found. This data represents a novel insight into the regulation of H_2S production during wine fermentation and offers a fresh perspective on the interplay between the sulfur assimilation pathway and cell metabolism.

Repurposing bisphosphonate drugs to kill yeast pathogens

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Yeast pathogens cause human diseases that range from superficial and largely cosmetic to severe, disseminated life-threatening infections. Certain species of *Candida* and *Cryptococcus* are particularly common and can be very difficult to treat. Azole-based antifungal drugs are a mainstay of therapy, particularly in resource-poor regions, but not all infections respond and resistance is an increasing problem. Drug synergy is one way of improving treatment as it can enhance efficacy and reduce the induction of resistance. To find targets that might work synergistically with fluconazole (FLC), we subjected *Cryptococcus gattii* to sub-lethal FLC treatment and analysed the proteomic response over time. We found upregulation of farnesyl pyrophosphate synthase (FPPS), an enzyme involved in the synthesis of squalene, which feeds into the ergosterol biosynthesis pathway that is subsequently targeted by FLC. FPPS can be inhibited by bisphosphonate drugs including alendronate (ALN) and zolendronate (ZOL), which are FDA-approved and marketed to treat osteoporosis. ZOL was synergistic with FLC across a range of yeast and mould pathogens. Mechanistic studies revealed synergy was mediated by squalene deprivation, resulting in membrane hyperfluidity and depolarisation, and in the case of *Candida glabrata*, complete elimination of the efflux pump activity that largely mediates FLC resistance. We modified ZOL by adding a 10-carbon alkyl tail and found a significant improvement in activity against a remarkably wide spectrum of fungi, with low toxicity to human cells. This repurposing of an available and approved drug can considerably shorten the timeline for producing an effective antifungal agent.

Relevance of nitrate assimilation by *Brettanomyces bruxellensis* in low nitrogen environments

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Brettanomyces bruxellensis possesses the rare (amongst yeast) ability to assimilate nitrate, which confers sufficient advantage for it to outcompete *Saccharomyces cerevisiae* in fermentation processes enriched in nitrate, such as fermentation of sugarcane for bioethanol. When nitrate is abundant, *B. bruxellensis* can grow and ferment more rapidly under anaerobic conditions, through reversal of the Custers' effect and concurrent acetate production. Surprisingly, some *B. bruxellensis* strains cannot use nitrate due to structural gene deletions. Could this be due to lack of advantage in environments with low nitrate concentrations?

Genomes of 189 *B. bruxellensis* strains were analyzed for presence/absence of nitrate assimilation genes, with 13 strains displaying at least partial deletion within the structural gene cluster. Previous studies have shown loss-of-heterozygosity (LOH) events across this cluster, that may have some impact on phenotype. We found a high proportion of strains with LOH (68%), that varied with regards to the initiation of LOH from 0-10kb upstream of the cluster. We are currently evaluating association of deletion and LOH events with different *B. bruxellensis* populations, and correlation with growth on nitrate.

To evaluate the relevance of nitrate assimilation during fermentation in low nitrate environments, one strain that displayed stronger growth on solid media with nitrate was selected. Preliminary experiments in anaerobic environments show this strain utilized significantly more glucose (p = 2e-16) and produced more acetic acid (p = 2e-16) when grown on nitrate vs ammonium, even at relatively low concentrations (0.042 g/L) of nitrogen.

Saccharomyces cerevisiae Hsp31p as a tool in the studies of Unconventional Protein Secretion

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As our group and others reported, the *Saccharomyces cerevisiae* Hsp31p protein belonging to the ubiquitous DJ-1/ThiJ/PfpI family is essential for survival in the post-diauxic growth phase and under diverse environmental stresses. Our previous work also showed that *HSP31* gene expression was controlled by multiple stress-related transcription factors, which mediated *HSP31* promoter responses to oxidative, osmotic, and thermal stresses, toxic products of glycolysis, and the diauxic shift. Hsp31p was shown to possess glutathione-independent glyoxalase III activity oxidizing methylglyoxal (MG) and to function as a protein chaperone. Yet despite the multitude of implicated activities, the exact biological role of this protein within budding yeast cells remained elusive.

Here we show that native Hsp31p is secreted to the periplasm, most likely by an unconventional pathway. Hsp31p cellular level increase in response to stresses affecting cell peripheries is controlled by HOG and CWI signalling pathways. Our search for attributes necessary for Hsp31p secretion revealed single amino acid residues within its polypeptide, indispensable for targeting the periplasm. Noteworthy, the modified, not secreted variants of Hsp31p could not protect the cell against MG stress. Identified regions of Hsp31p polypeptide were found to be located on the surface of the natively folded molecule as determined by X-ray crystallography. It suggests that Hsp31p secretion is driven by interactions through these regions with other proteins. Genome-wide search employing Hsp31p as a secretion marker allowed identifying a group of proteins potentially involved in its secretion. They may participate in poorly characterized unconventional secretion of proteins in *S. cerevisiae*.

Saccharomyces cerevisiae that grows without addition of vitamins

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Saccharomyces cerevisiae require a source of C, N, S, P, metal ions, and vitamins, in order to grow efficiently. Even though strains of this yeast species can synthesise B group vitamins, they grow slowly without addition of exogenous pantothenate, folate, inositol, niacin, pyridoxine, riboflavin, and thiamine. Using evolution, we have generated yeast strains that grow rapidly and efficiently without the addition of any vitamins. We call these novel strains "vitamin-free" yeasts. We will present data comparing the growth of standard laboratory strains alongside an exemplar vitamin-free strain. Ability of a yeast strain to grow without vitamins is of economic importance to the yeast biomass manufacturing industry, as vitamin additions represent significant cost. We will present data showing the kinetics, yield, and productivity of the vitamin-free strain growing in aerobic fed batch fermentations with, and without, vitamin additions. We will also present data on the chemical composition of this yeast biomass. Further work is underway to determine the genetic basis of vitamin-free growth in the evolved strain. This research includes genetic dissection, segregant analysis, transcriptomics, and DNA sequencing aimed at understanding Mendelian and quantitative effects.

Saccharomyces interspecific hybridisation delivers evidence of hybrid heterosis in winemaking

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Historically, interspecific hybridisation has been used in plant development programs to combine desirable fruiting and flowering traits from different species. While *Saccharomyces cerevisiae* wine yeast strains are the mainstay of modern commercial winemaking, recently, yeast breeding programs have utilised interspecific hybridisation within the genetically divergent *Saccharomyces* species to deliver novel phenotypes important to winemaking; traits ranging from yeast fermentation properties through to flavours and aromas in the final wine product.

Hybridisation of a robust *S. cerevisiae* wine yeast with *Saccharomyces uvarum* targeting the phenotype of lower volatile acidity production generated a hybrid strain capable of lower acetic acid production than either parent. Analysis of wines made by meiotic hybrid progeny and subsequent genomic sequencing of pooled high-acetic acid producers versus low-acetic acid producers revealed loss of the entire *S. uvarum* Chromosome 4 in high-producing acetic acid progeny, indicating that retention of this chromosome is complicit in the low-acetic acid production phenotype of the original hybrid. Targeted *S. uvarum* chromosome 4 deletions in the *S. cerevisiae* x *S. uvarum* hybrid identified potential regions of interest corresponding to genes involved in the HOG pathway adaption to osmotic stress.

Secondary genomic barcodes for improved resolution of inter- and intraspecies diversity among environmental yeast isolates

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ABSTRACTS

Budding yeasts (phylum Ascomycota, sub-phylum Saccharomycotina) are a diverse group of predominantly unicellular fungi that not only includes beneficial species used in food and beverage fermentations as well as other biotechnological processes but also encompasses some of the most common fungal pathogens that infect humans. Species assignment of individual yeast isolates is typically achieved by sequencing domains 1 and 2 (D1/D2) of the large subunit (LSU) 26S rRNA gene and/or the internal transcribed spacer (ITS), which is located between the LSU and small subunit (SSU) 18S rRNA genes.

However, these barcodes often do not contain sufficient variation to unambiguously assign a particular isolate to an already described species. Secondary genomic barcodes offer an alternative strategy to better resolve inter- and intraspecies relationships among yeasts. The present study investigated the use of intronic sequences from ribosomal protein (RP) genes as secondary genomic barcodes. Seven RP genes – *RPL14*, *RPL17*, *RPL18*, *RPL31*, *RPS7*, *RPS10* and *RPS23* – were found to be potentially suitable as secondary genomic barcodes for at least some taxonomic groups of budding yeasts. However, there was no instance where a single RP contained an intron in all surveyed genomes and therefore no single RP gene could be used as a "universal" genomic barcode for all budding yeasts. In addition, RP genes lacked introns altogether within the genera *Deakozyma*, *Spencermartinsiella*, *Starmerella* and *Tortispora*. The possibility of using intergenic regions of adjacent RP genes in a divergent orientation as alternative genomic barcodes in these genera was investigated and found to be feasible.

Selection of Indigenous *Pichia kluyveri* for Reliable Alcoholic Fermentation and Enhanced Fruity and Floral Wine Aroma

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Pichia kluyveri widely occurs at early stages during spontaneous fermentation; however limited research has been done on the oenological characteristics of indigenous *P. kluyveri* strains isolated from typical wine regions from China. Here, five indigenous *P. kluyveri* strains selected from spontaneous fermented wines from Ningxia and Gansu were characterised for their tolerance to wine-related stressors. All strains except FS-2-7 were able to tolerate 60% w/v glucose, low pH of 2.0, 16% v/v ethanol, extreme fermentation temperatures, and 500 mg/L SO_2. Following this, these strains were inoculated into a synthetic grape juice medium to test their fermentation performance and evaluate basic parameters of the final synthetic wine. Strain HS-2-1 was the first to initiate fermentation. Compared with the rest of the strains, HS-2-1 produced significantly higher amounts of total organic acids and less acetic acid. Thus, this strain was chosen for further characterisation in Cabernet Sauvignon fermentation trials co-fermented with at different ratios with *S. cerevisiae*. Whilst no significant inhibition on HS-2-1 growth was seen by *S. cerevisiae,* mixed fermentation also led to higher production of esters and higher alcohols compared to pure *S. cerevisiae* fermentation. Notably, concentrations of isopentanol, ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl 9-decenoate and ethyl lactate increased in line with the increased proportion of HS-2-1 in the inoculum. This study comprehensively investigated on the features of indigenous *P. kluyveri* strains, and highlighted potential application of strain HS-2-1 in winemaking by co-fermenting with *S. cerevisiae* for improving the fruity and floral aroma profile of the Chinese wine.

Selection of non-conventional yeasts for the reduction of alcohol content in wines

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High alcohol content in wines is a worldwide problem, the cause of which differs according to the wine production area. In response to this problem, several strategies have been developed to try to mitigate or reduce the alcohol content in wines, which can be applied to different stages of the biotechnological winemaking process. One of the strategies is the selection of non-*Saccharomyces* (NS) yeasts that, through their differentiated metabolism, can produce other metabolites to detriment of alcohol.

This work presents the results obtained from twenty-nine microvinifications in simil grape must carried out with native strains of the genera *Hanseniaspora, Starmerella*, and *Metschnikowia* (isolated from Uruguayan vineyards). The main parameters analysed were absence of aromatic defects, residual sugars, ethanol yield and glycerol content. Five native NS yeasts were selected for lower ethanol yields than *S. cerevisiae*, absence of aroma defects, and in some cases a glycerol production of about 6 g/L.

In a next step, the five selected yeasts will be studied in mixed cultures with a commercial *S. cerevisiae* strain, in order to select at least one mixed culture that minimizes the alcohol content. The resulting wines will be evaluated in terms of chemical composition, aromatic compounds, and sensory profile.

SO_2-production potential and early, transient acetaldehyde formation by *Saccharomyces cerevisiae*: implications for *Oenococcus oeni* co-fermentation

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The ability of wine yeast to produce SO_2 during fermentation is a well-recognised strain-dependent trait, occurring as an intermediate by-product during the assimilation of sulphate. *Saccharomyces cerevisiae* strains can be broadly classified as either low- or high-SO_2 producers, with most strains producing 10-30 mg/L SO_2; however, strains producing more than 100 mg/L SO_2 have been observed. Further, exogenous sulfite added to grape must can be consumed by wine yeast, and can repress the formation of SO_2 by low SO_2-producing strains. In parallel with SO_2 production, wine yeast also transiently produce acetaldehyde in the early fermentation stage, the peak concentrations of which are also highly yeast strain dependent. Such modulation of these metabolites can have significant impacts on malolactic starter culture performance and post-fermentation SO_2 management regimes, requiring greater knowledge of their potential formation amongst different winemaking yeast strains.

In this study, the SO_2 production potential of 94 genetically diverse *S. cerevisiae* strains from within the wine clade was assessed after fermentation in sulfited Chardonnay juice (41 mg/L initial total SO_2). The final SO_2 concentration of wines fermented by these strains varied by more than 10-fold from 20 to 212 mg/L. Importantly, approximately half of the strains either increased or decreased the SO_2 concentration relative to the initial concentration of SO_2 of Chardonnay juice. Further testing of a subset of strains in non-sulfited Chardonnay juice revealed a similar distribution of SO_2-production capability, and provides further insight into the phenomenon of early, transient acetaldehyde formation amongst low- and high-SO_2 producing yeast strains.

Softer wine with an improved combination of aeration and yeast strains

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The increase in the ethanol content of wines is among the many negative consequences of global warming on winemaking. And using yeast strains and fermentation conditions resulting in low ethanol yield is one of the most interesting strategies to deal with this problem. Within this strategy, the approach that would allow the clearest reduction in alcohol content is the respiration of part of the grape sugar by yeasts. Non-*Saccharomyces* species can be used for this purpose but show a limited survival, so reducing its potential impact. In turn, *Saccharomyces cerevisiae* shows a high production of acetic acid in aerated fermentation conditions (required for respiration). We have previously developed procedures using either combinations of non-*Saccharomyces* and *S. cerevisiae* starters, or a proprietary natural strain of *S. cerevisiae*, with unique metabolic properties. In both cases, fine tuning and automated control of oxygen availability was required to avoid excess volatile acidity. In this work, we have combined the two previous approaches, i.e., the use of *S. cerevisiae* PR1018 and that of non-*Saccharomyces*, namely *Metschnikowia pulcherrima*. The process developed is robust, since it tolerates various levels of aeration or yeast strain proportions, while aeration can be interrupted with no negative impact on quality. Under the selected conditions, volatile acidity was below 0.4 g/L, while an ethanol reduction of around 3% ABV was obtained (when compared to the standard anaerobic fermentation for the same grape must).

Synthesis, Assembling and Monitoring/Diagnostics of Nucleic Acids *in vitro* and *in vivo*

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Our work has successfully realized multiplexed optical coding of nucleic acids using multicolored nanostructures with precisely controlled emission wavelengths and intensity levels. This breakthrough enables massively parallel bioanalysis, allowing for simultaneous reading of coded DNA hybridization and target signals at the single-nanostructure level. The wavelength-and-intensity multiplexing capability of this technology opens up new opportunities in the synthesis, assembly, and monitoring/diagnostics of nucleic acids both in vitro and in vivo. In this research, we utilized yeast as a model system to explore synergies that enhance its flexibility in target selection, speed up binding kinetics, and improve sensitivity in hybridization detection. The insights gained from this study will not only contribute to the field of coding nucleic acids but also advance nucleic acid assembly techniques, revolutionize data storage methods, enhance the nano-bio interface, and expand our knowledge of yeast.

The interplay of yeasts in a complex environment of palm wine fermentation

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ABSTRACTS

Palm wine is a traditional alcoholic beverage that has economic values and socio-culture impacts in tropical countries. It has been produced traditionally for centuries with little information to shape production based on scientific measures. Palm wine is the product of a complex microbial ecosystem of palm sap tapped from various species of palm trees. Although yeasts dominate the majority of the fermentation processes from converting sugar to alcohol and secondary metabolites, the ecological interactions between microbial groups are one of the important keys on determining aroma and flavour structure. Ecological dynamics shift between *Saccharomyces* and non-*Saccharomyces* yeasts from the sap tapping process to the end of fermentation. The significant contribution of *Saccharomyces* to the complex microbial ecosystems is showed by the main product, ethanol, affecting the successional growth of other yeast species. The main product also modulates the progression of mixed acid fermentation involving lactic acid and acetic acid bacteria, especially in the final stages. The complex metabolic interplays reduce the shelf-life of palm wine and provide a challenge for maintaining a stable product under different storage techniques and packaging methods. Profiling microbial constituents can provide a better understanding of the fermentation ecology in spontaneous fermentation, that could suggest a fermentation model of managing microflora to maintain aroma and flavour signatures, product quality and increase marketing value of this important beverage.

The Yeast Deletion Collection at 25: a snapshot of progress and a view towards the future

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The yeast deletion collections represent a triumph of molecular genetics and scientific collaboration. These mutant strains carry precise start-to-stop deletions of ~6000 open reading frames in both haploid mating types and as heterozygous and homozygous diploids. The yeast deletion collection, or yeast knockout (YKO) set, represents the first and the only complete, systematically constructed deletion collection available for any eukaryotic organism. In the twenty-five years since its introduction, the YKO strains have been used in hundreds of laboratories and tested in over 10,000 screens. laboratories in >1000 genome-wide screens. Notable spinoff technologies include synthetic genetic array and HIPHOP chemogenomics. Despite its age, the YKO continues to provide new insights, and last year, we completed the first YKO screen in lunar orbit. This talk will review rich data provided by the YKO and explore what the next 25 years may hold.

The yeast toxicome: A potentially rich source of antifungals for food, agriculture, health and biotech

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Fungal pathogens are an emerging threat to human health and food security. Very few fungicides are available and resistance to these is rising. It is a long-standing challenge to develop new antifungals. As eukaryotic pathogens, fungi offer very few selective drug targets and we urgently need new strategies for antifungal development. Ascomycete yeasts – such as environmental isolates of *Saccharomyces cerevisiae* and related species – have evolved a large set of small protein toxins, so-called yeast killer toxins (or mycocins), to compete against fungi in the environment. Previous research revealed that these toxins exhibit diverse modes of action, thus, indicating that the yeast toxicome might constitute a rich source of functionally diverse but yet-untapped antifungals. In my talk I will exemplify my group's research on the molecular functioning of these yeast-derived toxins and their modularity and engineerability towards applications in food, human health and biotech/synbio.

Thermostable esterase expression and secretion using *Pichia pastoris*

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Esterases, a subgroup of the larger enzyme category of hydrolases, exhibit remarkable capabilities for the catalytic hydrolysis of ester bonds. This enzymatic activity is of paramount importance across diverse industrial sectors, facilitating the synthesis, modification, and degradation of ester-based compounds. Esterases are applied in fields such as chemical manufacturing, pharmaceuticals, food processing, and biofuel production, offering sustainability and versatility. Convenient tools for monitoring recombinant protein expression and secretion are also desired, and esterase enzymes provide a suitable tool, as esterase activity can be easily measured in both intracellular and extracellular fractions using simple colorimetric kinetic assays.

Esterase enzymes are derived from a range of natural sources such as microorganisms, plants, and animals. Diverse esterases exhibit a wide range of substrate specificities and reaction mechanisms, which can be enhanced and tailored to commercial application using synthetic biology and enzyme engineering. For large scale commercial applications, recombinant esterase enzyme production systems are most suitable.

In this study, we expressed and secreted an industrially useful and thermostable esterase E2 from *Alicyclobacillus acidocaldarius* (E2Aa) using the popular yeast *Pichia pastoris* as a recombinant protein production host. We compared a variety of promoter and secretion signal peptides to establish optimal recombinant protein expression and secretion. Our results demonstrated that both native *P. pastoris* and heterologous secretion signals were effective for the expression and secretion of E2Aa, and that E2Aa esterase activity was a useful tool for reporting recombinant protein expression and secretion in *P. pastoris*.

Trade-Offs and Trafficking: Enhancing Recombinant Protein Production in *P. pastoris*

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The methylotrophic yeast *Pichia pastoris* has been utilised to produce a variety of recombinant heterologous proteins from human, animal, plant, fungal and bacterial origins, including for use as biopharmaceuticals and sustainable food, flavour and fragrance compounds.[1] Secretion of recombinant proteins produced by *P. pastoris* into the culture medium offers several advantages including a protein folding pathway that allows disulphide bond formation and post-translational modifications which may increase functionality of the heterologous proteins, as well as much-simplified purification of recombinant proteins directly from the media supernatant without the need for cellular disruption. High levels of protein expression and secretion are possible, but as yield increases, trade-offs between growth, expression and protein trafficking through the endoplasmic reticulum develop, linked in some cases to glycosylation and secretion signals. We have used model recombinant proteins (e.g. fluorescent protein OFP, animal-free food proteins and enzyme phosphotriesterase OpdA) to examine the interplay of some of these factors in relation to recombinant protein expression and secretion in *Pichia pastoris*.

Augustin *et al*. 2023. Critical Reviews in Food Science & Nutrition, DOI: 10.1080/10408398.2023.2166014; Juturu, V. and J.C. Wu, *Heterologous Protein Expression in Pichia pastoris: Latest Research Progress and Applications*. 2018. **19**(1): p. 7-21.

Turning oleaginous yeasts into free fatty acid producers: the mystery of free fatty secretion

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The yeast *Starmerella bombicola* is known as a powerful producer of the biological detergent sophorolipids; molecules composed of a hydroxylated fatty acid linked to a glucose disaccharide, which are currently commercialized as biodetergents by several companies.

One of the strengths of the microbial production process of sophorolipids is the yeast's highly efficient lipid metabolism; one can even draw parallels between certain oleaginous yeasts like *Yarrowia lipolytica*. Hence, we exploited the lipidic potential of *S. bombicola* and converted it from a glycolipid production platform into a free fatty acid cell factory. Microbial fatty acid biosynthesis and derivates thereof have gained much attention as they provide a sustainable alternative for petrol- and plant oil- derived chemicals.

As also observed in other yeast cell factories, the free fatty acids spontaneously accumulate extracellular, rendering product recovery quite straightforward. However, it remains a riddle which biochemical mechanisms are involved in the crossing of the plasma membrane: no efflux proteins are identified so far, and also vesicular transport cannot be excluded.

We examined several candidate proteins based on findings from *Saccharomyces cerevisiae* and conducted transcriptomics and proteomics experiments where we also investigated an engineered fatty acid secreting *Y. lipolytica* strain[1]. In conclusion, the fatty acid export process seems to involve several actors and surprisingly, parallels between different yeast species cannot always be drawn. This later finding will be illustrated by the role of Pry proteins.

Uncovering the interplay between Copper and SO_2 tolerance in *Saccharomyces cerevisiae*

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Copper and SO_2 tolerance are two well-studied phenotypic traits of *Saccharomyces cerevisiae*. The genetic bases of these traits are the allelic expansion at the *CUP1* locus and reciprocal translocation at the *SSU1* locus, respectively. Previous work identified a negative association between SO_2 and copper tolerance in *S. cerevisiae* wine yeasts. To understand the genetic basis of copper sensitivity, we used bulk-segregant QTL analysis and identified genetic variation at the *SSU1* locus as a causative factor. This was confirmed through reciprocal hemizygosity analysis in a strain with 20 copies of *CUP1*. Transcriptional and proteomic analysis revealed that over-expression of *SSU1* didn't suppress *CUP1* expression or limit protein production. Instead, it induced sulfur limitation when exposed to copper. Furthermore, we observed that an *SSU1* over-expressing strain became more sensitive to moderately elevated copper concentrations in sulfur-limited conditions, indicating a burden on the sulfate assimilation pathway. Over-expression of *MET* 3/14/16, genes upstream of H_2S production in the sulfate assimilation pathway increased the

production of SO_2 and H_2S but did not improve copper sensitivity in an *SSU1* over-expressing background. We conclude that copper and SO_2 tolerance are conditional traits in *S. cerevisiae* and provide evidence of the metabolic basis for their mutual exclusivity.

Unfolded protein response biosensors for recombinant protein expression

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Komagataella phaffii is regarded as an attractive workhorse for the synthesis of valuable products. One of its key features as an industrial protein production host is its ability to secrete large amounts of recombinant proteins. However, secretion efficiency also depends on the target protein. Therefore, strain engineering and improvement are paramount for generating high-producing, industrially applicable strains. The unfolded protein response (UPR) significantly affects recombinant protein production and secretion in eukaryotic cell factories, including *P. pastoris*. This cellular stress response is triggered when unfolded proteins accumulate inside the endoplasmic reticulum (ER) lumen, exceeding the protein folding capacity of the ER. UPR modulates the transcription of UPRresponsive genes to restore ER homeostasis. The transcription of one of these UPR-responsive genes, KAR2, has been used as an indicator to evaluate the manifestation and strength of the UPR in yeast cells. Here, the putative promoter sequence of KAR2 was coupled with a fluorescent marker gene (eGFP) to generate a UPR biosensor strain. This strain was then used as a chassis for recombinant protein expression. An increase in the fluorescence signal was observed for the producing cells compared to the non-producing cells. The UPR biosensor strain gives a signal for folding stress and, thus, for target protein secretion, which can be used for high-producing clone selection during screening and can provide information to guide rational engineering strategies towards optimal heterologous protein production.

Unlocking the Secrets of Peptide Transport in Wine Yeast: Insights into Oligopeptide Transporter Functions and Nitrogen Source Preferences

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Oligopeptides from grape must represent a secondary source of nitrogen for yeasts to grow and carry out fermentation. *Saccharomyces cerevisiae* takes up oligopeptides from the environment through multiple oligopeptide transporters with different peptide length specificities. However, due to difficulties associated with the qualitative and quantitative measurement of peptides in natural matrices, peptide transporter specificities have been mostly researched in single peptide environments. Using a peptide mapping method, we monitored the relative consumption of peptides derived from a protein hydrolysate by a set of CRISPR-Cas9-engineered *S. cerevisiae* wine strains to study oligopeptide transporters from the Opt and Fot families. Results show that Opt2 can import peptides containing three to -at least-seven amino acid residues, which is a broader peptide length specificity than previously reported, while Opt1 was not functional as a peptide transporter in these strains. Fot1, Fot2 and Fot3, previously referred to as di-tripeptide transporters in *S. cerevisiae* wine strains, could also import tetrapeptides. The consumption order of peptides was determined by the peptide length as higher chain length peptides were taken up by Opt2 only after most di-tetrapeptides were depleted from the media. Altogether, Fot and Opt2 activity assured completion of the fermentation process without necessarily requiring ammonia or free amino acids. Analysis of peptide transporter gene expression during fermentation showed an effect of SO_4^{2-} not only on Opt1 but also on Fot, and supported the assumption of a possible interplay between Fot and Opt2 activities.

Whole Genome Sequencing of North American *Saccharomyces cerevisiae* strains isolated from spontaneous wine fermentations reveals a new Pacific West Coast Wine Clade

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Vineyards in wine regions around the world are reservoirs of yeast with oenological potential. We have isolated and sequenced *S. cerevisiae* strains from spontaneous fermentation of wine grapes from both British Columbian (BC) and Californian wine regions. Our phylogenetic analyses demonstrates that strains from BC and Californian vineyards/wineries fall into two major clades – the Wine/European clade and a new clade that we have designated as the Pacific West Coast Wine (PWCW) clade. The PWCW clade is an admixed clade that has high nucleotide diversity and shares genomic characteristics with North American oak clade strains but also has gene flow from the Wine/European clade. We have also sequenced *S. cerevisiae* strains isolated from Californian oak trees and find that they form a new subclade within the North American oak clade strains. PWCW strains are genetically more similar to California oak strains than North Carolina oak or Pennsylvania oak strains based on population admixture and Fst analysis. Strains isolated from Californian spontaneous fermentations also form subclades in the Wine/European clade, indicating regional population expansion. To identify alleles associated with wine-making traits we are carrying out phenotyping of PWCW, Wine/European and North American oak clade strains. Conditions tested include growth on single amino acid sources, carbon source utilization and resistance to potassium metabisulphite. We are also carrying out wine fermentation trials to compare the performance of BC and Californian wine strains in PWCW versus Wine/European clades.

Widespread divergence in protein stability between two *Saccharomyces* species

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The thermal growth limits of an organism constrain the environments in which it can evolve and its use in industry. Experimental evolution and bioengineering of enhanced thermotolerance has yielded limited results. Yet, over long-time scales thermophiles have evolved the ability to thrive at extreme temperatures. The proteomes of thermophiles are known to be more stable, but the mechanisms underlying their enhanced protein stability are not well understood due to the long-time periods over which stability has shifted. To investigate the mechanisms of thermal divergence between more closely related species, we measured the proteome stabilities of a pair of mesophilic *Saccharomyces* species, *S. cerevisiae* and *S. uvarum*.

We find that the majority of *S. cerevisiae* proteins are more thermostable than their *S. uvarum* orthologs. While this pattern persists in their interspecies hybrid, the magnitude of thermostability differences between orthologs is reduced. We attribute this to the hybrid cellular environment stabilizing the *S. uvarum* proteome, potentially mediated by protein – protein interactions. Our results demonstrate that thermal divergence is accompanied by changes in protein stability due to both the proteins' intrinsic structure as well the cellular environment and imply that increasing the thermal limits of an organism is constrained by the myriad of temperature dependent cellular functions carried out by the proteome.

Wine bioprotection with a specific *M. pulcherrima*: alternative to SO2 combining anti-spoilage and antioxidant properties

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Consumers are more and more asking for natural products. Non-*Saccharomyces* yeasts are a good alternative to chemical inputs such as SO2. Among them, *Metschnikowia pulcherrima* present interesting characteristic for bioprotection during pre-fermentative stages, allowing lower SO2 addition. Indeed, *Metschnikowia pulcherrima* ensures biocontrol against spoilage microorganisms during pre-fermentative stage (i) producing pulcherriminic acid leading to iron depletion, (ii) and colonizing the niche without media alteration thanks to low fermentative metabolism. However, SO2 is not only an antimicrobial agent, but also an antioxidant. Therefore, to combine both antioxidant and antimicrobial properties, a study of *Metschnikowia pulcherrima* biodiversity was conducted, and a specific *Metschnikowia pulcherrima* was selected among 100 other yeasts for its ability to consume oxygen. This specific non-Saccharomyces yeast, isolated in Burgundy, France, has been deeply characterized since its selection. Indeed, we confirmed its low fermentative capacity, good growth and survival in oenological conditions (even at low temperature), low nitrogen requirement, pulcherriminic acid production and high oxygen consumption rate. Moreover, we also demonstrated its huge ability to remove copper, an oxidation catalyzer, from the must. These antioxidant actions lead to visible color improvement when compared to no protection. Moreover, consequences on wine organoleptic quality have been confirmed with significant thiols increase with this specific *Metschnikowia pulcherrima* in comparison to a classical bioprotection tool during winery scale trials in Sauvignon Blanc.

Yarrowia lipolytica potential to valorize cricket powder into a versatile and safe food ingredient

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The principal aim of this research was to use *Yarrowia lipolytica* RO25 to obtain an innovative cricket powder hydrolysate to be used as versatile food ingredient endowed with high food safety, functionality, sensory and technological properties. The obtained hydrolysates were characterised, in comparison to a control, for the content of chitin, antimicrobial substances (short chain fatty acids, GABA) and health-promoting molecules (specific fatty acids and free aminoacids). Additionally, the ability of *Y. lipolytica* RO25 to reduce the allergenicity of cricket powder hydrolysate was evaluate in

order to obtain cricket-based foods with reduced allergenic potential. The hydrolysate was characterized, in comparison to the control, by a reduced chitin content and higher content of antimicrobial substances and functional compounds (arachidonic and linolenic acids, AABA, and BABA). Moreover, the hydrolysate obtained by the use of GRAS yeast was characterized by the presence of many volatile molecules and specific amino acids typical of ripened and fermented foods that can impart good sensory properties, as well as higher antioxidant activity with respect to the control. In addition, although cricket powder showed in vitro cross allergenic potential similar to that of shrimp, the use of *Y. lipolytica* RO25 showed reduction of allergenic potency. In conclusion the exploitation of a tailored biotechnological processes seems a good option to produce innovative and sustainable cricket-based ingredients to be uses in food formulation..

Yarrowia lipolytica strain potential to valorize cheese whey into sustainable adjunct for cheese sector

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Cheese whey disposal is a issue for dairy industries due to its high level of chemical and biochemical oxygen demand. However, it can still represent a source of nutrients (sugars, proteins and lipids) for microbial growth. *Yarrowia lipolytica* can grow in different environments, consuming hydrophilic and hydrophobic substrates, and tolerates high salt concentrations. Here, the ability of 20 strains of *Y. lipolytica*, isolated from different environments and characterized for their food-related enzymatic properties, were tested for their growth potential in three types of whey (caciotta, ricotta, squacquerone) to produce co-starter and adjunct cultures for the dairy industry. Among the strains evaluated, the best growers were PO1, PO2, and RO2, with the first one reaching up to 8.77 log cfu/mL in caciotta whey after 72 h of incubation. The samples were also characterized for their volatile molecule profiles by GC/MS/SPME and the samples were characterized by higher amounts of esters, acids, ketones and alcohols. Caciotta whey was the substrate allowing the production of the highest number of volatiles, due to the lipid and protein contents (around 15.4, 9.4 and 12.8 g/L in caciotta, ricotta and squacquerone whey, respectively) available for *Y. lipolytica*. Although further trials are required to optimize the processes and assess the feasibility at pilot scale, *Y. lipolytica* grown in untreated whey may open a novel field of research. In fact, the use of dairy waste as substrate to obtain *Y. lipolytica* biomass, to be used as starter or food adjunct in cheese, may represent a sustainable economic advantage.

Yeast and Drosophila, more than just a volatile relationship

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Insects and microorganisms are concomitant in nature and impact each other's survival and evolution. The common vinegar fly, *Drosophila melanogaster,* is known to harbour both desirable and spoilage microbes, which may be transported as part of this interaction. In this project, we investigated yeast traits that might affect this interaction, in particular attraction of and dispersal by flies. Yeast traits investigated include volatile aroma production (as flies have highly developed olfactory systems) and morphology. We hypothesized that some yeast aromas, not previously tested in this way, would impact fly behaviour and that filamentous morphology might enable yeast to adhere to flies enhancing dispersal.

Yeast are well known to produce volatile compounds that attract flies, including volatiles modulated in response to nutrients and yeast cell-cell signalling. We have analysed the attraction and repulsion of flies to volatiles not previously described as fly attractants in both T-maze and flask visitation assays and have found that in some cases fly behaviour is bimodal, depending on volatile concentration.

Yeast are also known to be capable of switching morphology to filamentous growth, presumably as a survival mechanism, but potentially also to enhance dispersal by insects. Filamentous and non-filamentous yeast were presented to flies and after incubation were quantified from the fly body, gut, and experimental arena. Results revealed that filamentous yeast were more widely dispersed. Fundamental knowledge of interactions between insects and microorganisms will contribute to a better understanding of aspects of evolution of these fascinating organisms.

Yeast applications in decarbonising fuel and food production

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The fossil fuel-based transportation sector is a major contributor to greenhouse gases (GHG). Renewable biofuels (ethanol and biodiesel) are more environmentally friendly replacements. First-generation ethanol produced by yeast fermentation of starchy substrates or sugar and molasses has expanded over the last two decades, but only offers partial reduction in GHG emissions relative to petroleum. Moreover, expanded use of food crops needed for ethanol to replace >100 million barrels of oil per day is not a sustainable use of arable land. Second-generation ethanol produced by yeast fermentation of lignocellulosic biomass offers an improved GHG reduction and land use alternative. The traditional idea of second-generation biorefineries is to convert biomass-derived six- and five-carbon sugars into ethanol, but the economic viability of these refineries is challenging. Biorefineries manufacturing multiple products will be more environmentally sustainable and economically viable than single-product models. Yeasts have long been used in the human and animal food chain and offer far more scope for valorising lignocellulosic biomass beyond ethanol. We describe a model biorefinery producing ethanol, nonrecombinant yeast biomass with high protein content for food applications, and lignin-rich "green" coal. This model provides significant reductions in GHG emissions, lower fossil energy use, less water consumption, and less land use relative to processes producing ethanol only. Opportunities for yeast applications in future biorefineries linked to renewable power-to-X, where X can be bioethanol, protein, sequestered carbon, or multiple carbon-carbon based synthetic fuels and chemicals will be presented.

Yeast extract exhibits great potential in mitigating alcohol-induced liver injury

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Previous research established that yeast extract (YE) has antioxidant, immune-boosting, and microbiota-regulating properties. However, there is lack of information regarding its function in alcoholic liver injury. This study seeks to obtain data that will help to address this research gap using a Wistar male rat experimental model. Histologic and biochemical analyses results showed that YE has no negative effects on the liver tissue of healthy rats, and can improve the antioxidant and anti-inflammatory capabilities, making it suitable for intervention research on alcoholic liver injury in rats. Both high and low doses of yeast extract were found to exert varying degrees of protective effects on alcoholic liver injury in rats, while also can improve intestinal flora dysbiosis. In the low-dose yeast extract (YEL) group, the abundance of *Peptococcus* and *Ruminococcus* decreased, whereas in the high-dose yeast extract (YEH) group, the abundance of *Peptococcus*, *Romboutsia*, *Parasutterella*, and *Faecalibaculum* decreased. Further analysis of differential metabolites and enriched pathways in the YEL group revealed a significant increase in the abundance of lysophosphatidylcholine (16:0/0:0), and the levels of histamine, adenosine, and 5'-adenine nucleotide elevated in the YEH group. These findings suggest that both high and low doses of YE can have different protective effects on alcoholic liver disease (ALD) rats, in addition to improving intestinal flora disorder. The high-dose YE has been found to more effective on oxidative stress, inflammatory response and metabolic regulation than low-dose YE .

Yeast on a specific nitrogen source diet: Optimization of Yeast Nitrogen Utilization for Enhanced Wine Fermentation

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Nitrogen deficiency in grape must is one of the main issues in winemaking since it can lead to slow fermentations. Development of fruity aromas in wine also depends on nitrogen content in grape must, making nitrogen utilization by yeasts a key point in biotechnological research for the wine industry. Grape must contain several nitrogen sources assimilable by *Saccharomyces cerevisiae* such as ammonium, amino acids and peptides, a secondary nitrogen source whose consumption by *Saccharomyces cerevisiae* during wine fermentation has previously shown clear benefits for vinification. In this work, Adaptive Laboratory Evolution (ALE) strategy was applied on three *S. cerevisiae* (A1 to A3) wine strains for 31 weeks. Twenty-seven populations derived from our ALE strategy were evaluated in fermentation under winemaking conditions using a Chardonnay grape must. Strains were isolated from selected evolved populations for further characterization of their industrial potential using different natural grape musts with different level of yeast assimilable nitrogen. The yeast strains displayed even lower requirements of nitrogen than the ancestral strain, being able to complete fermentation in significantly shorter times in all the natural musts tested and displayed different profiles of volatile compound production. These new phenotypes constitute an improvement in their fermentative capacities, which opens the gate to explore their use in winemaking.

Yeast secretome response to the endoplasmic reticulum stress with revealing of stress-induced Bip secretion

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The endoplasmic reticulum (ER) is a key site for the production of secreted proteins from yeast. ER stress occurs when the capacity of the ER to fold proteins becomes saturated. Many different environmental or intrinsic conditions can trigger ER stress, such as genomic- or antibiotic-induced defects in glycosylation, redox stress, and thermal perturbation. We used quantitative DIA LC-MS/MS proteomics to analyse the response of the

secreted yeast proteome (the secretome) to different ER stresses including lack of the *OST3* or *PMT1* genes required for efficient *N*- and *O*-glycosylation, addition of tunicamycin, dithiothreitol (DTT) or ethanol, and growth under high temperature. Our results showed changes to the secretome under different ER stresses, including changes to protein isoforms, abundance, and post-translational modifications. Intriguingly, we detected increased secretion of several protein folding chaperones in glycosylation stress conditions, suggesting a functional role for these proteins in maintaining client protein stability outside of the cell. ER stress induced by ethanol resulted in substantial changes to the abundance of enzymes involved in central carbon metabolism in the secretome. Surprisingly, defects in *N*-glycosylation and growth at high temperature resulted in similar changes to the secretome, with reduced secretion of cell wall glycoproteins. Proteomic analyses of the whole yeast cell and glycoproteomic analysis of yeast cell wall proteins, confirmed that changes in the secretome were driven by changes in the global yeast proteome. Our results show that the secretome of yeast is highly variable depending on growth conditions and intrinsic stressors, with implications for diverse applications, including beverage production and precision fermentation.

Yeasts as cell factories for the production of bacterial compounds - aminoriboflavin and roseoflavin

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The soil bacteria *Streptomyces davaonensis* and *Streptomyces cinnabarinus* synthesise a promising broad-spectrum antibiotic roseoflavin, its synthesis starting from the flavin mononucleotide and proceeding through an immediate precursor, aminoriboflavin, that also has antibiotic properties. Roseoflavin accumulation by the natural producers is rather low, while the accumulation of aminoriboflavin is negligible. Yeasts have many advantages as biotechnological producers relative to bacteria; however, no recombinant producers of bacterial antibiotics are known in yeast. The roseoflavin biosynthesis genes *rosB*, *rosC,* and *rosA* have been expressed in riboflavin- or flavin mononucleotides overproducing strains of *Candida famata* and *Komagataella phaffii (Pichia pastoris)*. Both strains accumulated aminoriboflavin, whereas only the latter produced roseoflavin. The accumulation of aminoriboflavin and roseoflavin by the recombinant strain of *K. phaffii* in a bioreactor reached 22 and 130 mg L^{-1}, respectively. For comparison, recombinant strains of the native bacterial producer *S. davaonensis* accumulated about one order less of roseoflavin, while no recombinant producers of aminoriboflavin were reported. Yeast strains, in addition to antibiotics, also overproduced riboflavin. It is interesting to note that most of riboflavin and antibiotics have been synthesised and accumulated in the cultural medium after growth cessation, i.e. in the stationary growth phase. Aminoriboflavin isolated from recombinant strains of *C. famata* inhibited the growth of the pathogenic bacteria *Staphylococcus aureus* (including MRSA) and *Listeria monocytogenes*.

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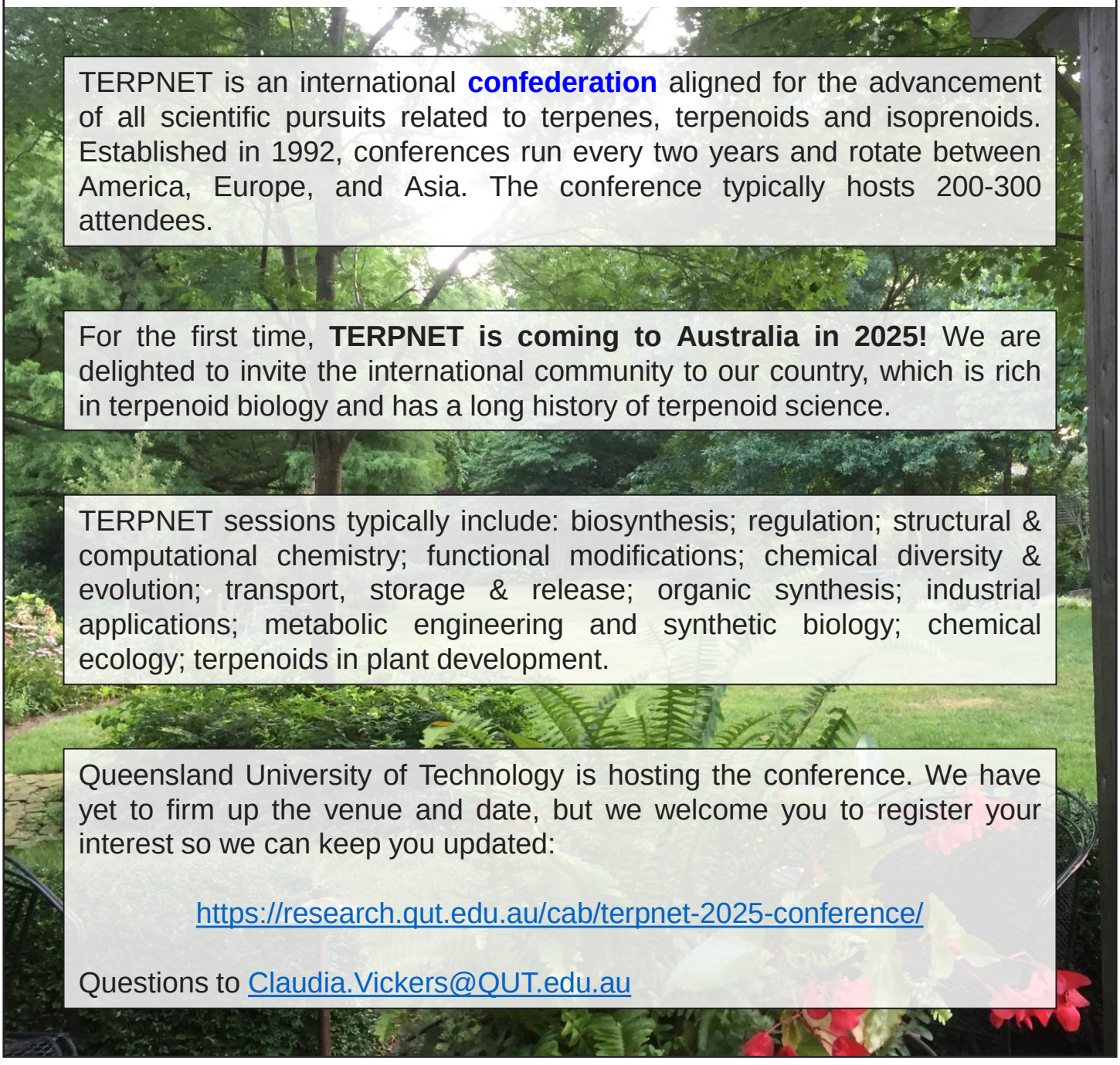

TERPNET is an international **confederation** aligned for the advancement of all scientific pursuits related to terpenes, terpenoids and isoprenoids. Established in 1992, conferences run every two years and rotate between America, Europe, and Asia. The conference typically hosts 200-300 attendees.

For the first time, **TERPNET is coming to Australia in 2025!** We are delighted to invite the international community to our country, which is rich in terpenoid biology and has a long history of terpenoid science.

TERPNET sessions typically include: biosynthesis; regulation; structural & computational chemistry; functional modifications; chemical diversity & evolution; transport, storage & release; organic synthesis; industrial applications; metabolic engineering and synthetic biology; chemical ecology; terpenoids in plant development.

Queensland University of Technology is hosting the conference. We have yet to firm up the venue and date, but we welcome you to register your interest so we can keep you updated:

https://research.qut.edu.au/cab/terpnet-2025-conference/

Questions to Claudia.Vickers@QUT.edu.au