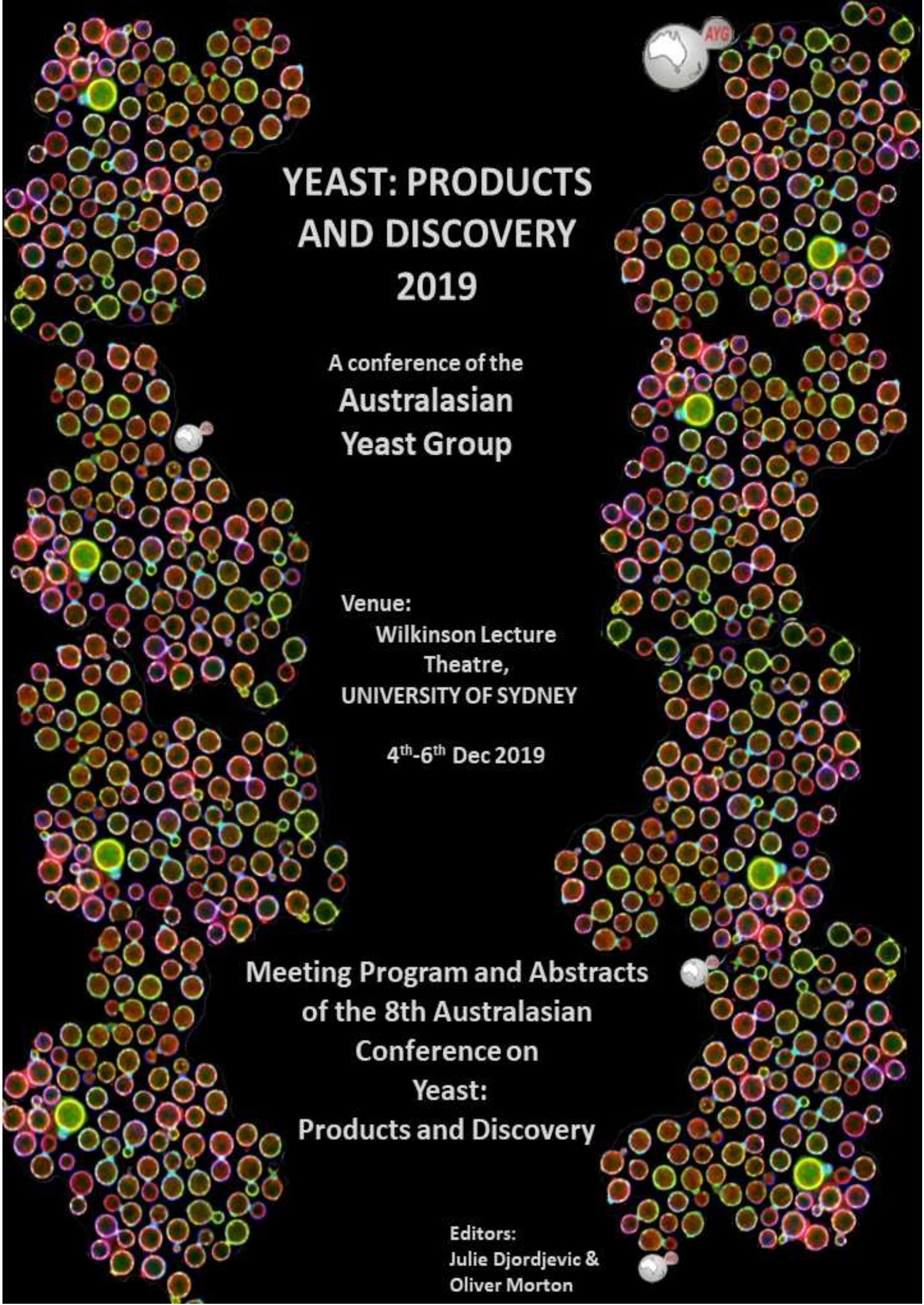

YEAST: PRODUCTS AND DISCOVERY 2019

A conference of the
Australasian Yeast Group

Venue:
Wilkinson Lecture Theatre,
UNIVERSITY OF SYDNEY

4th-6th Dec 2019

Meeting Program and Abstracts of the 8th Australasian Conference on Yeast: Products and Discovery

Editors:
Julie Djordjevic &
Oliver Morton

Local Organising Committee

Chair Local Organising Committee
Dee Carter
University of Sydney
dee.carter@sydney.edu.au

Chair Scientific Program
Julie Djordjevic
Westmead Institute for Medical Research
julianne.djordjevic@sydney.edu.au

Local Organising Committee
Oliver Morton (Secretary) Western Sydney University
O.Morton@westernsydney.edu.au

Marc Wilkins (Sponsorship) University of New South Wales
m.wilkins@unsw.edu.au

Heinrich Kroukamp (Treasurer) Macquarie University
heinrich.kroukamp@mq.edu.au

Justin Beardsley (Social Events) Marie Bashir Institute, Westmead Institute for Medical Research
justin.beardsley@sydney.edu.au

Program Organizing Committee

James Fraser, University of Queensland
Traude Beilharz Monash University
Austen Ganley The University of Auckland
Simon Schmidt Australian Wine Research Institute
Jiyoti Verma Monash University
Alex Andrianopoulos University of Melbourne
Oliver Rackham Perkins Institute and CHIRI, Curtin University
Vanessa Rossetto Marcelino Marie Bashir Institute
Laszló Irinyi Westmead Institute for Medical Research
Benjamin Schultz, University of Queensland
Anthony Borneman Australian Wine Research Institute
Evelyn Sattlegger, Massey University

YPD2019 gratefully acknowledges the invaluable support of the following companies and organizations:

The Australasian Yeast Group (AYG)
www.ayeastgroup.org/

The Australian Society for Biochemistry and Microbiology
www.asbmb.org.au

The Australian Society for Microbiology

The Australasian Mycological Society

AB Biotek
PARTNERS IN FERMENTATION™
A business division of AB MAURI
www.abbiotek.com

THE UNIVERSITY OF SYDNEY
Marie Bashir Institute
University of Sydney

Ramaciotti Centre for Genomics

SCIENTIFIC PROGRAM

Day 1 Wednesday 4th December

8:30 am-9:00 am Registration
9.00 am-10.00 am Opening remarks and Plenary Lecture

Chair: Dee Carter
Speaker: Sakkie Pretorius
Yeast 2.0 – building the world's first functional synthetic eukaryotic genome

10:00 – 10:30 am Morning Tea

10.30 am -12.00 Session I

"Industrial and Synthetic Fungi"

Chair: Traude Beilharz

Speakers:

1.1. Philip Bell (Microbiogen, NSW)
New industrial yeast for the biofuel industries

1.2. Victoria Haritos (Monash, Vic)
Engineering of yeast lipid biofactories

1.3. Heng Chooi (UWA, WA)
Strategies and tools for accessing the hidden treasure troves of fungi: bioactive molecules and biocatalytic enzymes

1.4. Nick Coleman (University of Sydney, NSW)
Taking the magic from mushroom to microbe: construction of an *E. coli* strain for psilocybin biosynthesis

12:00 – 1:00 pm Lunch

Chair: Justin Beardsley

1.00-3.00 pm Student Presentations and Posters Session I

3.00-5.00 pm Student Presentations and Posters Session II

4:30-7:00 pm Break with wine tasting from Tintilla vineyards (Hunter Valley winery) in the Great Hall or associated area – either the ante room or the courtyard in the Holme Building or outside in the Quad.

7:00 pm Dinner in the Great Hall

Great Hall

Day 2 Thursday December 5th

9.00-10.30 am Session 2

"Virulent Fungi"

Chair: Julie Djordjevic

Speakers:
2.1. Richard Cannon (University of Otago, NZ)
Candida auris an emerging multidrug resistant pathogen of humans

2.2. Oliver Morton (WSU, NSW)

Using infection models to study the interaction between *Aspergillus fumigatus* and the host.

2.3. Alex Idnurm (University of Melbourne, Vic)
Opposing roles of DNA repair systems in fungal virulence as assessed in *Cryptococcus* species

2.4. Jiyoti Verma (Monash, Vic)
Insights into chromatin dynamics and its role in fungal virulence.

10.30 - 11.00 am Morning Tea

11.00-12.30 Session 3

The Australian Society for Microbiology
bringing Microbiologists together

"The Microbiome and Biofilms"

Chairs: Heinrich Kroukamp and Vanessa Rosetto Marcelino

Speakers:
3.1. Megan Lenardon (UNSW)
A novel, *in vitro* model to study *Candida albicans* colonisation of the human colon.

3.2. Vanessa Rosetto Marcelino (MBI, University of Sydney and WIMR, NSW)
Novel approaches and challenges in fungal metagenomics

3.3. Bryan R. Coad (University of Adelaide, SA)
Antifungal surface coatings for preventing biofilm formation

3.4. Benjamin Schwessinger (ANU)
Pathogen Detection and the role of Microbial Communities in the phyllosphere during fungal infection of wheat.

12.30-1:30 pm Lunch

1.30- 3.00 pm Session 4

"Drug Discovery and Development"

Chair: Ana Traven

Speakers:

4.1. Luke Guddat (University of QLD)
Acetohydroxyacid synthase: an antifungal drug target

4.2. Claudia Simm (Monash, Vic)
New Strategies to combat the superbug *Candida auris*.

4.3. James McKenna (La Trobe, Vic)
Plant defensins work in synergy with caspofungin against Candida species and can kill *Candida auris*

4.4. Lorna Wilkinson-White (University of Sydney)
Exploiting inositol polyphosphate kinases for antifungal drug development

3.00pm- 3.30 pm Afternoon tea

3.30-5.00 pm Session 5

"**Networks and Regulation"**

Chair: Evelyn Sattlegger

Speakers:
5.1. Marc Wilkins (UNSW)
The Missing Link(s) in Proteomics: Network Discovery by Crosslinking Mass Spectrometry

5.2. Nikolay Shirokikh (ANU, ACT)
Rapid RNA-level responses to stress revealed by translation complex profile sequencing in yeast

5.3. Mark Bleackley (LaTrobe, Vic)
Proteomic characterization of *Saccharomyces cerevisiae* and *Candida albicans* extracellular vesicles to identify biomarkers and a potential role in antifungal drug tolerance.

5.4. Oliver Rackham (Perkins, CHIRI, Curtin University)
Using yeast to discover next generation tools for manipulating genes

5.5. Tim Tucey (Monash University) **(20 min)**
Inflammasome activation and metabolic control of innate immunity during fungal infections

5.00 pm SIG Meeting
7.00 pm Visit Marrickville Breweries

Day 3 Friday December 6th

9.00-10.30 am Session 6

"Yeast and Microfungi in the World"

Chair: Simon Schmidt

Speakers:

6.1. Cristian Varela (AWRI, Adelaide)
Discovering the indigenous microbiota associated with Australian Aboriginal and Torres Strait Islander fermentations

6.2. Manpreet Dhami (Manaaki Whenua Landcare Research, NZ)
Where the wild yeasts are: Reflections on how dispersal, environmental variability and competition shape metabolic potential of nectar yeasts

6.3. Laszlo Irinyi (WIMR)
Assessment of fungal diversity of Australian tree hollows in connection to the *Cryptococcus gattii* and *C. neoformans* species complexes

6.4. David Guest (University of Sydney, NSW)
The impact of fungal plant diseases on food security

10.30 - 11.00am Morning Tea

11-12.30 pm Session 7

"Genetics, Evolution and Resistance"

Chair: Benjamin Schultz

Speakers:

7.1. Alex Andrianopoulos (University of Melbourne, Vic)
Adaptation to the host niche through cell shape control in the human pathogenic fungus *Talaromyces marneffei*

7.2. Austen Ganley (University of Auckland, NZ)
Building Rube Goldberg machines in yeast to explore unnecessary complexity in biology

7.3. Erwin Lamping (University of Otago, NZ)
Challenges of membrane protein research in *Saccharomyces cerevisiae*.

7.4. Anthony Borneman
Is SO_2 tolerance in *Brettanomyces bruxellensis* a developing concern?

12.30-1.00 pm Concluding Remarks and Prizes (Julie Djordjevic)

Abstracts

Plenary Lecture

Yeast 2.0 – building the world's first functional synthetic eukaryotic genome

Tom Williams, Heinrich Kroukamp, Hugh Goold, Roy Walker, Ian Paulsen and **Isak S. Pretorius**

ARC Centre of Excellence in Synthetic Biology, Macquarie University, Sydney
Email: Sakkie.Pretorius@mq.edu.au

Historians of the future may well describe 2020 as the year that the world's first functional synthetic eukaryotic genome became a reality. Without the benefit of hindsight, it might be hard to completely grasp the long-term significance of a breakthrough moment in the history of science like this. The role of synthetic biology in the imminent birth of a budding *Saccharomyces cerevisiae* yeast cell carrying 16 man-made chromosomes causes the world of science to teeter on the threshold of a future-defining scientific frontier. The genome-engineering tools and technologies currently being developed to produce the ultimate yeast genome will irreversibly connect the dots between our improved understanding of the fundamentals of a complex cell containing its DNA in a specialised nucleus and the application of bioengineered eukaryotes designed for advanced biomanufacturing of beneficial products. By joining up the dots between the findings and learnings from the international *Synthetic Yeast Genome* project (known as the *Yeast 2.0* or *Sc2.0* project) and concurrent advancements in biodesign tools and smart data-intensive technologies, a future world powered by a thriving bioeconomy seems realistic. This global project demonstrates how a collaborative network of dot connectors – driven by a tinkerer's indomitable curiosity to understand how things work inside a eukaryotic cell – are using cutting-edge biodesign concepts and synthetic biology tools to advance science and to positively frame human futures (i.e. improved quality of life) in a planetary context (i.e. a sustainable environment). Explorations such as this have a rich history of resulting in unexpected discoveries and unanticipated applications for the benefit of people and planet. However, we must learn from past explorations into controversial futuristic sciences and ensure that researchers at the forefront of an emerging science such as synthetic biology remain connected to all stakeholders' concerns about the biosafety, bioethics and regulatory aspects of their pioneering work. This article presents a shared vision of constructing a synthetic eukaryotic genome in a safe model organism by using novel concepts and advanced technologies. This multidisciplinary and collaborative project is conducted under a sound governance structure that does not only respect the scientific achievements and lessons from the past, but that is also focussed on leading the present and helping to secure a brighter future for all.

Session 1

1.1. New industrial yeast for the biofuel industries

Philip Bell

Microbiogen Pty Ltd, Business Park, Unit E2, Lane Cove, 32 Sirius Rd, Lane Cove West NSW 2066

Yeast of the genus *Saccharomyces* are the most widely used industrial microbes underpinning industries worth in excess of $1 trillion annually. Large industries based on *Saccharomyces* include the traditional baking, brewing, and wine industries with over 1 500 000 tonnes of yeast biomass produced for the baking industry alone. In addition to these established industries, emergent industries such as the biofuels, biochemical, nutraceutical, and pharmaceutical industries are in many cases dependent upon *Saccharomyces* as a core component of their production system. Both the current first generation (crop based) and the emerging second generation (plant biomass based) fuel ethanol industries exploit the unsurpassed ability of *Saccharomyces* yeast to act as a catalyst in the conversion of sugars into ethanol with high yield and productivity. In this talk I will present an overview of Microbiogen's technology that has generated industrial yeast strains that are now producing > 25% of US bioethanol, and how it's A$8M 2nd generation program backed by an Australian Federal government ARENA grant is leading to the development of yeast that can enable the production of food and fuel from lignocellulosic biomass.

1.2. Engineering of yeast lipid biofactories

Jiang, W[1,2], Peng, H[2], He, L[1] and **Haritos, VS**[1]

1. Department of Chemical Engineering, Monash University, Clayton, VIC, 3800 Australia,
2. Department of Bioengineering, Imperial College London, London, United Kingdom
Email: Victoria.haritos@monash.edu

Bioproduction of chemicals and fuels is an important and growing segment of manufacturing and *Saccharomyces cerevisiae* is both widely used as production organism and as a metabolic model. Fatty acid-containing lipids are a class of moderate value, highly versatile chemicals produced by yeasts with application as fuels, cosmetics, lubricants and coatings. Over the past decade, metabolic lipid engineering of yeast has increased production levels of standard fatty acids enormously and these are now close to commercial reality. But we are still a long way from efficient cell factories producing a wider range of functional chemistries. We aim to develop yeast biofactories for new chemistries through enzyme discovery and engineering, informed by computational metabolic flux models and applying design-test-build-learn principles from synthetic biology. Here, cyclopropanated fatty acid is used as proof-of-principle that expands the current enzyme and metabolic pathway design space. While yeast naturally produce standard monounsaturated fatty acids such as oleic, palmitoleic and saturated forms stearic and palmitic, functionalised fatty acids such as cyclopropane fatty acids that possess a strained 3-membered ring in a saturated chain, present greater challenges. Our strategy to achieve high yield of cyclopropyl-containing lipid product includes expressing *Escherichia coli* cyclopropane fatty acid synthetase (EcCFAS) in yeast pre-engineered for increased lipid production. This step increased the yield of cyclopropyl fatty acid 4-fold in triglyceride (TAG). However, the cyclopropyl form was just 16% of total TAG fatty acid whereas the proportion in phospholipid was 40%. To further improve yield of this fatty acid, we have undertaken a systematic analysis of cyclopropane fatty acid synthesis, assessed the impact of expressing native and introduced lipid handling genes and pathway modifications, and examined potential substrate limitations. Together, this metabolic engineering strategy has greatly improved cyclopropyl production in yeasts and the approach is applicable to other similarly-synthesised high value exotic fatty acids.

1.3. Strategies and tools for accessing the hidden treasure troves of fungi: bioactive molecules and biocatalytic enzymes

Yit-Heng CHOOI

ARC Future Fellow - School of Molecular Sciences, University of Western Australia, Perth, 6009 WA.

A paradigm that has emerged for microbial drug discovery in the genomics era is that we are far from exhausting the chemical diversity encoded in the genomes of fungi, as the number of biosynthetic gene clusters in the genomes often greatly exceeds the number of secondary metabolites isolated from cultures in the laboratory. This is due to many of these specialised metabolite biosynthetic pathways are conditionally expressed, in response to varying biotic and abiotic factors in the environment. Inspired by this, we harness microbial biotic interactions to guide our genome mining effort for bioactive molecule discovery. Our lab has a special interest in molecules from fungi interacting with higher eukaryotic hosts, i.e. plants and animals, as these molecules could have potential agricultural and pharmaceutical applications. One of our strategies is to use transcriptomics to prioritise biosynthetic gene clusters that are expressed during fungal-host interactions and couple that with synthetic biology tools that enabled specific pathway activation and/or pathway reconstruction in heterologous expression system. To this end, we have developed a modular multi-gene episomal expression system for rapid reconstruction and elucidation of cryptic biosynthetic pathways in fungi. Using such strategy on

a wheat fungal pathogen, we have discovered a number of phytotoxic natural products previously not known to be produced by the fungus, some of which we have demonstrated to be important for its virulence. In the process, we have also uncovered a number of novel biosynthetic enzymes in these cryptic biosynthetic pathways, which could perform complex chemical transformations and have potential applications as biocatalysts for chemical synthesis. Finally, I will discuss the recent development in our lab on the development of CRISPR-based technology for accessing the hidden biosynthetic potential of fungi to accelerate bioactive molecules discovery.

1.4. Taking the magic from mushroom to microbe: construction of an *E.coli* strain for psilocybin biosynthesis

Ali, F., Caruana, M., Gonzaga, B., Hawkins, N., Magrath, I., Todd, E., and **Coleman N.V.**

School of Life and Environmental Sciences, University of Sydney

Psilocybin is the major psychoactive component of 'magic' mushrooms. This chemical is being investigated as a medicine for treatment of depression, addiction, and post-traumatic stress disorder. One of the major hurdles for research is in sourcing psilocybin; the wild mushrooms vary greatly in potency and are not easy to grow in captivity, and the compound is difficult to make via organic chemistry. We took a synthetic biology approach to solve this problem, by expressing the four genes from the psilocybin biosynthetic pathway in *E.coli*, which is easier to grow and scale-up than *Psilocybe* mushrooms. This project was done by a team of six undergraduates as part of the
The genes of the psilocybin pathway were synthesised as 'gBlocks' to remove internal restriction sites and allow codon optimisation of the genes. The first gene in the pathway (PsiH, a p450 hydroxylase) needs a partner enzyme, so the psiH gene was cloned into an IPTG-inducible expression plasmid (pCW) that also contains the human p450 reductase gene. The remaining three genes (psiD decarboxylase, psiK kinase and psiM methylase) were cloned into the cumate-inducible expression plasmid pUS250. All the genes were also cloned individually into the pET28 expression vector to confirm that soluble proteins were being made in *E.coli*. Successful expression of PsiD, PsiK, and PsiM was confirmed by SDS-PAGE and peptide mass spectrometry, but we could not confirm PsiH expression. E.coli cells containing the PsiDKM gene cluster were capable of converting 4-hydroxytryptamine to norbaeocystin and baeocystin. Although we did not detect psilocybin itself, it is notable that baeocystin does have psychedelic properties.
We successfully reconstructed several steps of psilocybin biosynthesis in *E.coli*, and now need to work on PsiH gene expression to complete the pathway from tryptophan through to psilocybin.

Session 2

2.1. *Candida auris* an emerging multidrug resistant pathogen of humans

Cannon, R.D., Aum, B., Lamping, E.

Sir John Walsh Research Institute, Faculty of Dentistry, University of Otago, Dunedin, New Zealand

Candida auris is a rapidly emerging human fungal pathogen that can cause fatal invasive fungal infections in the immunocompromised. *C. auris* was first identified in Japan in 2009, and the first case of nosocomial candidemia due to *C. auris* was reported in South Korea in 2011. *C. auris* poses a global health threat because of its transmissibility, its persistence in health care settings, and the prevalence of drug resistant strains. Some *C. auris* isolates are resistant to all three major antifungal classes; the azoles, polyenes, and echinocandins. Azole resistance can arise from mutations in the drug target, ERG11, but the contribution of increased expression of ABCG-type ATP-binding cassette (ABC) transporters to azole resistance in *C. auris* is unknown. The objective of this study was to determine which *C. auris* ABC genes might contribute to drug resistance by heterologous expression of candidate genes in *Saccharomyces cerevisiae* We identified the ABCG genes most similar to those in *C. albicans* responsible for azole drug resistance. We heterologously expressed these genes, *CDR1*, *CDR4, CDR41, SNQ2* and *SNQ21*, in *Saccharomyces cerevisiae* AD□□ and measured the antifungal drug susceptibilities they conferred. We confirmed that three genes, *CDR1*, *CDR41* and *SNQ2*, and possibly also *CDR4* and *SNQ21*, encoded multidrug efflux pumps. Cdr1, however, was by far the most effective, affirming it as the 'prime suspect' for playing a role in the drug resistance phenotype of *C. auris*.

2.2. Using infection models to study the interaction between *Aspergillus fumigatus* and the host.

C. Oliver Morton, Sam El-Kamand, Carl Ramirez, Marisa Zappia, Rogine Ligot.

School of Science and Health, Western Sydney University, Campbelltown Campus, NSW 2560.

Infection by opportunistic fungal pathogens have continued to increase, invasive aspergillosis (IA) is one of the most serious threats to immunocompromised individuals. IA is usually caused by *Aspergillus fumigatus* but other species within this genus are becoming more prevalent. The role of fungal virulence factors in disease outcome is still unclear given the immune status of the host.
IA is difficult to study in the human host, this has impeded the development of diagnostic tests and made it challenging to dissect fungal pathogenesis. As with many other infectious disease small mammals have

been used as model hosts to explore various aspects of IA. These present two problems, they are not human and there is increasing pressure to reduce the use of animals in research. Over several years there has been move to use different models to study IA. In this presentation the use of various models for studying IA will be discussed. These include purified human immune cells, a transwell model of the alveolar interface using human cell lines, *Trichomonas vaginalis* as a substitute for human immune cells, and meal worms as an infection model.
Using non-mammalian hosts provides a cheap and ethically favourable means of testing hypotheses related to fungal infection, such as virulence testing and drug testing. This allows for improved experimental design and reduced numbers of mammalian hosts in downstream experiments.

2.3. Opposing roles of DNA repair systems in fungal virulence as assessed in *Cryptococcus* species

Alexander Idnurm

School of BioSciences, University of Melbourne, VIC 3010

The fundamental nature of DNA in cells is reflected by a diverse array of mechanisms that are involved in the protection and repair of damage to this molecule. Research in the human pathogenic fungi has suggested that there are two conflicting roles of DNA repair enzymes, in allowing a level of sloppiness in replication for the rapid microevolution of new traits balanced by the need to protect DNA from too much damage. The talk will cover these two aspects of the impact of DNA repair on the virulence of the human pathogenic yeasts in the genus *Cryptococcus*. Recent findings have identified a mutation in the proof-reading component of DNA polymerase 3 as being central to a process once defined as phenotypic switching. Curiously, this mutation itself has no impact on virulence, but causes the rapid emergence of 'microevolved' strains with altered properties. Second, the *RAD23* gene was characterized in detail. Rad23, a known component of the nucleotide excision repair pathway that is required for *C. neoformans* virulence, has a second role in recycling proteins through the proteasome. By generating and testing alleles of *RAD23* impair in both processes, the role of *RAD2*3 in virulence was uncoupled from its role in DNA repair. These research directions thereby help extend from an original simple hypothesis that DNA repair is required for fungal disease, into a more comprehensive understanding of which components play specific roles in the biology of pathogenic yeasts and other fungi.

2.4. Insights into chromatin dynamics and its role in fungal virulence.

Jiyoti Verma[1], Qi Wang [1], Nikolina Vidan[1,2], Yanan Wang[1], Timothy M Tucey[1], Paul F Harrison[3], Michael See[3], Angavai Swaminathan[4], Karl Kuchler[5], Michael Tscherner[5], Jiangning Song[1], David R Powell[3], Mary Sopta[2], Traude H Beilharz[4] and Ana Traven[1]

[1]*Infection and Immunity Program and the Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton 3800 Victoria, Australia*
[2]*Department of Molecular Biology, Institute Rudjer Boskovic, Bijenicka 54, 10000 Zagreb, Croatia*
[3]*Bioinformatics Platform, Monash University, Clayton 3800 Victoria, Australia*
[4]*Development and Stem Cells Program and the Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton 3800 Victoria, Australia*
[5]*Medical University of Vienna, Center for Medical Biochemistry, Max Perutz Labs, Campus Vienna Biocenter, Dr. Bohr-Gasse 9/2, Vienna, Austria*

Metabolic regulation underpins all cell biology. Recent identification of multiple histone acylations diversifies transcriptional control by metabolism, and the challenge now is to understand their roles and regulation. Here, we report evidence of histone crotonylation in the human commensal and yeast pathogen *Candida albicans*, define the enzymes that regulate it, and show its dynamic control by host-relevant metabolic signals: carbon sources, microbiota-derived short-chain fatty acids butyrate and crotonate, and cell wall stress. Our findings shed new light on the functions of histone crotonylation and its readers, define regulation by metabolic cues, and suggest that eukaryotic microbiota use diverse histone acylations to regulate their biology and pathogenesis. This work is accomplished by transversal collaborations involving molecular biologists, biochemists and bioinformaticians from 4 labs and 2 research platforms (Bioinformatics Platform and Monash Animal Research Platform) at Monash and 2 international teams from Croatia and Austria.

Session 3

3.1. A novel, *in vitro* model to study *Candida albicans* colonisation of the human colon.

Lai, Y.W.[1], Prokop, M.D.[1], Griffiths, E.[1] and Lenardon, M.D.[1]

[1]School of Biotechnology and Biomolecular Sciences, UNSW Sydney, NSW, 2052.

The major fungal pathogen of humans, *Candida albicans*, colonises the gastro-intestinal (GI) tract of over 60% of the population. In severely ill or immune compromised patients, *C. albicans* can escape from the gut, disseminate through the bloodstream and cause systemic disease. Most research in the *Candida* field has focused on defining traits that contribute directly to virulence; there are comparatively few studies which have addressed how *C. albicans* colonises and persists in the gut. Furthermore, such studies have been performed mouse models devoid of resident GI bacteria, completely neglecting the major impact of the local microbiota on GI colonisation. This raises the key question: how does *C. albicans* persist in the GI tract in the presence of the normal gut microbiota?
To address this question, we have developed a novel *in vitro* two-phase anaerobic fermentation system that simulates the human colon microenvironment. This "colon microcosm" system supports the growth of human faecal microbiota in liquid anaerobic colon medium (phase 1) and *C. albicans* growth on agar plugs which are added to the medium to mimic the epithelial surface (phase 2). The impact of *C. albicans* upon the faecal microbiota is monitored by examining the planktonic phase (phase 1), whilst the effect of the microbiota on the growth of *C. albicans* is monitored after extracting *C. albicans* cells from the agar plugs (phase 2).
The results from RNASeq and competitive fitness assays will be presented which have been carried out to validate the model. Data from pilot studies will also be presented to illustrate the potentially exploitable impact of the human GI microbiota from healthy individuals on *C. albicans* growth. In the future, this model will be utilised to determine the adaptive and evolutionary mechanisms that enable *C. albicans* to persist in the colon in the presence of the GI microbiota.

3.2. Novel approaches and challenges in fungal metagenomics

Vanessa R. Marcelino

Marie Bashir Institute for Infectious Diseases and Biosecurity, The University of Sydney and The Westmead Institute for Medical Research

Culture-independent high-throughput sequencing approaches (metagenomics) are revealing an uncharted diversity of microorganisms with important functions for the health of their associated host or environment. Yeasts, molds and other microbial eukaryotes are diverse, widely distributed, and play important functional roles in these environments, but are far less studied than their bacterial counterparts. A new bioinformatics pipeline developed within our group can circumvent a key methodological limitation – the lack of reference databases of complete eukaryotic genomes – and facilitates the inclusion of fungi in metagenomic studies. We applied our pipeline to characterize the gut microbiome of wild birds, and we found an abundant and diverse community of micro-eukaryotes, with fungal taxa composing 50% of the family-level diversity in those samples. Our work opens numerous possibilities to identify fungi in mixed communities with metagenomics.

3.3. Antifungal surface coatings for preventing biofilm formation

Naderi, J.[1], Lamont Friedrich, S.J.[1], Chakraborty, A.[1], Giles C.[1], Traven, A.[2], Griesser, H.J.[1], **Coad, B.R.**[1,3]

1. Future Industries Institute. The University of South Australia. Mawson Lakes SA 5095.
2. Infection and Immunity Program and the Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton VIC 3800.
3. School of Agriculture, Food & Wine, The University of Adelaide, Urrbrae SA 5064.

Introduction: My group is broadly interested in plant and animal fungal pathogenesis; more specifically, how pathogens sense and respond to the chemical and physical properties of interfaces/surfaces. In this talk, I will focus on investigations into prevention of fungal biofilm formation on medical device implant materials caused by *Candida spp.* Implant coatings incorporating azoles and polyene antifungal drugs effectively prevent or delay biofilm formation on surfaces because these drugs are gradually released from the coating. However, results from experiments using permanently attached echinocandins as antifungal surface coatings have produced intriguing results.
Hypothesis, Methods & Results: We have gathered evidence that supports the hypothesis that yeasts that contact surfaces bearing covalently attached echinocandins are inhibited despite no diffusive release of drug from the surface. We have confirmed chemical attachment of caspofungin, anidulafungin and micafungin to surface coatings using instrumental analysis and quantified strong inhibition (> 10^6 CFU/ml) of *Candida spp* with no biofilm formation. We have investigated whether elution of these drugs could explain an antifungal effect in solution; however, the evidence from surface patterning experiments and SEM imaging does not support this view. Additionally, since surfaces remain active after being exposed several times to fresh fungal challenges (i.e. they can be reused), we believe that drugs are not being cleaved by hydrolases. Inhibition of the β-1,3 glucan synthase complex is likely involved because the resistance to

echinocandins caused by FKS mutations in *C. albicans* persists whether tested on surfaces or in solution.
Conclusion: A surface-chemistry approach for fabricating antifungal surface coatings has produced highly effective materials for eliminating fungal cell adhesion on contact and preventing biofilm formation. Promising results with echinocandins warrant further testing to assess clinical translatability and elucidate what we believe is a novel mechanism of action for this drug class.

3.4. Pathogen Detection and the role of Microbial Communities in the phyllosphere during fungal infection of wheat

Yiheng Hu[1]*, Gamran Green[1], Andrew Milgate[2], Laszlo Irinyi[3], Wieland Meyer[3], Eric Stone[1], John Rathjen[1]* and **Benjamin Schwessinger[1]***

[1] Research School of Biology, The Australian National University, Canberra, ACT, Australia;
[2] Wagga Wagga Agricultural Institute, New South Wales Department of Primary Industry, Wagga Wagga, NSW, Australia;
[3] Molecular Mycology Research Laboratory, Center for Infectious Diseases and Microbiology, Faculty of Medicine and Health, Sydney Medical School, Westmead Clinical School, The University of Sydney, Sydney, NSW, Australia.

Fungal diseases of plants are responsible for major losses in agriculture, highlighting the need for rapid and accurate identification of fungal plant pathogens. Disease outcomes are influenced by diverse microbial communities known as microbiomes at sites of infection. We conducted the first use of metagenomics sequencing with a portable sequencer as a method for detecting fungal pathogens from wheat (*Triticum aestivum*) in a standard molecular biology laboratory. The data revealed that our method is robust and capable of diagnosing stripe rust (caused by *Puccinia striiformis* f. sp. *tritici*), septoria blotch (caused by *Zymoseptoria tritici*) and yellow spot (caused by *Pyrenophora tritici repentis*). We also identified the bacterial genus *Pseudomonas* co-present with *Puccinia* and *Zymoseptoria* but not *Pyrenophora* infections. One limitation of the method is the over-representation of redundant wheat genome sequences from samples and therefore a low coverage of pathogen species which affects the identification accuracy. We are improving fungal identification by testing different classification strategies on a mock fungal community. Here I will present recent progress in our method development and the biological insight gained during this process. Our work outlines a new approach for detection of a broad range of plant pathogens and associated microbes using a portable sequencer, providing the basis for the development of a high-throughput, large scale and on-site plant disease monitoring system.

Session 4

4.1. Acetohydroxyacid synthase: an antifungal drug target

Luke W Guddat

School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, 4072, Queensland, Australia.

Acetohydroxyacid synthase (AHAS) is the first enzyme in the branched chain amino acid biosynthesis pathway. This pathway is present only in plants, bacteria and fungi making it an attractive target for drug and herbicide discovery. Indeed, more than 50 commercial herbicides have been developed that target this enzyme and are in high demand for crop protection. We have shown that several AHAS inhibitors developed as commercial herbicides, are powerful accumulative inhibitors of *Candida albicans* AHAS (K_i values as low as 800 pM) and have determined high-resolution crystal structures of this enzyme in complex with several of these herbicides. In addition, we have demonstrated that chlorimuron ethyl (CE), a member of the sulfonylurea herbicide family, has potent antifungal activity against five different *Candida* species and *C. neoformans* (with MIC_{50} values as low as 7 nM)[1]. Furthermore, in these assays, we have shown CE and itraconazole (a P450 inhibitor) can act synergistically to further improve potency. Finally, we have shown that in *Candida albicans* infected mice, CE is highly effective in reducing pathogenic fungal burden in the lungs, liver and spleen, thus, reducing overall mortality rates. Therefore, in view of their low toxicity to humans, AHAS inhibiting herbicides represent a new class of antifungal drug candidates[1].
Reference [1] Garcia, M. D., Chua, S. M. H., Low, Y. S., Lee, Y. T., Agnew-Francis, K., Wang, J. G., Nouwens, A., Lonhienne, T., Williams, C. M., Fraser, J. A., and Guddat, L. W. (2018) Commercial AHAS-inhibiting herbicides are promising drug leads for the treatment of human fungal pathogenic infections, *Proc Natl Acad Sci USA 115*, E9649-E9658.

4.2. New Strategies to combat the superbug *Candida auris*.

Simm, C. and Traven, A.

Department of Biochemistry and Molecular Biology, Monash University Building 76, 19 Innovation Walk Monash University, Clayton 3800, VIC, AUSTRALIA

It is increasingly being recognized that fungal infections pose a significant and growing threat to human health, and that the current armoury of antifungal drugs is inadequate. Most antifungal drugs such as amphotericin B and fluconazole were developed decades ago and target either ergosterol or cell wall biosynthesis. Limited

target spectrum, efficacy and substantial host toxicity turn systemic fungal infections into a deadly human disease with mortality rates of up to 50%. Increasing resistance towards these “old drugs”, as well as the emergence of intrinsically resistant fungal pathogens such as *Candida auris* means that the development of new antifungal therapeutic strategies is paramount. For this project, we conducted a phenotypic screen against *C. auris* using compounds of the Pathogen box (Medicines for Malaria Venture (MMV) foundation), a collection of 400 small drug-like molecules directed against neglected diseases. Several compounds with antifungal activity against *C. auris* were identified and minimal inhibitory concentrations were determined for 8 candidate compounds. Multiple complementary techniques are employed to identify the mechanism of action of the most efficacious compounds. The investigation of novel targets will give us insight into pathogenicity and virulence factors of this new human pathogen.

4.3. Plant defensins work in synergy with caspofungin against Candida species and can kill *Candida auris*.

McKenna, J. A, Bleackley, M. R., van der Weerden N. L., Anderson M. A.
La Trobe Institute for Molecular Science, Bundoora, Victoria, Australia.

Plant defensins are small peptides that are a major component of the innate immune system of plants. Many have broad spectrum activity against a range of plant as well as human fungal pathogens (Parisi et al 2019). We have been investigating the potential of plant defensins for treatment of Candida infections both alone and in combination with clinical antifungals from the echinocandin family. More recently, we have assessed the activity of a range of plant defensins against the emerging fungal pathogen *C. auris.*
Candida species are among the most common human commensal organisms, because they can colonize almost all mucosal surfaces. They only cause mild localized infections in healthy individuals, but their unrestricted growth can cause life threatening systemic infections in the immunocompromised. We have identified several plant defensins that act in synergy with caspofungin. Sub-inhibitory concentrations of defensin decrease the MIC of caspofungin by as much as 32-fold against several Candida species including *C. albicans*, *C. krusei*, *C. glabrata* and *C. parapsilosis*.
Plant defensins also inhibit and kill *C. auris*. We have tested a range of defensins for their activity against three *C. auris* strains that were isolated in Australia; each with different antifungal susceptibility profiles. One of these strains is resistant to echinocandins and one is resistance to azoles. While all defensins inhibited the growth of *C. auris* to varying degrees, one defensin was significantly more active. This defensin completely inhibited the growth of *C. auris* at 2-3µg/mL including strains that were fluconazole or echinocandin resistant. We are currently studying the mechanism of action of this defensin to understand how successful treatments for fungal disease might be developed.

4.4. Exploiting inositol polyphosphate kinases for antifungal drug development.

Lorna Wilkinson-White[1], Desmarini Desmarini[2,3,4], Sophie Lev[2,3,4], Tania C. Sorrell[2,3,4], Philip Thompson[5] and Julianne Teresa Djordjevic[2,3,4].

[1]Sydney FBDD Facility, Sydney Analytical, University of Sydney 2006 NSW.
[2]Centre for Infectious Diseases and Microbiology, Westmead Institute for Medical Research, Westmead, 2145 NSW.
[3]Westmead Clinical School, Faculty of Medicine and Health, University of Sydney at Westmead, 2145 NSW
[4]Marie Bashir Institute for Infectious Diseases and Biosecurity, University of Sydney, 2006 NSW
[5]Monash Institute for Medical Research, Parkville, 3052 Victoria.

Invasive fungal disease poses a serious threat to human health, affecting 300 million people and causing 1.5 million deaths annually. Current therapies are toxic, sub-optimal or poorly absorbed, and anti-fungal resistance is emerging.

Using *Cryptococcus neoformans* as a model, our team demonstrated that the fungal kinase, Arg1, is critically linked to fungal virulence and invasiveness. Arg1 deletion leads to symptom-free infection and rapid clearance of *C. neoformans* in mice. Therefore, small molecule inhibitors of Arg1should achieve similar outcomes for any fungal pathogens with Arg1 orthologs, including *Candida albicans* and *Aspergillus fumigatus*. The team has used rational design to synthesise a lead compound (PO2) that inhibits the fungal kinase more effectively than its closest human homologue, IPMK, providing proof-of-concept that selective targeting of Arg1 is feasible. We have also used a fragment based drug discovery (FBDD) approach, with the aim of identifying novel molecules and/or binding sites that could inhibit Arg1. The development of more potent and selective small molecule inhibitors of Arg1 from both of these starting points is being pursued to create a novel class of antifungal agent.

Session 5

5.1. The Missing Link(s) in Proteomics: Network Discovery by Crosslinking Mass Spectrometry

Tara Bartolec [1] , Daniela-Lee Smith [1] , Ignatius Pang [1] , You DanXu [2], Joshua Hamey [1] , **Marc Wilkins** [1]

1. Systems Biology Initiative, School of Biotechnology and Biomolecular Sciences, UNSW
2. School of Chemistry, UNSW

Saccharomyces cerevisiae has the most comprehensively characterised protein-protein interaction network, or interactome, of any species. This has been generated through multiple, systematic studies of protein-protein interactions by two-hybrid techniques and through multiple, systematic studies of affinity-purified protein complexes. Despite the quality of this interactome, a question remains as to the extent of interactions that are yet to be detected. These may include interactions that are weak or transient, or those involving proteins that are not amenable to study as two-hybrid fusion proteins. Large scale crosslinking-mass spectrometry (XL-MS) has become possible through the development of cleavable crosslinkers, MS3 methods for peptide analysis and new software. To date, these methods have been applied to analysis of mammalian cells and tissues, to some organelles and to a number of bacterial systems however they have not been applied to yeast - despite it providing a valuable system for benchmarking. Here we used XL-MS to study intact yeast nuclei, using DSSO, MS3 analysis and XlinkX. Linear peptides identified ~3,300 nuclear-associated proteins, generating the most comprehensive proteome of this yeast organelle to date. A total of ~2,500 crosslinked spectral matches were found, resulting in ~1,350 unique lysine-lysine crosslinks. Of these, 65% were intra-protein crosslinks and 35% were inter-protein crosslinks. Approximately one-third of intralinks mapped to PDB structures, 93% which were less than the <30Å distance constraint. Interestingly, intralinks were found for 437 proteins with no existing structural data. In some cases, such intralinks could refine ab initio structural models. Application of stringent score cutoffs to interlinks yielded a high confidence nuclear interactome. Strikingly, almost half of the interactions were not previously detected by two-hybrid or AP-MS techniques. Multiple lines of evidence existed for many such interactions, whether through replicates, literature or ortholog interaction data. We conclude that XL-MS is a powerful means to measure protein-protein interactions that can complement two-hybrid and AP-MS techniques.

5.2. Rapid RNA-level responses to stress revealed by translation complex profile sequencing in yeast.

Shirokikh, N.E.[1], Janapala, Y.[1], Horvath, A.[1], Beilharz, T.[2], Hannan, K.[3], Eyras, E.[1], Hannan, R.[3], Preiss, T.[1,4]

1. *Department of Genome Sciences, The John Curtin School of Medical Research, The Australian National University, Canberra, ACT, Australia*
2. *Monash Bioinformatics Platform, Monash University, Melbourne, Vic, Australia*
3. *Australian Cancer Research Foundation Department of Cancer Biology and Therapeutics, The John Curtin School of Medical Research, The Australian National University, Canberra, ACT, Australia*
4. *Victor Chang Cardiac Research Institute, Sydney, NSW, Australia*

From synaptic plasticity including memory functions, to adaptation to immediate environments and stresses, eukaryotic cells rely on fast-paced translational control. To understand how the cells mitigate adverse conditions or acquire and maintain an adapted phenotype, it is critical to expose the underlying regulation of protein synthesis encoded in messenger (m)RNA. Yeasts depend on glucose as a primary nutrient and acutely respond to change of its levels. Glucose stress adaptation relates to yeast robustness in bioproduction and pathogenicity, as well as it is an archetypical eukaryotic reaction to a changed environment. Yeast response to glucose deprivation is dramatic and manifests as a multi-stage, complex process eventually leading to reprogramming of the exposed cells[1]. However, primary responses initiating this reprogramming cascade are incompletely understood[1].
To detect and investigate translational responses *in vivo*, we developed translation complex profile sequencing (TCP-seq)[2,3]. TCP-seq uniquely allows to resolve all translation intermediates, including those of fast-paced processes such as mRNA scanning, and visualize start codon recognition events and ribosomal recycling directly in live cells[4]. We combine TCP-seq with translation initiation factor-selective purification of protein synthesis complexes to discern how glucose starvation response is initiated, maintained and released in yeast.
We observe previously uncharacterised extremely rapid reactions of yeast cells to glucose removal, with the initial response discernible at a 20-second time point. Many 'housekeeping' mRNAs are inhibited during this response; however, a subset of mRNAs remains highly translated or is specifically activated. Much of these mRNAs are involved in gluconeogenesis and energy control, suggesting that yeasts first try to ‘wait over’ glucose depletion and rely on the existing mRNAs to adjust their metabolism, before making more permanent changes to the gene expression profiles. Our data demonstrate the complexity of cellular genetic responses to stimuli and may reveal primary responses serving as triggers to the subsequent genetic control.

References

1. Janapala, Y., Preiss, T., **Shirokikh, N.E.** (2019)

International Journal of Molecular Sciences 20, 4043. Control of Translation at the Initiation Phase During Glucose Starvation in Yeast.
2. ●Archer, S. K., ●**Shirokikh, N. E.**, Beilharz, T. H., Preiss, T. (2016)
Nature 535, 570-574. Dynamics of ribosome scanning and recycling revealed by translation complex profiling.
3. ●**Shirokikh, N. E.**, ●Archer, S. K., Beilharz, T. H., Powell, D., Preiss, T. (2017)
Nature Protocols 12, 697-731. Translation complex profile sequencing to study the in vivo dynamics of mRNA-ribosome interactions during translation initiation, elongation and termination.
4. **Shirokikh, N. E.**, Preiss, T. (2018) *Wiley Interdisciplinary Reviews RNA* 9, e1473. Translation initiation by cap-dependent ribosome recruitment: Recent insights and open questions.

5.3. Proteomic characterization of *Saccharomyces cerevisiae* and *Candida albicans* extracellular vesicles to identify biomarkers and a potential role in antifungal drug tolerance.

Mark Bleackley, Charlotte Dawson, Kening Zhao, Suresh Mathivanan, Marilyn Anderson

La Trobe Institute for Molecular Science, La Trobe University, Bundoora, Victoria, Australia, 3086

Extracellular vesicles (EVs) are small, lipid encapsulated particles containing proteins, nucleic acids and metabolites that are produced by all organisms. Our knowledge of their biogenesis and their function in intercellular communication stems largely from studies in mammalian cells and their role in pathogenesis of Gram-negative bacteria. Yeast EVs are a major pathway of non-classical protein secretion and have been implicated in basic cellular physiology, pathogenesis and hypervirulence. Proteomic characterization of yeast EVs revealed that many of the canonical biomarkers used in mammalian systems, including the highly conserved ESCRT proteins, are not present. However, *S. cerevisiae* strains with deletions in ESCRT proteins produce fewer EVs and these EVs have markedly different proteomic profiles to the wild type strain supporting a role for ESCRT in EV biogenesis in yeast. This led us to identify proteins that could be used as EV biomarkers in yeast. Isolation and proteomic characterization of EVs from three *C. albicans* strains grown as planktonic cultures, together with one strain grown as a biofilm, led to identification of 22 proteins that are potential EV markers. These included a subset of proteins with four transmembrane domains that are topologically similar to mammalian tetraspannins, some of which are highly conserved EV markers in mammalian cells. These markers have application in the identification of EVs and tracking the EVs during purification or in a biological system such as an infection model. EV cargo also included proteins that may enhance resistance to antifungal drugs such as the efflux pumps Cdr1/2, the 1,3 β-glucan synthase Fks1 (as well as its regulator Rho1) and the major cell wall chitin synthase Chs3. We discovered that yeast EVs protect yeast against the antifungal activity of caspofungin and a plant antifungal defensin supporting a role for EVs in enhancing tolerance to antifungal molecules.

5.4. Using yeast to discover next generation tools for manipulating genes

Oliver Rackham

Harry Perkins Institute of Medical Research and Curtin Health Innovation Research Institute, School of Pharmacy and Biomedical Sciences

Curtin University, Western Australia.

The ability to alter the genomes of living cells is key to understanding how genes influence all the functions of organisms and will be critical to modify living systems for useful purposes. However, this has long been limited by the technical challenges involved in genetic engineering. Recent advances in gene editing have bypassed some of these challenges but they are still far from ideal. We have used a variety of genetic selection systems that enable the re-engineering of gene modulating systems via life/death selections in yeast. We have expanding the RNA recognition code of Pumilio and FBF homology (PUF) proteins in yeast, enabling the design of RNA-binding proteins with programmable specificities. Furthermore, in recent work we have created synthetic proteins from another family of RNA-binding repeat domain proteins: the pentatricopeptide repeat (PPR) proteins. These artificial proteins have revealed the code for RNA binding by natural PPR domains and provide unique tools for manipulating cellular RNAs. Furthermore, we found that these proteins can be used to selectively manipulate ssDNA and show that these proteins can be designed to potently inhibit telomerase, a crucial cancer target. Furthermore, we have capitalised on these synthetic biology methods to engineer CRISPR/Cas9 gene editing systems in yeast. The design of proteins that can bind any RNA or DNA sequence of interest and modulate its function will be important to elucidate the mechanisms by which genes are controlled and for building genetic circuits with programmable properties in synthetic biology.

5.5. Inflammasome activation and metabolic control of innate immunity during fungal infections.

Tucey, TM[1], Verma, J[1], Lo, TL[1], Robertson, A[2], Naderer, T[1] and Traven, A[1]
1. Infection and Immunity Program and the Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton 3800, Victoria, Australia
2. School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, Australia

Candida albicans is a commensal yeast and normal component of the human microbiome. However, the use of heavy antibiotics, indwelling devices, or immunosuppressive therapies can cause colonization to escalate into systemic infections with high mortality. Macrophages are one of the first lines of defense following *C. albicans* infection. *C. albicans* controls its interaction with macrophages through a morphological switch between yeast and hyphal cells, and this triggers NLRP3 inflammasome activation, pyroptotic cell death and egress of the pathogen. We have recently demonstrated that another key aspect of this interaction involves the metabolic competition for glucose, which becomes an essential nutrient for *C. albicans*-infected macrophages later in infection. In our latest work, we compare a collection of 20 clinical isolates of *C. albicans* during infection of macrophages.
Through quantitative live cell imaging, we find that activation of the inflammasome pathway occurs by an elaborate network of morphogenetic and metabolic switches in pathogen and host. We define the mechanistic requirements for metabolic activation of the inflammasome by *C. albicans*. We also find that pyroptosis is only observed with some clinical isolates of *C. albicans* and only under specific experimental conditions, whereas metabolic inflammasome activation is dominant across the phenotypically and phylogenetically diverse isolates. This study redefines the mechanistic models of inflammasome responses to *C. albicans* and suggests that immunometabolic status is the core sensor of the commensal-pathogen transition in microbiota.

Session 6

6.1. Discovering the indigenous microbiota associated with Australian Aboriginal and Torres Strait Islander fermentations

Cristian Varela[1,2], Lucien Alperstein[2], Joanna Sundstrom[2], Kathleen Cuijvers[1], Maggie Brady[3], Vladimir Jiranek[2], Anthony Borneman[1]

[1]*The Australian Wine Research Institute, PO Box 197, Glen Osmond SA 5064, Australia*
[2]*Department of Wine and Food Science, University of Adelaide, PMB 1 Glen Osmond, SA 5064, Australia*
[3]*Centre for Aboriginal Economic Policy Research, College of Arts and Social Sciences, The Australian National University, Acton, ACT 2601, Australia*

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 before the arrival of the first Europeans, Aboriginal people produced several fermented drinks including *mangaitch* from flowering cones of a banksia plant and *way-a-linah* from Eucalyptus tree sap. In the Torres Strait, Islanders learned from Filipinos how to make a fermented drink *Tuba* from coconut palm syrup. Numerous microorganisms, including fungi, yeast and bacteria, present on the surface of fruits and grains, are responsible for the conversion of the sugar present in these materials into ethanol. Here we describe native microbial populations associated with the spontaneous fermentation of sap from the 'cider gum' *Eucalyptus gunii*, a Eucalyptus tree indigenous to the remote Central Highlands of Tasmania, and the yeast microbiota associated with flowers, fruits and palm trees from Erub Island in the Torres Strait. Amplicon-based ITS phylotyping has shown numerous microbial species in cider gum samples, with fungal species differing greatly to those associated with winemaking. Phylotyping also revealed several fungal sequences which do not match known fungal genomes suggesting novel yeast species. Similarly, many different yeast species were identified from plants, flowers and fruits from Erub Island. These findings highlight the vast microbial diversity associated with native Australian plants and beverages.

6.2. Where the wild yeasts are: Reflections on how dispersal, environmental variability and competition shape metabolic potential of nectar yeasts.

Dhami M.K.

Biodiversity & Conservation, Manaaki Whenua Landcare Research, Lincoln 7640, New Zealand

Wild yeasts occupy a variety of niches, from whale excrement to floral nectar. Such niches can vary in their nutritional value and environment exerting unique selective pressures on their occupants. Floral nectar is a carbohydrate-rich, nitrogen-poor niche, that can be highly variable due to pollinator-driven yeast dispersal and competition from other microbes. How do *Metschnikowia reukaufii* yeasts (*Saccharomycetales*) then dominate this variable and nutrient-limited nectar niche? Using regional population genetics surveys, experiments and simulations, we explore how mechanisms such as dispersal, environmental variability and
competition, shape the metabolic potential and persistence in *M. reukaufii*. We find that duplication of amino acid transporters, such as *GAP1* and *PUT4,* combined with modifications to stress tolerance pathways in *M. reukaufii,* facilitate persistence against nutrient limitation and variability. We find no evidence that *M. reukaufii* population is
dispersal-limited across the sampled regions. Further, the divergence of metabolic capacity across the population allows the species to successfully compete with other microbes at the regional scale. Our results exemplify how wild yeasts can thrive under conditions of nutrient
limitation and environmental variability that are prevalent across all niches.

6.3. Assessment of fungal diversity of Australian tree hollows in connection to the *Cryptococcus gattii* and *C. neoformans* species complexes

Laura J. Schmertmann[a,b,c*], **Laszlo Irinyi[b,c,d*]**, Richard Malik[e], Jeff R. Powell[f], Wieland Meyer[b,c,d], Mark B. Krockenberger[a,d#]

[a]Sydney School of Veterinary Science, B14 McMaster Building, The University of Sydney, Sydney, New South Wales, 2006 Australia
[b]Molecular Mycology Research Laboratory, Centre for Infectious Diseases and Microbiology, The University of Sydney - Westmead Clinical School, Faculty of Medicine and Health, Sydney, New South Wales, Australia
[c]The Westmead Institute for Medical Research, Westmead, New South Wales, Australia
[d]Marie Bashir Institute for Infectious Diseases and Biosecurity, The University of Sydney, Sydney, New South Wales, Australia
[e]Centre for Veterinary Education, B22, Sydney School of Veterinary Science, The University of Sydney, Sydney, 2006, NSW, Australia
[f]Hawkesbury Institute for the Environment, Western Sydney University, Penrith, NSW, 2751 Australia

Cryptococcosis is a fungal infection caused by members of the *Cryptococcus gattii* and *C. neoformans* species complexes. The *C. gattii* species complex has a strong environmental association with eucalypt hollows (particularly *Eucalyptus camaldulensis*), which may present a source of infection. It remains unclear whether a specific mycobiome is required to support its environmental survival and growth. Conventional detection of environmental *Cryptococcus* spp. involves culture on differential media, such as *Guizotia abyssinica* seed agar. Next-generation sequencing (NGS)-based culture-independent identification aids in contextualising these species in the environmental mycobiome. Samples from 23 Australian tree hollows were subjected to both culture- and amplicon-based metagenomic analysis to characterise the mycobiome and assess relationships between *Cryptococcus* spp. and other fungal taxa. The most abundant genera detected were *Coniochaeta*, *Aspergillus* and *Penicillium*, all being commonly isolated from decaying wood. There was no correlation between the presence of *Cryptococcus* spp. in a tree hollow and the presence of any other fungal genus. Some differences in the abundance of numerous taxa were noted in a differential heat tree comparing samples with or without *Cryptococcus*-NGS reads. The study expanded the known environmental niche of the *C. gattii* and *C. neoformans* species complexes in Australia with detections from a further five tree species. Discrepancies between the detection of *Cryptococcus* spp. using culture or NGS suggest that neither is superior *per se* and that, rather, these methodologies are complementary. The inherent biases of amplicon-based metagenomics require cautious interpretation of data through consideration of its biological relevance. Further improvements in NGS, such as whole genome shotgun and long-read sequencing, together with appropriate data analysis pipelines and the extension of reference databases, should significantly contribute to better characterisation and understanding of such complex microbial community structures.

6.4. The impact of fungal plant diseases on food security

Guest, D.

The University of Sydney

Microbial pathogens, pests and weeds cause huge losses to agricultural production and environmental damage. “Fungi”, including the oomycetes, are responsible for crop failures, yield losses and unsafe food. The impact of fungal plant disease on humans is invariably compounded by socioeconomic, political and human health factors. I will present examples of famines precipitated by fungi, describe the events leading to famines and disease outbreaks and discuss features of fungal biology that enable them to escape conventional attempts at management. I will propose an interdisciplinary “One Health” strategy to better manage the impacts of fungal plant pathogens on food security.

Session 7

7.1. Adaptation to the host niche through cell shape control in the human pathogenic fungus *Talaromyces marneffei*

Weerasinghe, H., Payne, M., Gupta, A. and **Andrianopoulos, A**.

Genetics, Genomics and Systems Biology, School of BioSciences, University of Melbourne, VIC 3010. Australia

Talaromyces marneffei (*Penicillium marneffei*) is an important fungal pathogen of humans, in particular those who are immunocompromised. *T. marneffei* has the capacity to alternate between a hyphal and a yeast growth form, a process known as dimorphic switching. The strongest extrinsic trigger for dimorphic switching is in response to temperature. *T. marneffei* grows in the hyphal form at 25°C and in the yeast form at 37°C. The hyphal form produces conidia that are likely to be the infectious agent and believed to establish infection after inhalation. The yeast growth form is the pathogenic form found in infected patients. These yeast cells exist intracellularly in the mononuclear phagocyte system of the host. The aim of this work is to understand the mechanisms for specifying cell type and to understand how these impact on pathogenicity.
High throughput transcriptomic analyses identified hyphal and yeast cell specific pathways during growth of *T. marneffei*. Coupled with genomics studies we

characterised a number of candidates that were predicted to play a role during growth in the host. A number of the genes were shown to play critical roles during yeast cell morphogenesis inside host cells. Cell shape is critical for *T. marneffei* to survive within host cells without prematurely killing them. The results suggest that *T. marneffei* has evolved a unique set of strategies to establish and maintain its morphological state in the host, which is central to pathogenicity.

7.2. Building Rube Goldberg machines in yeast to explore unnecessary complexity in biology

Danielle Maddock, **Austen R.D. Ganley**, and Ant Poole

School of Biological Sciences, University of Auckland, New Zealand

Biology makes frequent use of metaphors. A common one is portraying cellular systems as highly engineered solutions resulting from the relentless force of natural selection maximising performance. However, the extent to which this metaphor accurately represents reality is debated, with molecular evolutionists in particular proposing that biological systems have a great deal of 'unnecessary' complexity. Such unnecessary complexity is proposed to arise through chance events that lack adaptive benefit, a process is known as Constructive Neutral Evolution (CNE), and these are predicted to be prevalent in populations prone to genetic drift. Proposed examples include the seemingly gratuitous number of proteins that form the eukaryotic mitochondrial ribosome, and the convoluted processes of RNA editing and splicing. If correct, Rube Goldberg machines (deliberately absurd contraptions that perform simple tasks via a large number of steps) might be a better metaphor for cellular systems. However, there is a lack of clear experimental evidence demonstrating the validity of the CNE model. This has led to scepticism regarding its generality, and to suggestions that we simply fail to understand the adaptive benefits in cases of seeming unnecessary complexity. Here we will present our work building an unnecessarily complex multi-protein machine in yeast. This machine was constructed via initially spurious, non-adaptive interactions between unrelated proteins identified using the yeast 2-hybrid system. These proteins derive from all three domains of life to minimize the likelihood that adaptive interactions underpin our results. We will present the design and construction of these 'Rube-Goldbergesque' protein machines, and will anticipate the outcomes of experimental evolution designed to explore how nature reacts to complexity that is demonstrably unnecessary. This project will provide an experimental test of CNE, and illustrate the dynamics through which convoluted molecular systems evolve.

7.3. Challenges of membrane protein research in *Saccharomyces cerevisiae*.

Lamping, E.

Sir John Walsh Research Institute, Faculty of Dentistry, University of Otago, Dunedin NZ; erwin.lamping@otago.ac.nz

Biological membranes are stunningly complex mixtures of spatiotemporally separated lateral microdomains comprising certain types of proteins embedded in a hydrophobic bilayer of certain types of asymmetrically distributed lipids. Thus, membrane proteins often require additional proteins and certain types of lipids to express and function properly in a heterologous host. Our experience suggests that this is one of the major challenges when studying heterologous membrane proteins in the eukaryotic model organism *S. cerevisiae*. The range of membrane proteins of interest that we expressed and studied in *S. cerevisiae* over the past two decades includes bacterial, fungal, and human multidrug efflux pumps and the antifungal drug target Erg11, an essential enzyme of the ergosterol biosynthesis pathway, from major plant and human fungal pathogens including various mould and yeast species. Examples of critically important protein-protein and protein-lipid interactions for proper expression, folding and/or function of these proteins in our heterologous *S. cerevisiae* host, AD$\Delta\Delta$, will be discussed.

7.4. Is SO_2 tolerance in *Brettanomyces bruxellensis* a developing concern?

Caroline Bartel[1], Cristian Varela[1], Chris Curtin[2], **Anthony Borneman[1]**

[1]The Australian Wine Research Institute, PO Box 197 Glen Osmond, South Australia, 5064. Australia
[2]Department of Food Science and Technology, Oregon State University, Wiegand Hall, 3051 SW Campus Way, Corvallis, OR 97331, USA

Brettanomyces bruxellensis is a wine spoilage yeast that produces the volatile phenols 4-EP and 4-EG, which contribute to wine characteristics described as "barnyard" and "band-aid". Commonly *B. bruxellensis* is controlled through the addition of SO_2. Current winemaking trends, that minimise SO_2 additions, may provide levels that are insufficient to kill *B. bruxellensis*. These sub-lethal concentrations may give rise to strains with increased tolerance to SO_2.

In order to investigate the potential for *B. bruxellensis* to evolve tolerance to SO_2, a two-step approach was taken that combined experimental adaptive evolution to increasing, sub-lethal concentrations of SO_2., with a comparison of SO_2 tolerance levels between modern-day and historical isolates of *Brettanomyces*.

Adaptive evolution was shown to be able to increase SO_2 tolerance increased for the two main genetic-groups of *B. bruxellensis* found in wine, with up to 6-fold

more SO_2 tolerance observed in some 100 generation isolates compared to the parent. Growth kinetics of these isolates showed a reduced lag phase during growth in SO_2concentrations up to 1.0 mg/L mSO_2.

To determine if Australian industrial isolates of *B. bruxellensis* were potenitally increasing their tolerance to SO_2 over time, over 200 *B. bruxellensis* isolates were obtained from across three time periods (2000-04, 2010-14 and 2016-18). The isolates from 2000-04 and 2010-14 were obtained from the AWRI Culture Collection, while the 2016-18 isolates were acquired from 24 wine samples from 10 different wineries. Results indicate that the mean maximum SO_2 tolerance remained largely unchanged from 2000-04 to 2010-14 but had greatly increased for the period of 2016-18. The distribution of SO_2 tolerance had widened, with more isolates clustering above the mean. Characterisation of the most tolerant isolates confirmed their high SO_2 tolerance. These isolates were able to grow at higher concentrations than two strains considered as highly tolerant.

Our results demonstrate that *B. bruxellensis* has the potential to develop SO_2 tolerance in industry. Further sampling from more wineries is required to confirm this finding, however results suggest that the wine industry should carefully consider alternative strategies for controlling *B. bruxellensis*.

Student presentations

1. Sequence level characterization of the evolution of resistance to antimicrobial peptides in *Candida albicans*

Amanda McColl, Mark Bleackley, Marilyn Anderson.

La Trobe Institute for Molecular Science, La Trobe University, Science Dr, Bundoora VIC 3083

Over the last few decades, the emergence of resistance to commonly used antifungal molecules has become a major barrier to effective treatment of fungal infections. Resistance combined with the increased incidence of fungal diseases has created the need for new antifungals. Antimicrobial peptides (AMPs) are one potential solution. They differ from azoles and echinocandin antifungals because they do not act by inhibiting a single enzyme but have multiple targets. They may interact with the fungal cell wall and membrane and in some cases enter the fungal cytoplasm and target multiple cellular processes leading to cell death.

A key step in the development of novel antifungals is an understanding of the potential for the fungus to develop resistance. Our previous work in *S. cerevisiae* demonstrated that AMP resistance could develop as a result of mutations in genes that function in osmoregulatory processes. Here, we have expanded on our initial study in *S. cerevisiae* by performing a similar experiment in *C. albicans*. We have used the model plant defensin NaD1, the human cathelicidin LL-37 and the clinically relevant azole Itraconazole in serial passages with the fungal pathogen *C. albicans* to examine the evolution of resistance to these molecules. Enhanced tolerance was developed in 11 independent lines for each antifungal. Passaging of *C. albicans* in the absence of antifungal was also performed to control for mutations that improved fitness in our assay medium. *C. albicans* strains did develop tolerance to the antimicrobial peptides, except at a slower rate compared to Itraconazole. Genomic DNA from all the resistant lines was purified and sequenced using a NextSeq system. The sequence data is being passed through a bioinformatic pipeline to identify mutations that are associated with AMP resistance and will be presented at YPD.

2. Identification and Characterisation of sPEPs in *C. neoformans*

Erpf P.E. & Fraser J.A.

Australian Infectious Diseases Research Centre, School of Chemistry & Molecular Biosciences, The University of Queensland, Brisbane, Queensland, Australia

Short open reading frames (sORFs) with coding potential have been found in a variety of species across a wide range of genomic locations, including transcripts previously classified as noncoding, 5' and 3' UTRs of transcripts for known proteins, and in overlapping alternate reading frames of encoded genes. Characterisation of sORF-encoded peptides (sPEPs) from humans to yeast has revealed their importance in biological processes ranging from morphogenesis, embryogenesis and ion trafficking to hormone signalling, translational regulation, and DNA repair and replication. Some of the best examples of sPEPs have come from the model ascomycete *Saccharomyces cerevisiae* and are conserved in a variety of other fungal species, including members of the phylum Basidiomycota, to which *Cryptococcus neoformans* belongs. Our laboratory is interested in this encapsulated pathogenic yeast as it causes life threatening infections in the immunocompromised with the aid of virulence traits that enable survival in the human host. We hypothesise that sPEPs within *C. neoformans* may play a role in virulence, however their identification, validation, and characterisation to date has been limited due to a lack of standardised protocols. We have developed an enrichment process that permits sPEPs detection within a protein sample and implemented proteogenomics to provide an insight into the validity of predicted and hypothetical sORFs annotated in the *C. neoformans* genome. In addition to this, novel sORFs within 5' and 3' UTRs of known transcripts have also been discovered using the same proteomic preparation. The molecular genetic characterisation of sORFs that reside within these regions is understandably problematic. However, our development of a new dominant recyclable genetic marker utilizing the *amdS* gene from *Aspergillus nidulans* prevails these problematic features by facilitating in the investigation of sPEPs' roles in the virulence of this important fungal pathogen.

3. Function of Ost3p and Ost6p in and out of glycosylation

Zhou, C.[1] and Schulz, B.L.[1,2,3]

[1] *School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia 4027, Queensland, Australia.*

[2] *Australian Infectious Disease Research Centre, The University of Queensland, St Lucia 4027, Queensland, Australia.*

[3] *Centre for Biopharmaceutical Innovation, Australian Institute of Bioengineering and Nanotechnology, The University of Queensland, St Lucia 4027, Queensland, Australia.*

N-glycosylation plays an essential role in protein folding and functions in eukaryotic cells. Transfer of the glycans to the selected asparagine residues in peptides is catalysed by oligosaccharyltransferase (OTase), which is a multimeric complex consists of eight subunits. Ost3p and Ost6p are mutually exclusive subunits in yeast OTase, defining two substrate-specific OTase isoforms. Oxidoreductase activity of Ost3p/6p mediated

by the N-terminal thioredoxin domain is important for efficient glycosylation. Absence of both Ost3p/6p in yeast causes underglycosylation at a subset of glycosylation sites, and therefore resulting in severe growth defects of the yeast. However, abnormally fast-growing yeast strains were frequently produced by the *OST3/6* double deficient strains. This study aims at ascertaining the potential reasons that enable the yeast lacking both Ost3p and Ost6p to improve growth without recovering glycosylation. To realize that, whole genome sequencing was performed on the *OST3/6* double deficient yeast and the isolated revertants which show a stable growth improvement phenotype. Mutations located inside coding regions (CDS) of functional genes with high detection qualities were identified and further confirmed by Sanger sequencing. Three genes were finally identified to contain stable and heritable point mutations. While knockout of the whole genes in the *OST3/6* double deficient yeast failed to improve growth, other approaches will be required later for testing the effects of the point mutation itself. In addition, mitochondrial genome loss was observed in two of the revertants, and confirmed by growth spotting assays performed on medium using non-fermentable glycerol as the only carbon source. The correlations between mitochondrial genome loss and growth rescue of the *OST3/6* double deficient yeast will also be investigated in the future.

4. Cell Wall and Whole Cell Proteomes Define Flocculation and Fermentation Behaviour of Yeast

Edward D. Kerr [1,2], Duin C. McDiarmid [1,2], James A. Fraser [1,2], and Benjamin L. Schulz [1,2,3]
[1] *School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia 4072, Australia*

[2] *Australian Infectious Diseases Research Centre, School of Chemistry & Molecular Biosciences, The University of Queensland, St Lucia 4072, Australia*

[3] *Centre for Biopharmaceutical Innovation, Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, St Lucia 4072, Australia*

Flocculation is one of the most important characteristics of brewing yeast as it allows for the easy and cheap removal of cells after fermentation. The genes responsible for both the Flo1 and NewFlo flocculation phenotypes are well characterized. However, the relationship between Flo protein abundance and flocculation efficiency is poorly understood. In this present study, we used mass spectrometry proteomics to compare the cell wall and whole cell proteomes of commercial yeast strains with diverse flocculation behaviours. We found that the relative abundance of Flo1/5 or Flo10 in the cell wall was correlated with the ability of these yeast strains to flocculate. Analysis of whole cell proteomes identified differences in the proteomes of yeast strains and identified the potential for high metabolic diversity. Characterization of the cell wall and whole cell proteomes during fermentation showed high levels of Flo10 in cells that settled early during fermentation. Our data reveal the diversity of the cell wall and global proteomes of brewing yeast, highlighting the potential biochemical diversity present in yeast that can be utilized in the production of fermented beverages.

5. The nuclear protein interactome of budding yeast revealed by large scale cross-linking mass spectrometry.

Tara K. Bartolec[1], Daniela-Lee Smith[1], Chi Nam Ignatius Pang[1], You Dan Xu[2], Joshua J. Hamey[1], Marc R. Wilkins[1]
[1] *Systems Biology Initiative, School of Biotechnology and Biomolecular Sciences*
University of New South Wales NSW 2052 Australia.
[2] *Centre for Advanced Macromolecular Design, School of Chemistry, University of New South Wales, Sydney, New South Wales 2052, Australia*

In cross-linking mass spectrometry (XL-MS), crosslinkers covalently link specific amino acids in proteins within spatially constrained reactions. Mass spectrometry (MS) is then used to identify these linkages either within or between proteins. It is a powerful technique which provides information beyond just identification of protein interactors or complex components, but also structural information on the interaction interface or of an individual protein's fold. Recent technological advances have enabled its application to complex samples, facilitating the study of cellular protein-protein interaction (PPI) networks as well as uncovering the *in vivo* structural proteome. *Saccharomyces cerevisiae* has the most comprehensively characterised protein interactome of any eukaryote; A pressing question is to understand how large-scale XL-MS can confirm and extend this interactome.

Here, intact yeast nuclei were subject to crosslinking with DSSO, generating the first yeast large scale XL-MS dataset with over 2,000 unique residue pairs. A method for optimising inter-protein score cut-offs was developed by leveraging the extensive resource of existing known yeast interactions, which led to a high confidence nuclear interactome with over 200 unique PPIs. Strikingly, even after stringent quality control, almost half of the interactions were novel. Additionally, intra-protein crosslinks provided extensive structural constraint data of *in vivo* protein conformations, which (1) validated both experimentally derived structures and homology models, and (2) were successfully used to guide construction of novel models.

This work contributes to pertinent discussion on large scale XL-MS quality control for the study of protein interactomes. Furthermore, it illustrates the utility of XL-

MS in uncovering novel biological insights, even when applied to comprehensively studied model organisms like yeast.

6. Musical Beer; the impact of audible sound on fermentations.

Harris, A; Ganley, A; Jeffs, A;

The University of Auckland, School of Biological Sciences,
3A Symonds Street, Auckland, 1010

Sound is ubiquitous in nature and industry, however, its impact on microbes is largely unknown. Recently, the biological effects of audible sound, with a frequency between 20 hertz (Hz) to 20 kHz, have been investigated in yeast. It was found that audible sound positively influences the growth of both *Saccharomyces cerevisiae* and *Candida albicans*. Furthermore, different frequencies of sound altered secondary metabolism of *S. cerevisiae*, producing distinct metabolic profiles. In the current study we are investigating the effects that the two key components of audible sound, frequency and intensity, have on the fermentation characteristics and metabolism of a commercial *Saccharomyces cerevisiae* strain US-05, with the ultimate aim of producing a novel sonic beer. To limit background noise we utilized the University of Auckland's anechoic chamber, which significantly reduces interference from external sounds. Initially, the audible spectrum was divided into four bands, with a silent control. Fermentations were conducted in triplicate in minimal media with maltose, at industry concentration, as the sole carbon source. The general fermentation characteristics, growth rate, sugar consumption and ethanol production were compared to identify an 'active' frequency band. Subsequently, this active frequency band was exposed to three different intensities, to compare the intracellular and extracellular metabolism during exponential growth. Overall, sound was shown to increase the specific growth rate when compared to silence by up to 40% in lower frequencies whereas higher frequencies showed very little change. Applying different sound intensities to fermentations resulted in distinct intra- and extracellular metabolite profiles, from which we could identify fermentations with up to 87% accuracy. Together these results suggest sound, both frequency and intensity, could be used to influence *S. cerevisiae* fermentations in the brewing industry. Furthermore, combining metabolomics with gene expression could provide insight into the mechanism through which sound acts.

7. Lactoferrin and Amphotericin B Synergise Against Yeasts

Fernandes KE[1], Lai Y[1], Wilkins MR[2] & Carter DA[1]
[1]*School of Life and Environmental Sciences, University of Sydney, NSW, Australia;*
[2]*School of Biotechnology and Biomolecular Sciences, University of New South Wales, NSW, Australia*

Natural products represent a significant source of novel antifungal compounds, and an increasing number are being identified from mammalian sources including milk proteins. One such compound is lactoferrin (LF), a multifunctional iron-binding glycoprotein long known to have antimicrobial activity against a range of pathogenic microorganisms including fungi. However, substantial differences in activity have been reported for LF in the same or closely related fungal species, ranging from reasonably to not at all active.
To clarify this, a comprehensive evaluation of the antifungal spectrum of activity of three defined sources of LF was undertaken across 22 yeasts and 24 mould species using CLSI methods. The potential of LF for synergistic interactions was then tested with 6 commonly used antifungal drugs using checkerboard assays and determined by Fractional Inhibitory Concentration Index (FICI). Finding a synergistic pairing in LF and amphotericin B (AMB), combination therapy of LF+AMB against yeasts was evaluated *in vivo* using a *Galleria mellonella* infection model. The effect of LF+AMB treatment on morphological changes associated with virulence was examined by looking at capsule and cell size changes in *Cryptococcus* and biofilm and hyphal formation in *Candida.*

LF exhibited broad-spectrum antifungal action both alone and in combination with AMB against yeasts, however its efficacy varied substantially across moulds. The synergistic pairing of LF+AMB was effective in an *in vivo* model and potent against *Cryptococcus* and *Candida* virulence factors, and therefore has great potential to be further developed as a novel antifungal treatment.

8. Auxin-mediated conditional gene inactivation in metabolic engineering: flux redirection, metabolic regulation, and growth arrest in terpene-production yeast

Lu, Z [1, 2], Peng, B [1, 3], Dumsday, G [4], Vickers, C [1,3]

1. *Australian Institute for Bioengineering and Nanotechnology (AIBN), The University of Queensland, Brisbane, QLD 4072, Australia*
2. *School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD 4072, Australia*
3. *CSIRO Synthetic Biology Future Science Platform, GPO Box 2583, Brisbane, QLD 4001, Australia*
4. *CSIRO Manufacturing, Clayton, VIC 3169. Australia*

Metabolic engineering enables yeast to produce many valuable metabolites applicable as fuels, food, and pharmaceuticals. To produce the target molecules at a high yield, requires redirecting and redistributing metabolic flux through multi-aspect engineering of metabolic and regulatory networks. Metabolic perturbation through gene inactivation is a method employed to understand metabolism and regulation for metabolic engineering. However, for essential genes, gene deletion can be lethal. Therefore, a tool is required

to assess the roles of essential genes in metabolism under normal cultivation conditions. Here, we exploited auxin-inducible protein degradation to deplete a target protein and evaluate metabolic effects. We reconstructed the auxin inducible protein degradation mechanism in Saccharomyces cerevisiae by expressing F-box protein TIR1 from rice Oryza sativa and characterized a variety of auxin-inducible degron (AID) tags for labelling target proteins. N-terminal fusion of Skp1p to TIR1 coupled with the Cup1p-AID* tag minimized the non-auxin-dependent decrease in target protein level. This tool can be used to deplete specific cytosolic and nuclear proteins. Applying this system in terpene-producing yeasts, we demonstrated that: (1) depleting essential farnesyl pyrophosphate synthase Erg20p redistributed metabolic flux at geranyl pyrophosphate node to favor the production of monoterpene limonene; (2) depleting hexokinase-2 Hxk2p, an moonlighting protein modulating glucose metabolism and glucose regression, boosted the production of sesquiterpene nerolidol from 1.8 g/L to 3.5 g/L in flask cultivations; (3) depleting essential acetyl-CoA carboxylase Acc1p arrested cell growth, whereas the cells still maintained nerolidol production without the specific production rate compromised. In conclusion, auxin-mediated conditional gene inactivation, being the tool for investigating perturbation effects of gene inactivation, can be incorporated as a system-wide mechanism in metabolic engineering for metabolic control and regulation.

9. Determination of the cellular targets of a novel anthracycline using chemical genomic approaches in yeast.

Akram, Z[1]., Capon, R[2]., Stewart, M[2]., Hill, L[2]., Ryan, B[2]., Vajjhala[2], P., Boone, C[3]., Lopez, A[3]., Harrich, D[4]., Lewin, S.R[5]., Mak, J[6]., Munn, A.L[1,2].

1: School of Medical Science, Menzies Health Institute Queensland, Griffith University (Gold Coast campus), Parklands Drive, Southport, QLD 4222, Australia.
2: Institute of Molecular Bioscience, The University of Queensland, St. Lucia, QLD 4072, Australia.
3: Banting and Best Department of Medical Research and Department of Medical Genetics and Microbiology, Terrence Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, 160 College Street, Toronto, Canada
4: QIMR Berghofer, 300 Herston Rd, Brisbane QLD 4006, Australia.
5: Department of Infectious Diseases, Alfred Hospital, Prahan, VIC 3004, Australia.
6: The MacFarlane Burnet Institute for Medical Research and Public Health, Prahan, VIC 3004, Australia.

Anthracyclines are chemotherapeutic agents commonly used to treat a broad range of malignancies. Although effective, these drugs present serious complications, most notably cardiotoxicity. The novel anthracycline A1 that is the product of Australian *Streptomycete* like available anti-cancer drugs. Our aim was to identify the cellular targets and thereby the mode-of-action of anthracyclines, therefore we employed the chemical genomic approach. This approach provides insight into possible mode-of-action of the compound by revealing which of the genes in yeast influence the sensitivity of yeast cell growth to the presence of the compound. We have used wild type haploid yeast cells and 4,700 individual gene knockout mutant strains constructed in the BY4741 genetic background for this genome-wide chemical genomics screen. Consequently, 54 mutant strains that display hypersensitivity to A1 were identified using this chemical genomic approach. This novel A1 anthracycline has no sugar moiety, which suggests it may be less cytotoxic and have a distinct mode of action than other anthracyclines. In this manner, we have selected 12 yeast gene deletion mutant strains (*SOD1*, *SOD2*, *CTR1*, *CCS1*, *RGP1*, *MAC1*, *XRS2*, *RAD1*, *RAD2*, *RAD10*, *RAD50* and *RAD52*) identified as hits in this chemical genomics screen to directly confirm their hypersensitivity to A1. Complementation of the A1-sensitive phenotypes of the deletion strains with the wild-type genes proves that the sensitivity of the strain to A1 is due to the gene deletion. The genes that are targets of A1 belong to pathways including DNA repair, RNA metabolism and chromatin remodelling. In addition, proteins with mitochondrial, vacuolar, and ribosomal functions are also targets of A1. Most of the identified target proteins have mammalian homologues that participate in conserved pathways. Our data may prove useful to achieve a better understanding of the effects of anthracyclines on cells and this understanding may enable the development of anthracyclines with reduced off-target cytotoxic effects.

10. The role of upstream phosphorylation in the regulation of histone methylation

Separovich, R.J.[1], Hamey, J.J.[1], Wilkins, M.R.[1]

Systems Biology Initiative, School of Biotechnology and Biomolecular Sciences, The University of New South Wales, Sydney, New South Wales 2052, Australia

Histone methylation is a central means by which gene expression is controlled. In the lower eukaryote, *Saccharomyces cerevisiae,* histone methylation is regulated by a reduced, but evolutionarily conserved set of methyltransferases (Set1, Set2, Set5, Dot1) and demethylases (Jhd1, Jhd2, Rph1, Gis1). While the catalytic activity and specificity of these enzymes have been established, knowledge of how they themselves are regulated by post-translational modification is surprisingly limited. Consequently, the regulatory network of histone methylation in yeast remains unknown and is also unknown in all other eukaryotes. To this end, we aimed to comprehensively characterise

the modifications occurring on the eight yeast histone methyltransferases and demethylases *in vivo.* This was achieved by purification of these proteins, and their analysis by targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS). With respect to phosphorylation, to date, we have identified modification sites on the histone methyltransferases Set2 (12 sites), Set5 (14 sites) and Dot1 (11 sites), and the demethylases Jhd1 (one site), Jhd2 (two sites) and Rph1 (19 sites). Of these 62 phosphorylation sites, 35 validate those observed previously in high throughput phosphoproteomic screens, and 27 sites are novel. To determine the upstream kinases responsible for the phosphorylation, and potential regulation of these enzymes, mass spectrometric analysis was employed to monitor levels of histone methylation in kinase knockout yeast strains. As a proof of concept, quantification of H3K79 methylation in the knockout cells established twenty-five kinases that are not responsible for the regulation of Dot1 methyltransferase activity. The screening of all other non-essential kinases is in progress. We plan to extend this methodology to the other yeast histone methyltransferases and demethylases in order to comprehensively integrate these enzymes into intracellular signalling pathways, and ultimately facilitate the assembly of the first regulatory network of histone methylation in eukaryotes.

11. Interactive peptides, the undisclosed communication networks of pathogenic polymicrobial biofilms

Gemma Deakin[1,2], Thomas Jeffries[1,2], Michael Phillips[1], Oliver Morton[1]

1: School of Science and Health, Western Sydney University Australia. 2: Western Microbial Systems Laboratory, Western Sydney University Australia.

Polymicrobial biofilms influence the severity of an infection, which poses challenges for treating infection, especially in immunocompromised individuals. This leads to the question, 'how do pathologically relevant bacteria, yeast, and fungus interact? The mechanisms behind the interactions were investigated using mass spectrometry of peptides extracted from the extra polymeric substances (EPS) that the bacterial and fungal members of pathogenic polymicrobial biofilm produce in response to each other.

12. "Lazy" yeast and cell-to-cell variation in microbial recombinant protein production.

Hu, K.K.-Y. [1†], Suri, A. [1], Dumsday, G.J. [2], Haritos, V.S. [1]

1 *Department of Chemical Engineering, Monash University, Clayton, VIC 3800, Australia*
2 *CSIRO Manufacturing, Clayton VIC 3800, Australia*
[†]e-mail: nguyen.ho@monash.edu

In their natural environment, cell-to-cell variation in microbial communities arises as a counter-measure against natural selection, wherein metabolic diversity among individuals increases population robustness and survival rate against adverse conditions. In a bioprocess, however, phenotypic variations among cultured microbial cells are unfavourable, as they increase the fluctuation in process performance and productivity, making it less predictable and controllable. The ability to control and minimise performance variations between individual cells thus represents an opportunity for the improvement in productivity and yield in biotechnology. Here we investigated the well-studied biotechnology yeast, *Saccharomyces cerevisiae* under recombinant expression of fluorescent proteins as productivity measures, to examine mechanisms and external factors causing cell-to-cell variation. Fluorescence microscope imaging and flow cytometry were used for single cell analysis and product quantification. Our investigation has demonstrated that recombinant fluorescent protein expression varies greatly between yeast cells, especially where protein expression is plasmid-based. In this situation, variation in expression is elevated to where it becomes bimodal and ≥30% of the cells have no detectable fluorescent protein during the culture period – the so called "lazy" yeast. Furthermore, the cells that do express fluorescent proteins, the variance (%rCV) of the measured single-cell fluorescent intensity is consistently above 50% for all tested systems. Causes of cell-to-cell variation in recombinant protein expression include: inherent "noise" in promoter regulation, insufficient uptake of promoter inducing agents, plasmid loss and asymmetrical plasmid inheritance during cell duplication. Each of these was addressed in our study and eliminated as the root cause although plasmid loss may contribute. Recent results point to inherent physiological differences between the fluorescent protein-expressing and the non-expressing cohorts in response to differing loads of metabolic burden. Further investigation of this hypothesis is underway. Knowledge of the mechanisms underlying bimodal recombinant protein expression will provide insight into strategies to improve the overall performance and productivity of microbial cultures.

13. Mechanistic investigation of vanillin toxicity to *Saccharomyces cerevisiae* using ambr15 microbioreactors and flow cytometry.

Suri, A. [1†], Hu, K.K.-Y. [1], Peng, H.D. [2], Haritos, V.S. [1]

1 *Department of Chemical Engineering, Monash University, Clayton, VIC 3800, Australia*
2 *Department of Bioengineering, Imperial College London, United Kingdom*
[†]e-mail: ankita.suri@monash.edu

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is a popular flavoring agent with a growing global market. It is sourced via chemical treatment of wood or may be synthesised within cells of genetically engineered yeast or bacteria. In both the engineered organisms and yeast exposed to cellulosic ethanol fermentation, vanillin has

been shown to be a potent toxicant and inhibitor of microbes. Recent investigation of toxicity of vanillin to yeast based on transcriptomics and gene knockouts have concluded that vanillin causes effects on protein production or increased radical oxygen species (ROS) generation. Other studies indicate the exposed yeast maintain full metabolic function. To investigate the mechanism and potency of vanillin toxicity to *Saccharomyces cerevisiae* BY4741, we cultivated the control strain (empty pESC-Ura vector) and those expressing modified Turquoise Fluorescence Protein (mTFP) in the Sartorius ambr®15 fermentation microbioreactor system, at 30 °C for up to 72 hours in the presence of vanillin at initial concentrations of 2 to 10 mM in batch mode. Single-cell TFP expression level along with other cellular physiology indicators such as membrane integrity, ROS level and mitochondrial membrane potential were measured using Beckman Coulter CytoFlex flow cytometer. No cytotoxic effect of vanillin was observed on yeast at any of the concentrations tested. The cells were metabolically active with intact membranes and had active mitochondria and showed no signs of elevated ROS nor inhibition in protein production. Only cell division and biomass accumulation were affected, wherein concentrations >2 mM induced potent yeast growth inhibition. Along with the extended lag phase, lower yeast growth rate (μ_{max}) and increased oxygen usage were observed in a concentration-dependent manner indicating vanillin conversion requires additional metabolism. The microbioreactor system coupled with flow cytometry are a powerful combination for examining substrates, toxicants and inhibitors of microbes in a high throughput reproducible manner and here determining vanillin to be a specific inhibitor of yeast cell division.

14. Using yeast to decipher human Gcn1-Gcn2 interaction, and its suitability as drug target

Susanne Gottfried*, Evelyn Sattlegger

*School of Natural and Computational Sciences, Massey University, Auckland *Albany Campus Gate 4, Building 11*

susannegottfri@gmail.com, +64211818557

The eIF2α protein kinase Gcn2 (“General control non-depressible” 2) is present in virtually all eukaryotic cells, from yeast to human. It helps cells to cope with nutrient starvation. Under amino acid starvation, Gcn2 phosphorylates the factor eIF2α, which subsequently stimulates the translation of the transcription factor Gcn4. Gcn4 then induces the expression of stress-response genes involved in amino acid biosynthesis. Activation of Gcn2 requires binding of an uncharged tRNA to its HisRS-like domain, and this depends on the physical interaction between Gcn2 and Gcn1. Gcn1-Gcn2 complex formation is mediated by the N-terminal RWD domain in Gcn2 and the C-terminal RWD-binding domain (RWDBD) in Gcn1. Gcn2 malfunction is implicated in various diseases such as cancer and Alzheimer’s. Cancer cells take advantage of Gcn2 and are dependent on Gnc2 to satisfy their high nutritional demand. As healthy cells do not critically depend on Gcn2, this makes Gcn2 a promising target for drug development.

In order to find suitable drug targets to fight or prevent Gcn2 associated diseases it is crucial to understand Gcn2 function in detail. Our aim is to investigate human Gcn1-Gcn2 interaction using yeast as a model organism. Site-directed mutagenesis and *in vivo* Gcn2 activity screening allowed us to conduct a more in depth characterisation of Gcn1-Gcn2 interaction.

Our results showed again high evolutionary conservation of Gcn1-Gcn2 interaction, confirming that yeast is a prime model for answering human biomedical questions.

15. Investigating dynamics of rDNA copy number at intra-species level.

Sharma D[1], Hermann-Le Denmat S[1,2], Matzke NJ[1], Hannan KM[4], Hannan RD[4], O’Sullivan JM[3], Ganley ARD[1]

1. School of Biological Sciences, University of Auckland, Auckland, New Zealand

2. Ecole normale supérieure, PSL Research University, F-75005 Paris, France

3. Liggins Institute, University of Auckland, Auckland, New Zealand

4. CRF Department of Cancer Biology and Therapeutics, John Curtin School of Medical Research, Australian National University, Canberra, ACT, 2601, Australia

In most eukaryotes ribosomal DNA (rDNA) genes exist as a tandem array(s) of repeats with high variability in copy number within and between species. The rDNA is central to many critical cellular activities for which its multi-copy nature is believed to be important. However, while the mechanism of rDNA copy number variation is understood, little is known about rDNA dynamics at the population level, partly because of difficulties in measuring copy number. In particular, while different species maintain different “set” rDNA copy numbers, it is not known if this holds true for different populations within the same species. To address this question, we developed a novel approach to measure rDNA copy number from whole genome sequence data using the most frequent (modal) coverage. We validated this method with *Saccharomyces cerevisiae* strains having known, different rDNA copy numbers, and then applied it to calculate the copy numbers of 789 *S. cerevisiae* strains using sequence data from the 1002 Yeast genome project. Interestingly, we found no correlation between phylogeny and rDNA copy number, unlike what is seen above the species level in fungi. We rule out ploidy differences driving this lack of correlation. Another explanation, consistent with different mean copy number across these strains (~92 copies)

compared to laboratory *S. cerevisiae* (150-200 copies), is that environmental differences drive rDNA copy number differences. However, our analyses do not provide strong support for this explanation, either. Therefore, our results are consistent with all *S. cerevisiae* populations having a single 'set' rDNA copy number, suggesting that the genetic differences that drive copy number differences only manifest above the species levels, at least in *S. cerevisiae*. This further suggests that variation in rDNA copy number in other systems, such as tissue-specific differences in humans, may simply be the result of stochastic copy number variation.

16. Bioprospecting Australian wine microbiota.

Hartmann L A[1,2], Schmidt S A[1], Borneman A R[1]

[1] The Australian Wine Research Institute, PO Box 197, Glen Osmond (Adelaide) SA 5064, Australia, [2] University of Adelaide, School of Biological Sciences, North Terrace Campus, Adelaide SA 5000

Like all plants, grapevine is host to a plethora of microorganisms. Unlike other plants, these micro-organisms are carried over via the grape into winemaking. While *Saccharomyces cerevisiae* is the main driver for alcoholic fermentation, it has become apparent that the other microbes can have a significant impact on fermentation and resulting wine. Few of these micro-organisms have been successfully isolated and their contributions during fermentation are poorly understood. A potential component of this influence is the production of glycoside hydrolases not produced by *S. cerevisiae* but are used by other micro-organisms to afford access to plant resources whilst surviving on the plant. During winemaking, glycoside hydrolases have the potential to affect the breakdown of complex sugars in plant cell walls, with the potential to aid juice extraction and clarification, and mediating the release of flavour and aroma compounds from glycosyl linkage.

A combination of metagenomics and synthetic biology were used to explore the enzymatic potential of two grapevine derived environments, an unfermented Chardonnay grape must and a mixed varietal grape marc (MVGM). These environments contained distinct microbial communities. The Chardonnay must primarily contained fungal genomes whereas the MVGM was dominated almost exclusively by bacteria. Numerous novel glycoside hydrolases were identified from both metagenomes. In order to assess suitability as wine processing aids, a subset of β-glucosidases and polygalacturonases were chosen for heterologous expression in the yeasts *Pichia pastoris* and *Saccharomyces cerevisiae* as a potential "in-wine" platform. When expressed in *P. pastoris*, the two polygalacturonases of fungal origin exhibited promising levels of activity at wine pH and the equivalent *S. cerevisiae* strains were used to ferment synthetic grape must containing polygalacturonic acid to test activity in a wine-like context. These enzymes could be leveraged to improve juice extraction and the clarification during winemaking.

17. Investigating the role of phosphorylation in the regulation of the *S. cerevisiae* lysine demethylase Rph1 using LC-MS/MS

Wong, M.W.M.[1], Separovich, R.J.[1], Hamey, J.J.[1], Wilkins, M.R.[1]

[1]Systems Biology Initiative, School of Biotechnology and Biomolecular Sciences, The University of New South Wales, Sydney, Australia

A nucleosome consists of DNA wrapped around an octamer of histone proteins. Histone proteins are subject to various post-translational modifications which affect chromatin states to enable local and genome-wide chromatin functions, such as transcription, DNA replication and chromosome condensation. While the identities and activities of histone modifiers have been well studied, their regulation is still poorly understood. Such is the case with histone lysine methylation, where methyl groups are added by methyltransferases and removed by demethylases. These enzymes are often highly phosphorylated, and this may serve to regulate their function. Here, we investigated the effect of phosphorylation on the enzymatic activity of *Saccharomyces cerevisiae* lysine demethylase Rph1, which acts on lysine 36 of histone 3 (H3K36). We genomically engineered phospho-mimic and phospho-null amino acid substitutions at two well characterised phospho-sites of Rph1, serine 412 and serine 575 (S412/575). By monitoring H3K36 methylation levels using an mass spectrometry-based approach, we investigated the regulatory role of phosphorylation at these sites on the activity of Rph1. The biological effect of S412 and S575 phosphorylation was determined by the quantification of all three H3K36 methylation states in strains containing wild-type and S412A, S412D, S575A, S575D Rph1 mutants. We observed no significant change in H3K36 methylation between mutant and wild-type Rph1 expressing strains, suggesting that S412 and S575 do not independently function as regulatory phosphorylation sites in Rph1 and challenging previous observations. However further investigation is required to determine whether these sites exhibit a regulatory effect when phosphorylated in combination with other Rph1 phosphorylation sites, or under different conditions. Our investigation demonstrates the utility of mass spectrometry as an effective tool for investigating the regulatory role of Rph1 phosphorylation.

18. Purine biosynthesis as an antifungal target

Chua, S.M.H., Wizrah, M.S.I., Luo, Z., Kobe, B, Fraser, J.A.

School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane QLD 4072

Introduction: *Cryptococcus neoformans* is a major fungal pathogen that causes life-threatening systemic mycoses. The current antifungals employed to treat this disease have not changed significantly for the past 25 years despite the high mortality and morbidity. Therefore, there is an urgent need to develop new antifungal drugs. An area of interest for finding new antifungal targets is the purine biosynthesis pathway. Many effective drugs have been developed based on the purine metabolic pathway, however there are limited studies that utilise this pathway as a source of targets for antifungal discovery. The purine biosynthesis pathway consists of eleven sequential enzymatic steps to form IMP, an intermediate for formation of ATP and GTP. Over the course of evolution of the eukaryotes, several gene fusion events have occurred resulting in the formation of bifunctional or trifunctional enzymes in higher order organisms. An example of this is bifunctional GAR synthetase/AIR synthetase, which catalyses steps two and five of the purine biosynthesis pathway. In humans, this has undergone has an additional gene fusion to create a trifunctional enzyme that includes GAR transformylase.
Hypothesis and Aims The gross differences between the fungal and human enzymes could potentially be exploited in the development of fungal specific inhibitors. The enzyme has been characterised using molecular genetics, enzymology and structural biology techniques.
Results and conclusion Our work proves that the enzyme is required for *de novo* adenine and guanine production and is essential for virulence in a mouse inhalation model, showing its potential as an antifungal target. Each domain of the protein has been heterologously expressed and crystallised. The GARS domain crystal had a preliminary diffraction of 1.8 Å and the AIRS domain crystal had a preliminary diffraction of 2 Å, and reveals differences from the human enzyme that could be exploited in antifungal drug development.

19. Antifungal activity of honey against dermatophytes: Is H_2O_2 the whole story?

Guttentag, A.[a] Krishnakumar, K.[a] Cokcetin, N.[b] and Carter, D.A.[a]

[a]*School of Life and Environmental Sciences and the Marie Bashir Institute for Infectious Diseases and Biosecurity, University of Sydney, New South Wales, Australia*
[b]*ithree Institute, University of Technology Sydney, New South Wales, Australia*

Dermatophytes are fungi that cause superficial infections of keratinised tissues, collectively termed 'tineas'. Current therapeutic options often require long treatment times and struggle to resolve these common ailments. Previous work has found certain Australian jarrah honey, which contains glucose oxidase and produces H_2O_2 upon dilution, is particularly effective against dermatophytes. The aim of this study was to investigate whether honey components in addition to H_2O_2 mediate this potent antifungal activity and to determine the specific mechanism/s of action against *Trichophyton rubrum*, a common dermatophyte species. Removal of H_2O_2 using catalase treatment increased the minimum inhibitory concentration (MIC) of honey against three dermatophyte species, however, quantification using a colourimetric assay found the level of H_2O_2 present was 8–15 fold lower than expected based on inhibition by H_2O_2 alone. Microscopic analysis of conidia treated with honey and stained with calcofluor white indicated that honey inhibits conidial germination, which was not seen with following treatment with H_2O_2 in a sugar solution. Electron microscopy of mature hyphae treated with honey found these were substantially damaged and collapsed, while DCFDA, a fluorophore that detects reactive oxygen species, suggested the treated hyphae were not affected by significant oxidative stress. Together, these findings indicate that H_2O_2 and sugar cannot account for the remarkable anti-dermatophyte properties of Australian jarrah honey and suggest the presence of a novel honey component that may be a very effective antifungal.

20. AICAR transformylase/ IMP cyclohydrolase: An antifungal drug target.

Wizrah, M.S.I.W., Chua, S.M.H., Luo, Z., Kobe, B, Fraser, J.A.

School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane QLD 4072

Introduction Cryptococcal meningitis is one of the most dangerous fungal infections caused by *Cryptococcus neoformans*. Immunocompromised individuals are susceptible to the fungus and annually, 1.1 million incidences of cryptococcal meningitis infections are recorded with significant number of deaths cases. Current treatment uses anti-fungal, but they are associated with obstacles such as toxicity, high costs, variability in its effect, the need for frequent hospitalization, and drug resistance. Pioneering a treatment that is more immunologically, financially, and technically efficient is a rising urgent need. The purine metabolic pathway has been the target for drug design since this pathway composed of a series of processes supplies the cell with ATP and GTP essential as an energy source and for important cell functions. Evidence supports targeting enzymes in this pathway serves as a therapeutic agent to effectively treat antifungal infections.
Hypothesis and Aims There is a need to develop new antifungal treatments. The purine biosynthesis pathway has proven to be a potential target to develop the promising antifungal drugs since ATP and GTP production is required for several cell functions. Within this pathway several enzymes could potentially serve as antifungal targets, where their inhibition may lead to loss of virulence of the fungal pathogen.
Results and conclusion characterisation of AICAR transformylase/IMP cyclohydrolase-from *C. neoformans*

was performed. The amino acid similarity between *C. neoformans* ATIC and that present in other eukaryotes, including humans, was 73.2 percent. A higher similarity was observed in regard to *C. albicans and S. cerevisae*, being 76.2% and 76.9% respectively. Crystals were cryoprotected with either glycerol before diffraction, data was collected at the Australian Synchrotron and the structure of C. neoformans ATIC was solved to 2.7Å.

21. Applying dual DNA barcoding to the identification of clinically relevant *Candida* species.

Minh Thuy Vi Hoang[1,3], Laszlo Irinyi[1,2,3], Sharon C. A. Chen[1,2,5], Tania C. Sorrell[1,2,3], the ISHAM Barcoding of Medical Fungi working group, and Wieland Meyer[1,2,3,4]

[1]*Molecular Mycology Research Laboratory, Centre for Infectious Diseases and Microbiology, Faculty of Medicine and Health, Sydney Medical School, Westmead Clinical School, The University of Sydney, Sydney, NSW, Australia.*
[2]*Marie Bashir Institute for Infectious Diseases and Biosecurity, The University of Sydney, Sydney, NSW, Australia.*
[3]*Westmead Institute for Medical Research, Westmead, NSW Australia.*
[4]*Westmead Hospital (Research and Education Network), Westmead, NSW, Australia.*
[5]*Centre for Infectious Diseases and Microbiology-Laboratory Services, Institute for Clinical Pathology and Medical Research, NSW Health Pathology, Westmead, NSW, Australia.*

Rapid and accurate diagnosis is essential for effective management of mycoses. DNA barcoding has been established as the gold standard for the identification of pathogenic fungi. It is based upon the selection of short, standardized DNA regions – DNA barcodes – that are divergent at the species level. The dual barcoding scheme is composed of the primary fungal DNA barcode, the internal transcribed spacer region (ITS1/2), and the secondary fungal DNA barcode, the *translational elongation factor 1α*. To enable clinical implementation of DNA barcoding the ISHAM Barcoding Database was established, providing quality-controlled reference DNA barcode sequences. The genus *Candida* contains major global fungal pathogens with three of the five most pathogenic *Candida* species being contained within the *Lodderomyces* clade. Additionally, rising occurrence of antibiotic resistance is being observed, e.g. *C. auris, C. krusei* (*Pichia kudriavzevii*) and *C. glabrata*. The current study aimed to complement the primary DNA barcodes (ITS1/2) with the generation of secondary reference barcode sequences of *Candida* species for the ISHAM Barcoding Database. The study also aimed to evaluate the ability of the dual DNA barcoding scheme to identify the species of the *Lodderomyces* clade. Barcoding sequences were amplified from pure fungal cultures and commercially sequenced. The resulting sequences were submitted to the ISHAM Barcoding Database. 84 secondary barcode sequences were generated representing 45 *Candida* species. Barcoding gap analysis was performed to evaluate the usefulness of the dual barcoding scheme to distinguish between the species of the *Lodderomyces* clade. No barcoding gap was produced for the primary barcode, however, the secondary barcode and the combination of both barcodes generated barcoding gaps indicating an accurate identification. As such, the dual DNA barcoding scheme, in combination with the expansion of the ISHAM Barcoding Database, establishes a highly accurate and rapid identification system for routine diagnosis of *Candida* species.

22. Multi-Locus Sequence typing of Canine *Pneumocystis* isolates.

Lana Pasic[1], Richard Malik[2], Laszlo Iriny[1], Wieland Meyer[1]

[1]*Molecular Mycology Research Laboratory, Center for Infectious Diseases and Microbiology, Marie Bashir Institute for Emerging Infectious Diseases and Biosecurity, Faculty of Medicine and Health, Sydney Medical School, Western Clinical School, The University of Sydney, Westmead Hospital (Research and Education Network), The Westmead Institute for Medical Research, Westmead, NSW*
[2]*Centre for Veterinary Education, The University of Sydney, Sydney, New South Wales, Australia*

Pneumocystis spp. are known to have the potential to cause host species-specific life-threatening fungal diseases, manifesting as *Pneumocystis* pneumonia (PCP), in a variety of mammals, including rabbits, mice, rats, horses and dogs. *Pneumocystis carinii f.sp. 'canis'* (*P. canis*) is the fungal pathogen causing infections specifically in dogs. Based on our previous studies focusing on the optimization of the Multi-Locus Sequence Typing (MLST) scheme, which successfully amplified human *Pneumocystis* (*P. jirovecii*) DNA, we have now extended the application of this scheme to genotyping *P. canis*, with the final aim of building a universal, consensus *Pneumocystis* MLST scheme. Five isolates from dogs with active *Pneumocystis* infections were subjected to MLST typing. As part of this process, the ISHAM MLST scheme, consisting of the β-tubulin, *mt26s*, DHPS and ITS loci, was compared to a newly developed scheme consisting of the *CYB, SOD, mt26s* loci. Both schemes were able to effectively amplify and sequence all targeted loci from *P. canis*. Due to difficulties in amplifying the ITS loci within the ISHAM MLST scheme, the newly developed MLST scheme performed with a marginally higher success rate and is therefore recommended as the preferred scheme. Genotypic analysis of the sequences showed that all five isolates were identified as separate sequence types with both schemes. Phylogenetic analyses comparing human and dog samples showed that *P. canis* differed by more than 10% from the human pathogen *P. jirovecii*. To determine the ability of the developed MLST scheme to be applicable to all *Pneumocystis* species, further studies are planned on a

broader range of mammalian species to develop a 'one-size-fits-all' approach to *Pneumocystis* genotyping. In addition, next generation sequencing (NGS) using the Nanopore MinION devise was undertaken for two of the dog isolates. MinION sequencing identified *Pneumocystis canis* in both samples but did not allow for genotyping of the involved fungal strains based on the NGS data obtained.

23. The *Cryptococcus neoformans* SAGA: the epigenetic impact of the transcriptional coactivator on virulence in a global fungal pathogen

Stephenson, C. J. Erpf, P. and Fraser, J.A.

School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane QLD 4072

Introduction *C. neoformans* is an opportunistic pathogenic fungus which poses a threat to the global immunocompromised population. Upon infection this pathogen induces a plethora of virulence traits which enable it to evade host immune systems, causing *meningoencephalitis and death*. These virulence traits are highly regulated by a complex interplay between the environment, transcriptional machinery and genomic elements. Therefore, understanding any mechanism which influences these pathways are areas of significant interest, and can aid in the development of novel drug strategies. Transcriptional regulatory proteins, which have a widespread impact on gene regulation, make ideal candidates for drug therapies as they can interfere in numerous pathways. A primary candidate for this level of pleiotropic regulation is the SAGA complex, a 20-protein transcriptional coactivator, conserved across eukaryotes. Interactions between this complex and regulation of stress response genes have widely been established in *S. cerevisiae.* A handful of studies focussing on the HAT module within SAGA has linked this complex to hypovirulence within *C. neoformans*, however these studies were conducted in a hypervirulent background.
Aims Virulence within *C. neoformans* is intricately tied to the ability of the pathogen to induce an array of stress response pathways upon infection. The SAGA complex which plays an overarching role in these pathways' regulation should have an observable impact on virulence. Targeting these genes, through a combination of genetics and proteomic approaches could identify novel regulatory pathways, providing avenues for fungal specific drug therapies against cryptococcal infections.
Results and conclusions The HAT module has been deleted within the H99O strain to reassess the virulence effect of this module outside of the influence of the hypervirulent background. The recreated strains demonstrate a diverging phenotype to the original mutants. We have also demonstrated that the deubiquitinase (DUB) module of SAGA plays an overarching role in modulating virulence within *C. neoformans*, with hypervirulence observed within a murine model when any component of the DUB module is removed from the complex.

24. Irregular Cells and Relapsing Infection in *Cryptoccocus*

Iceton, A,[*] Fernandes, K,[*] Perfect, J[#] and Carter, D.[*]

[*]*School of Life and Environmental Sciences and the Marie Bashir Institute for Infectious Diseases and Biosecurity, University of Sydney, New South Wales, Australia*
[#]*Department of Medicine, Duke University Medical Center, Durham, North Carolina, USA*

Cryptococcus is a pathogenic encapsulated yeast causing a significant disease burden, especially in developing countries due to the prevalence of immunosuppressive disease. Under stressful conditions typically encountered during mammalian infection, *Cryptococcus* can form pleomorphic cell types that have been found to be associated with various clinical parameters. Among these, irregularly shaped and elongated cells seen in some strains are positively associated with relapsing infection and the requirement for additional lumbar punctures, and negatively associated with patient death, suggesting these may be promoting chronic, low-grade infection that is difficult to clear. These may be "persister" cells, which have been characterised in various micro-organisms and are characterised by low metabolic activity, and reduced drug uptake and susceptibility. The aim of this study is to characterise the irregular *Cryptococcus* cells and determine how these may bypass treatment and cause relapsing infection. Strains with various amounts of irregular cells have been characterised using microscopy, and these will be tested for susceptibility to various stresses. Cell morphology and metabolism will be examined using scanning electron microscopy and fluorescence microscopy with various metabolic dyes. Finally, proteomic analyses will be used to gain insight into their molecular biology. This investigation will help determine the role of the irregular cell type in relapsing infection, and may have implications for the treatment of recalcitrant fungal infections.

25. Using bisphosphonates to overcome azole resistance in *Candida*

Kane, A, Carter, D

School of Life and Environmental Sciences, Faculty of Science, University of Sydney

Systemic infection by *Candida* results in at least 350 000 deaths a year, and candidiasis of the oral and vaginal mucosa affects the lives of millions. Almost 10% of *Candida* isolates causing bloodstream infections are resistant to azole-derived antifungals, with a majority of these

belonging to the species *Candida glabrata*. With azoles becoming less effective there is a need for strategies that can synergise and restore their antifungal activity. We previously identified up-regulation of farnesyl pyrophosphate synthetase (FPPS) in response to azole treatment. FPPS is part of the mevalonate pathway, responsible for the synthesis of squalene that feeds into the ergosterol biosynthesis pathway, which is targeted by triazoles. FPPS is inhibited by bisphosphonates, an FDA-approved class of anti-osteoporotic pharmaceuticals. Here we demonstrate that bisphosphonates have antifungal activity against various species of *Candida*, including highly azole-resistant strains of *Candida glabrata* and *Candida krusei*. Checkerboard microdilution plates were used to show that bisphosphonates can synergise with azole-based antifungals and result in fungicidal activity. By supplementing inhibited cells with exogenous squalene, we demonstrated that squalene deprivation was responsible for the observed synergy. Time-course fluorescence assays demonstrated that combinations of bisphosphonates and azoles result in increased membrane depolarisation, reduced efflux, and the accumulation of toxic reactive oxygen species. Furthermore, in certain combinations, azoles + bisphosphonates were able to damage biofilms and prevent the acquisition of resistance. We conclude that bisphosphonates are an exciting lead towards a new, effective, broad-spectrum antifungal treatment strategy.

26. Methanol assimilation in native and synthetic strains of *Saccharomyces cerevisiae*

Monica I. Espinosa [1,2], Kaspar Valgepea [3,4], Ricardo A. Gonzalez-Garcia [3], Colin Scott [2,5], Isak S. Pretorius [1], Esteban Marcellin [3], Ian T. Paulsen [1]* and Thomas C. Williams [1,2]*

[1] ARC Centre of Excellence in Synthetic Biology, Department of Molecular Sciences, Macquarie University, Sydney, Australia

[2] Synthetic Biology Future Science Platform, CSIRO, Sydney, Australia

[3] Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, St. Lucia, Australia

[4] ERA Chair in Gas Fermentation Technologies, Institute of Technology, University of Tartu, Tartu, Estonia

[5] Biocatalysis and Synthetic Biology Team, CSIRO, Canberra, Australia

Microbial fermentation for chemical production is becoming more broadly adopted as an alternative to petrochemical refining. Fermentation typically relies on sugar as a feed-stock. However, one-carbon compounds like methanol are a more sustainable alternative as they do not compete with arable land. This study focused on engineering the capacity for methylotrophy in the yeast *Saccharomyces cerevisiae* through a yeast xylulose monophosphate (XuMP) pathway, a 'hybrid' XuMP pathway, and a bacterial ribulose monophosphate (RuMP) pathway. Through methanol toxicity assays and ^{13}C-methanol-growth phenotypic characterization, the bacterial RuMP pathway was identified as the most promising synthetic pathway for methanol assimilation. When testing higher methanol concentrations, methanol assimilation was also observed in the wild-type strain, as ^{13}C-ethanol was produced from ^{13}C-methanol. These results demonstrate that *S. cerevisiae* has a previously undiscovered native capacity for methanol assimilation and pave the way for further development of both native and synthetic one-carbon assimilation pathways in *S. cerevisiae*.

27. Australian sourdough yeasts and bacteria: identity, diversity and their influences on the bread aroma

Di Liu, Michela Winters, Daphne Panayotides, Annli Tee, Anna Wittwer, Kate Howell*

School of Agriculture and Food, Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Parkville, Vic, Australia

Yeast and bacterial communities inhabit a sourdough starter to make artisanal bread. This study identifies species found in Victorian sourdoughs and shows whether the interactions of micro-organisms derived from Australian sourdough starters provide some of the positive flavour, and aroma properties to bread by using defined sourdough cultures as the sole leaven in bread production. Four different yeast species were identified as *Saccharomyces cerevisiae*, *Kazachstania humilis*, *Kazachstania bulderi*, and *Pichia membranifaciens*. Furthermore, a total of seven *Lactobacillus* species were isolated, two *Acetobacter* species, one *Kocuria*, and one *Bacillus*. When bread was made combining these yeasts individually and in combinations with lactic

acid bacteria also isolated from these sourdough starters, the bread aroma profiles were more distinctive, with enriched compounds associated with sour aromas produced, and preferred by sensory panels. In conclusion, the use of defined mixed cultures as the leaven in bread making, by exploiting the microbial diversity of artisanal Australian starters, can produce bread with distinctive and attractive aromas.

28. Does intraspecies variation in *Aspergillus fumigatus* affect infection outcomes? A phenotype/genotype study using an insect model.

El-Kamand, S[1], Papanicolaou, A[2], Morton, CO[1]

[1]Western Sydney University, School of Science and Health, Campbelltown Campus, NSW 2560, Australia

[2]Hawkesbury Institute for the Environment, Western Sydney University, Hawkesbury Campus, NSW 2753, Australia

*Aspergillus fumig*atus is a saprophytic soil-fungus and an opportunistic human pathogen. This mould reproduces asexually using spores that readily become airborne. In immunocompromised individuals, inhalation of *A. fumigatus* spores can lead to a pulmonary infection termed 'invasive aspergillosis' (IA). Despite extensive research on human immunity and treatment, the relative contribution of fungal genetic and phenotypic variation to infection outcome is yet to be determined. In this study, we sought to determine the pathogenic relevance of the intraspecies variation in *A. fumigatus*. Clinical isolates were assayed for relevant phenotypic traits such as radial growth rate, UV resistance and amphotericin-B resistance. Comparative genomics approaches were used to identify phylogenetic relationships and common single nucleotide variants. These data were integrated with virulence data generated in an invertebrate infection model, using *Tenebrio molitor* larvae (mealworms), to determine the relevance of fungal variation to clinical outcomes, identify potential virulence factors and build our understanding of *A. fumigatus* pathogenesis in invasive aspergillosis. It was found that isolates from cases of IA grew more slowly in nutrient-rich environments than colonising isolates. IA isolates were also less virulent in the infection model. The correlation between clinical origin and modelled virulence suggests a contribution of fungal biology towards clinical outcomes. The low modelled virulence of IA isolates relative to colonisers suggests the biology of IA isolates may be optimised for overcoming clinical challenges not modelled in *T. molitor* larvae.

Other abstracts

Development of a flow cytometry-based competitive fitness assay to validate a novel, *in vitro* model for *Candida albicans* colonisation of the colon.

Prokop, M.D.[1], Lai, Y.W.[1] and Lenardon, M.D.[1]

[1]School of Biotechnology and Biomolecular Sciences, UNSW Sydney, NSW, 2052.

The most common source of life-threatening systemic infections caused by *Candida albicans* is the reservoir of the fungus living commensally within the patient's gastrointestinal (GI) tract. Due to the limitations of existing animal models, little is known about how *C. albicans* interacts with the GI microbiota during GI colonisation. We have therefore developed a novel *in vitro* two-phase anaerobic fermentation system, the colon microcosm, that simulates the human colon microenvironment and can be exploited to determine the adaptive mechanisms that enable *C. albicans* to persist in the colon in the presence of the GI microbiota.

As part of the validation of this new model, a flow cytometry-based competitive fitness assay has been developed and optimised. *C. albicans* wild-type and mutant strains constitutively expressing either green (GFP) or red (dTomato) fluorescent proteins have been constructed. Pairs of strains are being competed against each other for 48 h in the colon microcosm. The abundance of each strain at t=0 and t=48 h will be assessed by flow cytometry and used to determine relative fitness of the two strains in colon simulating conditions. The results from these assays will be compared to published data from comparable experiments performed in mouse models of *C. albicans* GI colonisation to determine where our model sits in relation to the current literature.

An inexpensive, rapid, efficient protocol for the identification of *Saccharomyces cerevisiae* mating types.

Arras SDM[1], Mitsugi-McHattie L[1], **Hermann-Le Denmat S[1]**, Woods MA[2], Johnson CE[1] and Ganley ARD[1]

[1]School of Biological Sciences, University of Auckland, Auckland, New Zealand
[2]Institute of Natural and Mathematical Sciences, Massey University, Auckland, New Zealand

The budding yeast *Saccharomyces cerevisiae* is a long-established, widely known genetic model. It can be maintained in haploid and diploid forms, making it an exceptional genetic system with which to perform genetic crosses. Such crosses are rather straightforward, as long as the mating type and ploidy of the strains are known. Haploid *S. cerevisiae* cells are either *MAT*a or *MAT*α mating type, and determination of mating type (or ploidy) is classically performed by crossing with test *MAT*a and *MAT*α strains and observing mating through the formation of diploid 'schmoo' cells under the microscope. However, visible mating formation can be ephemeral and is typically only observed in a small number of cells, reducing the robustness of the assay. Another approach is PCR, but this is relatively expensive and laborious. Here we validated a simple, cheap and robust alternative method to enable rapid identification of *S. cerevisiae* mating types. When cells of opposite mating type are mixed in liquid media, they undergo a change in density and/or flocculation that can easily be detected visually in a culture tube, as a 'creeping' cloud up the sides of the culture vessel. Mixtures of cells of the same mating type or with a diploid strain(s) can easily be distinguished, as they just settle out over the same time duration. This method does not require specialized equipment, is robust to different media, different *S. cerevisiae* strains, and the different proportions of the two strains. Furthermore, it can be scaled to 96-well plate format, and the 'creeping' cloud phenotype is observable for several days and thus is less transient than 'schmoo' formation. The simplicity and robustness of this method makes it ideally suited for routine verification of *S. cerevisiae* mating type and could form an effective approach to screen for genes that play a role in the phenotype.

The inositol pyrophosphate IP_7 is critical for PHO pathway activation in *Cryptococcus neoformans*

Desmarini Desmarini[1,2,3], Sophie Lev[1,2,3], Dorothea Fiedler[4], Tania C Sorrell[1,2,3], and Julianne Teresa Djordjevic[1,2,3]

[1]Centre for Infectious Diseases and Microbiology, The Westmead Institute for Medical Research, Sydney, NSW, Australia,
[2]Sydney Medical School-Westmead, University of Sydney, Sydney, NSW, Australia,
[3]Marie Bashir Institute for Infectious Diseases and Biosecurity, University of Sydney, Sydney, NSW, Australia,
***[4]Leibniz-Forschungsinstitut für Molekulare Pharmakologie im Forschungsverbund Berlin e.V. (FMP)** Campus Berlin-Buch Robert-Roessle-Str. 10 13125 Berlin, Germany.*

In the human fungal pathogen *Cryptococcus neoformans,* activation of the phosphate signalling and acquisition (PHO) pathway and production of the inositol pyrophosphate 5-PP-IP5 (IP_7) by the IP_6 kinase, Kcs1, are essential for fungal dissemination to the host brain and induction of fatal meningitis. Activation of the PHO pathway is triggered by a cyclin-dependent kinase (CDK) complex comprised of the CDK (Pho85), a cyclin (Pho80) and a CDK inhibitor (Pho81). Given that Pho81 contains an SPX domain and SPX

domains can bind inositol pyrophosphates in eukaryotic cells, we hypothesized that IP_7 interaction with the SPX domain of Pho81 is essential for PHO pathway activation.

To investigate this, we used sequence alignment to identify a lysine surface cluster (KSC) in the SPX domain of Pho81 putatively involved in forming electrostatic interactions with IP_7. We then used site-directed mutagenesis to modify these lysine residues to alanine, and affinity chromatography and Western blotting to determine whether they enable Pho81 to bind to a custom-made IP_7 affinity resin. We also determined the effect of disrupting Pho81-IP_7 interaction on PHO pathway activation and virulence in a mouse inhalation model of cryptococcosis.

Our results show that IP_7 physically associates with the SPX domain of Pho81 via the KSC and that this association is essential for PHO pathway activation. This is in contrast to the model yeast, *Saccharomyces cerevisiae*, where IP_7 suppresses PHO pathway activation. Despite the IP_7 binding-defective strain having a similar virulence profile to the WT strain *in vitro,* it was unable to cause disease in mice. Our results highlight the occurrence of evolutionary changes in PHO pathway regulation that contribute to the virulence repertoire of a fungal pathogen of medical significance and that disruption of IP_7-Pho81 interaction is a potential antifungal strategy.

Notes

Notes