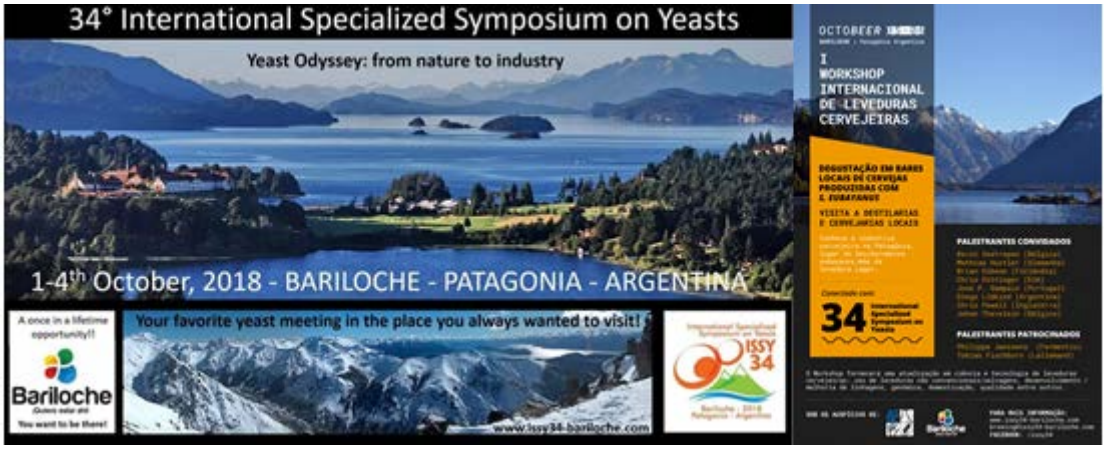
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Identifying new ideas for an improved 'microbial toolkit' for Australian winemaking: Attendance of ISSY34 - 'Yeast Odyssey from Nature to Industry' and 1st International Workshop on Brewing. (Bariloche, Argentina)



REPORT TO WINE AUSTRALIA FOR TRAVEL

Project Number: **WAT1708**

Principal Investigator: **Michelle Walker**

Research Organisation: **The University of Adelaide**

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WAT 1708 Project Title: Identifying new ideas for an improved 'microbial toolkit' for Australian winemaking: Attendance of ISSY34 - 'Yeast Odyssey from Nature to Industry' and 1st International Workshop on Brewing. (Bariloche, Argentina)

Author: Michelle Walker

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Abstract

The travel bursary enabled Dr Michelle Walker to attend the 34th International Specialized Symposium of Yeasts (ISSY34) and the 1st International Workshop on Brewing held in Bariloche, Patagonia (Argentina) in early October 2018. The ISSY34 symposium showcased important scientific advancements in yeast fundamental and applied research. Included was a workshop on yeast genomics and taxonomy dedicated to the late Cletus Kurtzman, 3 keynote lectures, 8 sessions (65 oral presentations and 187 e-posters). Following from ISSY34, 2 days were dedicated to the 1st International Workshop on Brewing Yeasts attended by academics and brewers. The workshop was conducted in English with spontaneous Spanish translation, and included a tour of the four main local Craft breweries and a distillery. The locally discovered yeast *Saccharomyces eubayanus* was showcased in several locally produced beers and the bread provided at the conference. Attendance allowed Dr Walker to be updated on the latest advances in science related to fermentation (beer, wine, dough, bioethanol), present her research (on yeast derived colour modulation and hydrogen sulfide production during wine fermentation), as well as reaffirm research acquaintances and establish new ones.

Executive Summary

- Attended the 34th International Specialized Symposium of Yeasts (1-4 October, 2018) and the 1st International Workshop on Brewing (5-6 October, 2018), held in Bariloche, Patagonia (Argentina).

- Gave an oral presentation in the Session 8B/ Yeasts in Food and Beverage. Industrial Fermentation. The talk entitled '*Sulfate transport mutants affect hydrogen sulfide production during alcoholic fermentation*' reported on research undertaken as part of Wine Australia Projects UA1101, UA1302.

- Gave an e-poster (3 min talk) in the E-poster 4 session on research related to colour modulation during red wine fermentation. The e-poster entitled '*Investigation of yeast genes required for the modulation of colour in model red wine*' related to projects funded by Wine Australia (UA1405, UA1302).

- Fostered new and current linkages with researchers from Canada, Japan and France. The conference was attended by Dr Phillipe Marullo (University of Bordeaux) and Professor Steve Olivre (Univesity of Cambridge) who have established collaborations within Professor Jiranek's Group, and Professor Takagi, who will visit the group in November to discuss potential collaborations on proline-stress tolerance related research and hosting of ISSY37 in 2022.

Key Activities and Outcomes

General Introduction

The International Symposium on Specialised Yeasts is a major international conference held annually, dedicated to the genetics and application of non-conventional yeasts. This year, ISSY34 was organised by the South American contingent, led by Dr Diego Libkind and was held in Bariloche, a city located within the Nahuel Huapi National Park in Argentina's Patagonia. ISSY34 attracted 290 delegates, from South America, Europe, USA, Japan and South Africa. Students and early career researchers was encouraged through the provision of 24 fellowships (part and full).

The 6th South American Meeting on Biology and Biotechnology of Yeasts proceeded ISSY34, providing the opportunity for Spanish speaking PhD students to present their research as short talks in their first language. This was followed by a half-day workshop on impact of genomics on yeast taxonomy and systematics, which was held in honour of Cletus Kurtzman, who was regarded as the founder of modern yeast taxonomy. The symposium itself was divided into 8 sessions covering the latest research on the ecology and biodiversity of yeasts, their current and potential industrial application, including a section on traditional and industrial fermentations. Rosan Schwan gave the 'Graham Fleet Memorial Lecture', reporting on the use of starter cultures, as yeast and bacteria, or yeast (up to 6 species) to replace traditional fermentations which rely on the indigenous microbiota, and the change from small basket, box type vessels to stainless steel tanks (capable of holding 50 - 100 kg) for industrial scale up of cocoa production.

Participants were given the opportunity to discuss their research as e-posters (3-5 min talks) *in lieu* of paper posters; a total of 187 e-posters were presented. The inaugural meeting of the 1st International Brewing Workshop was held after ISSY34

in the same venue. Lectures were held on the first day (in English with 'real-time' translation for Spanish speaking industry members and academics). Four craft breweries (Berlina, Blest, Wesley and Manush) and a Distillery (Madoc; Appendices 1, 3) were toured on the second day. At each facility, the brewing process, etc was discussed with the head brewer. Different beer styles were tasted, including those made with *Saccharomyces eubayanus* (which have characteristic phenolic 'clove' flavours (due to 4-vinyl guaiacol derived from the yeast).

Research relevant to Wine Australia's 5 Year Strategic R, D&E Plan 2015-2020, namely Strategy 6: Enhanced yeast and bacterial performance will be discussed.

Presentations relevant to yeasts and/or winemaking

Several presentations were devoted to yeast diversity and natural variation, with the potential application in fermentation technology. Chris Hittinger (Uni. Wisconsin-Maddison, USA) spoke on the y1000 sequencing project (y1000plus.org) and how scientists now have a better understanding of the evolution of yeasts belonging to the *Saccharmycotina* subphylum, including the *Saccharomyces stricto senso* clade; to which *Saccharomyces cerevisiae* belongs; this yeast historically has been associated with alcoholic fermentation, whether wine, beer, sake or cider. The discovery of *Saccharomyces eubayanus* (POF+ve, maltotriose –ve), and more recently *Saccharomyces jurei*, has allowed the generation of new hybrids with *Sacharmoyces cerevisiae* through hybridisation coupled with mutagenesis and adaptive evolution both for understanding evolution and domestication and as 'fit for purpose' yeasts. Whole genome sequencing together with CRISPR-cas9 gene editing have allowed the identification of genetic changes in an evolved strain or hybrid, and transfer to other yeasts, to see whether the genes are actually responsible for the improved phenotype.

Jean-Marc Duran (TNW-Delft, Netherlands) spoke on the generation of POF-ve, maltiotriose +ve) *S. eubayanus* isolates with improved maltose consumption and modified ester, diacetyl acetate and higher alcohol production as 'industry ready' strains or added genetic diversity in breeding programs, whilst Tom Hittinger, Kevin Verstreppen (KUL Belgium) and Brian Gibson (VTT, Finland) described their work on making beer hybrids to meet the demand for specific beer styles by brewers. Verstreppen is also involved in the 'Beerome' project, which

aims to objectively describe and compare beers (a total of 250 beers using chemical and sensory analysis) and then to be able to predict beer aroma and appreciation from the chemical analysis, with the aim of making better beer. By phenotyping and genotyping more than 150 yeasts, he hopes to offer the brewing industry yeast 'tailored' to a specific beer style.

Jessica Noble (Lallemand, Canada) gave a talk on the techniques being used by Lallemand in the generation of new wine yeasts. She spoke on clonal selection and hybridisation as ways of exploiting the natural diversity and combining properties of different yeast (identified through genetic mapping), and directed evolution to expand upon this genetic diversity through selection of beneficial mutations resulting in improved phenotype. Three new yeasts were examples of these technologies: Lalvin Tango Malbec™ (yeast selection), Lalvin ICV Okay® (directed crossing) and Lalvin IONYS$_{WF}$™ (evolutionary engineering). Phillipe Marullo (Univ. Bordeaux, France) spoke on the influence of the mitochondrial DNA inheritance, gene conversion and ploidy level on phenotypic variability and how this can impact on the genetic determination of complex traits, such as fermentation kinetics (ploidy), *ENO2* expression in glycolysis (gene conversion) and cold tolerance (mitotype) and importantly, transferal into new genetic backgrounds.

Several e-posters were devoted to the use of indigenous grape yeasts such as *Aureobasidium pullulans*, *Wickerhamomyces anomalus*, *Metschnikowia pulcherrima*, *Starmerella bacillaris*, *Cryptococcus magnus*, and to a lesser extent *Hanseniaspora uvarum*, as biocontrol agents in grape must and postharvest storage of table grapes. These yeasts were effective against *Aspergillus, Penicillium*, *Alternaria spp* and *Botrytis cinerea,* under laboratory conditions (on plates or isolated grape berries). Scott Simonin (Université de Bourgogne Franche-Comté) reported on *Torulaspora delbrueckii* being able to inhibit microbial diversity and numbers when implanted into white juice prior to fermentation without affecting performance. Eloise Arrarte (Universidad de la República, Uraguay) reported on *Debraryomyces hansenii* isolates from the sub-Antarctic region, reduced disease incidence of *Penicillium expansum* in cold stored apples and pears, whilst Julián Rafael Dib (Universidad Nacional de Tucumán, Argentina) spoke on the efficacy of *Clavispora Lusitaniae* (isolate 146) against *P. digitatum* on cold stored lemons. These investigations hold promise in terms of providing an alternative to sulfur dioxide as an antimicrobial (and antioxidant in wine making) but this needs to be further explored. If

these yeasts could be used to control microbial spoilage, without any adverse effect to humans, the potential benefits of being able to store grapes out of season, could include extended wine production and year-round availability of domestically sourced table grapes sourced.

Several presentations related to the use of non-*Saccharomyces* to improve wine complexity. *Hanseniaspora vineae*, an apiculate yeast with strong fermentation capability, is being used to provide added complexity to both red and white wines produced under cool temperatures (Francisco Carrau, Universidad de la República, Uruguay). *H. vineae* is thought to ferment and survive in grape musts, because of the presence of a *SSU1*-like transporter (Neža Čadež; Univ.Ljubljana, Slovenia) and 8 copies of alcohol dehydrogenase gene, favouring ethanol production (F. Carrau, Uruguay). Rosalba Lanciotti (Univ. Bologna, Italy) has looked at the potential of *Saccharomyces eubayanus* (used in commercial brewing e.g. Heineken's wild lager beers, H-41, H-32 and H35), for white wine production. *S. eubayanus* was compared to *S. cerevisiae* Vin 13 in chardonnay fermentations (10L; flash pasteurised juice) at 10◦C and 16◦C. Faster fermentation kinetics and increased viability were observed with *S. eubayanus* CBS 12357 compared to Vin13. Whilst no significant differences in alcohol content were found between species, *S. eubayanus* reduced the volatile acidity and free SO_2 content (although total SO_2 was higher). Changes in volatile composition (measured by GCMS) were not reflected in the sensory profile. Of interest is flavour modulation through the use of yeasts normally associated with a particular fermentation, for example, whilst beers are traditionally made using ale and lager yeasts, sake, wine and cider yeasts have also been used to produce new beer styles with distinct and different flavour profiles.

The production of quality wine by spontaneous and mixed-culture fermentations relies on the successful implantation of *S. cerevisiae*, non-*Saccharomyces* and/or bacteria. The understanding of how these microbial partners interact, and their nutrient requirements for growth is important. Javier Alonso-del-Real (IATA-CSIC, Spain) used a model based approach to understand the interactions between *S. cerevisiae* wine strain T73 and *S. kudriavzevii* CR85, allowing the design of co-culture conditions where *S. k.* CR85 was not outcompeted by T73 due to competition for nutrients. Carole Camarasa (INRA, France) also highlighted the importance of understanding nitrogen demand of both non-*Saccharomyces* and *S. cerevisiae*, and to supplement appropriately to prevent poor implantation of *S. cerevisae*, commonly used to complete alcoholic fermentation. Yeast-bacterial interactions

featured as an evolutionary driver in the generation of new improved strains. Florian Bauer (Univ. of Stellenbosch, South Africa) described work on evolving *Lactobacillus plantarum* IWBT B063 in a chemostat through co-culture with yeast strains EC1118, Vin13 or Cross Evolution, whereby the bacteria were successively grown with yeast, removed by filtration and re-inoculated with the same yeast strain. The evolved bacteria were subsequently shown to be faster in growth than the parent. Nerve Zhou (Lund Univ. Sweden) has also used experimental co-evolution, whereby *Lachancea thermotolerans* (a weak-Crabtree yeast, which does not readily produce ethanol under aerobic conditions) was grown in competition with 5 bacterial species of increasing ethanol tolerance. Afterwards, the evolved yeast were observed to undergo fermentation, producing ethanol at levels inhibitory to the bacteria. This ability to undergo aerobic fermentation is considered a Crabtree positive trait, as found in *S. cerevisae*. Competitive co-evolution provides an interesting alternative to ethanol as an evolutionary driver, whereby increasing ethanol concentrations are found to increase mutation rate within a population, through the action of error-prone DNA polymerases rather than DNA polymerase, Pol1 during DNA replication and cell division (Karin Voordeckers, KUL).

Maitreya Dunham (Univ. Washington) spoke on the practise of yeast re-pitching in beer fermentation. Brewers usually re-pitch up to 5-10x, until they notice changes in attenuation or flavour, and the yeasts are discarded and a new culture propagated. Dunham collaborated with craft brewer, who would re-pitch up to 50 times in the process of making 12 beer styles. Dunham sequenced the genomes of the yeast population sampled over 28 re-pitches (a total of 84 generations or doublings). By the 28th pitch, not only were nucleotide changes seen in genes such as BAT1 (involved in branch-chain amino acid synthesis) but also partial chromosomal duplication. Small scale brewers were advised not to pitch more than 10-15 times, given that mutations are present in the population by 10 pitches, whether good, bad or both. Furthermore, serial growth methods like growing out frozen stocks could lead to mutations accumulating, so that after time, one might not have the same strain as originally purchased. Undoubtedly, evolutionary biology is alive and well in breweries and wineries, where yeasts are being adapted to worts, juices and during cold storage.

The abstracts are available on the conference website (https://www.issy34-bariloche.com/wp-content/uploads/2018/09/Book-of-abstracts.x56048.pdf).

Outputs as outlined in the original application

The original proposal was to attend and participate in ISSY34 and the 1st International Brewing Workshop, and present some of the research funded by Wine Australia. Dr Walker gave an oral presentation entitled '*Sulfate transport mutants affect hydrogen sulfide production during alcoholic fermentation'* in Session 8B/ Yeasts in Food and Beverage. Industrial Fermentation. An e-poster entitled '*Investigation of yeast genes required for the modulation of colour in model red wine*' was also given in E-Poster Session 4 (see Appendix 2). The work reported was undertaken as part of completed projects UA1101, UA1302 and UA1405. Both presentations were well received and led to discussion with several researchers on the genetic basis of several fermentation related traits. Hydrogen sulfide and sulfite production remains topical, especially with regards to production of sulfite, and tolerance to sulfite and the link to microbial competition.

E-posters were also given by 2 research collaborators on work undertaken with the Jiranek group which was in part funded by Wine Australia and/or the ARC training Centre for Innovative Wine Production. The first, entitled '*Large scale sulfite response study reveals specific adaptation of B. bruxellensis populations through human-related selection*' was presented in E-Poster Session 1 by Dr Isabelle Masneuf-Pomarede (Univ. Bordeaux). The second entitled '*Oenological phenomes of Lachancea thermotolerans reflect patterns of domestication and allopatric differentiation*' was presented by Dr Marina Bely (Univ. Bordeaux) in E-Poster Session 5. The abstracts can be found in Appendix 2.

Dr Walker gave a short presentation on the key findings to the Jiranek group and provided the abstracts (electronic and printed form), together with some of the slides of the key presentations to supplement the abstracts. In addition to this report, a magazine article will be prepared, detailing the key learning points from the conference programme, should Wine Australia consider it necessary.

Whilst establishing new collaborations was one of the proposed activities, this is not always possible without funding to support the research. Attendance of ISSY34 and the 1st International Brewing Workshop was still beneficial in establishing and reaffirming connections with others in related fields, including Dr Jean-Luc Legras

(INRA, France), Dr Philippe Marullo (Bordeaux University, France) and Professor Steve Oliver (Cambridge University, UK). Phillipe Marullo (Bordeaux) and Professor Steve Oliver (Cambridge University, UK) have had previous PhD collaborations with the Jiranek group. Professor Takagi (NAIST, Japan) who works on proline utilisation and stress tolerance will visit Professor Jiranek in November to discuss potential new collaborations and the hosting of ISSY37 in 2022.

Guidance was sought in relation to NGS analysis from Maitreya Dunham's lab, Washington University), who have extensive experience as evolutionary biologists, working on brewing yeasts. Bioinformatics analysis of sequenced yeast genomes is essential to understanding the genetic basis of improved or novel attributes exhibited by newly evolved strains or non-*Saccharomyces* isolates. Informal discussions with Jessica Noble (Lallemand, Canada) and Jean Luc Legras (INRA, France) were undertaken regarding strain improvement and genetic basis behind nitrogen utilisation respectively. Lallemand have used to classical breeding, mutagenesis and evolutionary engineering approaches to generate new yeasts to the wine and brewing industry. Dr Legras has identified genes involved in N usage, and was interested in our research on fermentation under N limiting conditions. From talking with Drs Noble and Marullo, back-crossing is an essential step, requiring a means to track the alteration, when generating new strains through mutagenesis or adaptive evolution.

Benefit of attendance with regards to personal development

The 2 events provided an excellent opportunity to update current knowledge in wine microbiology, specifically on non-*Saccharomyces* as potential yeast starter cultures in fermentation processes. Dr Walker's current research (UA1708) aims to provide the wine industry with new yeast starter cultures including non-*Saccharomyces*. ISSY34 was a relevant extension, as it brought together academic and industry researchers worldwide, to discuss the latest research linked to biotechnological application, including fermentation technology. Relevant topics included genetic and metabolic improvement of yeasts, industrial application of non-*Saccharomyces*, natural variation, and applied opportunities, to name but a few. In addition, attendance of a workshop targeted at brewing was added value and still relevant; the program being organised immediately after ISSY34 in Bariloche.

Benefit of attendance with regards the Australian Wine Sector

R & D in wine microbiology is an ongoing priority in the wine sector, as a means of improving winery performance through improved yeast and bacterial performance.

The conference provided an opportunity to talk with experts and discuss approaches such as adaptive evolution, genetics including CRISPR gene editing, with reference to non-*Saccharomyces*. The use of non-*Saccharomyces* (with *Saccharomyces* or bacteria) as starter cultures in fermentation and biocontrol agents proved topical, as was the use of adaptive evolution to generate new *Saccharomyces* strains and hybrids. Whole genome sequencing featured in many presentations, not only in terms of evolution of ascomycete yeasts, many of which are fermentative, but also understanding the genome alterations taking place during adaptive evolution in the laboratory.

Conference attendance, especially on an international platform such as ISSY34, not only allows Australia’s wine research sector to be showcased, but is valuable in highlighting how other countries incorporate research and development to address the challenges faced by today’s wine sector. As such, ISSY34 can be considered relevant as an extension and outreach program to Wine Australia's 5 Year Strategic R, D&E Plan 2015-2020.

Appendix 1: Program Schedule

6ta Jornadas Sudamericanas de Biología y Biotecnología de Levaduras (spanish/portuguese)	34° International Specialized Symposium on Yeasts (ISSY34)					1st International Workshop on Brewing Yeasts	
30th Sunday	**1st Monday October**		**2nd Tuesday**	**3rd Wednesday**	**4th Thursday**	**5th Friday**	**6th Saturday**
CRUB - UNComahue	NH Edelweiss, Llao-llao		NH Edelweiss	NH Edelweiss	NH Edelweiss	NH Edelweiss	
						8:15 - 9:00 Registration	
8:30 - 9:00 Registration							
9:00 - 9:30 Opening	8:30 - 16 hs ISSY34 registration at NH Edelweiss.	**Cletus Kurtzman´s** Yeast Taxonomy and systematics workshop: The impact of genomics. 9 - 13 hs. Place: NH Edelweiss Hotel	9:00 - 10:00 Keynote Lecture 1: K. Verstrepen	9:00 - 10:30 Session 5: **Evolutionary genomics and domestication of yeasts**	9:00 - 10:45 Session 8A: **Yeasts in food and beverages. Traditional fermentations**	9:00 - 10:30 Welcome, Lectures Verstrepen, Hittinger and Sampaio	
9:30 - 10:00 Invited speaker Pietro Buzzini							
10:00 - 10:30 Oral Session 1			10:00 -10:30 coffee break	10:30 -11:00 coffee break	10:45 -11:15 coffee break	10:30 -11:00 break/posters	Visiting patagonian Breweries and distilleries 10:00 - 19:30 BERLINA Brewery WESLEY Brewery BLEST Brewery MANUSH Brewery MADOC Distillery
10:30 - 11:00 Coffee break			10:30 - 12:00 Session 1: **Ecology and Biodiversity of yeasts**	11:00 - 12:30 Session 6: **Genetic and metabolic improvement of yeasts**	11:15 - 13:00 Session 8B: **Yeasts in food and beverages. Industrial fermentations**	11:00 - 12:30 Lectures Walker, Powell and Dunham	
11:00 - 12:00 Oral Session 2							
12:00 - 12:30 Invited speaker Marcois Morais			12:00 - 13:00 Lunch	12:30 - 13:30 Lunch		12:30 - 14:00 lunch + Expo	
12:30 - 13:30 Lunch				12:30 - 14:30 ICY Commissioners lunch	13:00 - 14:00 lunch		
13:30 - 14:30 e-posters 1			13:00 - 14:00 e-posters 1	13:30 - 14:30 Sponsored talks			
14:30 - 15:30 Oral Session 3		14:00 - 17:00 h Free transfers from NH Edelweiss with sightseeing stops to LLAO LLAO Hotel (Opening Ceremony)	14:00 - 15:30 Session 2: **Industrial applications of non-conventional yeasts.**	14:30 - 15:00 e-posters 3	14:00 - 15:00 e-posters 5	14:00 - 16:00 Lectures Libkind, Fischborn, Janssens, Thevelein	
15:30 - 16:00 Coffe break			15:30 -16:00 coffee break	15:00 - 16:30 Session 7: **Yeast in Biorefineries**	15:00-15:30 coffee break		
16: 00 - 17:00 e-posters 2			16:00 - 17:00 Session 3: **Natural variation and applied opportunities**		15:30 - 16:30 e-posters 6 + beers	16:00 -16:30 break/posters	
17:00 - 17:45 Oral Session 4			17:00-18:00 Session 4: **Bioprospection of extremophiles**	16:30 -17:00 coffee break	16:30 - 17:30 Keynote Lecture: Larrondo	16:30 - 18:30 Lectures Daran, Gibson, Huztler, and Prahl	
17:45 - 18:15 Invited Speaker Amparo Querol				17:00 - 17:45 Graham Fleet´s Lecture: Schwan	17:30 - 18:30 CLOSING CEREMONY		
18:30 - 20:00 Closure - Drinks		17:00 - 20:00 Opening Ceremony. Location: LLAO LLAO Hotel	18:00 - 19:00 e-posters 2 + beers	17:45 - 18:45 e-posters 4 + beer		18:30 - 19:00 closing ceremony	
				19:30 - 23:00 Conference dinner. BEC	19:00 CRAFT BEER PUB´s TOUR	19:00 Craft Beer´s tour	

Appendix 2: Oral and E-poster Presentations

SULFATE TRANSPORT MUTANTS EFFECT HYDROGEN SULFIDE PRODUCTION DURING ALCOHOLIC FERMENTATION

Michelle E. Walker[1], Jin Zhang[1], Krista Sumby[1], Andrea Lee[1], Anne Houlès[2], Sijing Li[1], Tommaso Watson[1], and Vladimir Jiranek[1]

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Abstract ID: 198
Type: Oral

Hydrogen sulfide is the most prominent volatile sulfur compound (VSC) in wine that causes unfavourable reductive aromas; with H_2S levels directly related to yeast metabolism in response to nitrogen and sulfur availability. In grape juice, sulfate is the most abundant inorganic sulfur compound, which is taken up by yeast through two high-affinity sulfate transporters, Sul1p and Sul2p and a low affinity transporter, Soa1p. Sulfate contributes to H_2S production, through the reduction of sulfate (SO_4^{2-}) to sulfite (SO_3^{2-}) and sulfide (S^{2-}) via the Sulfur Assimilation Pathway (SAP). The SAP is central to the production of the essential sulfur containing amino acids, methionine and cysteine, as well as the antioxidant, glutathione. Yeast strains with limited H_2S production and the ability to preserve desirable thiol derived sensorial characteristics are highly sought after in winemaking. We report on the isolation of six wine yeast strains that produce limited or no H_2S during fermentation. These strains are derived from commercial wine yeast Lalvin EC1118 (E178, E211, E212, E214) and Maurivin Distinction® (D13, D25) on YPD agar plates containing the toxic sulfate analogues potassium dichromate and sodium selenate after treatment with ethyl methanesulfate. The strains were evaluated in 100 mL juice and synthetic grape juice or 10 L juice fermentations and shown to yield no or reduced H_2S and SO_2. Sequencing of the sulfate transporter genes (*SUL1*, *SUL2* and *SOA1*) revealed four identical non-synonymous mutations in the *SUL1* gene in all strains except D25. D13 had an additional mutation, whilst E211 and E214 each had an extra (non-identical) mutation in the *SUL2* gene. These findings allude to the importance of Sul1p in H_2S production and raise the possibility of the mutations being the basis for strain improvement.

INVESTIGATION OF YEAST GENES REQUIRED FOR THE MODULATION OF COLOUR IN MODEL RED WINE

Michelle Walker[1], Tommaso Watson[1], Miguel Roncoroni[2], Chen-Wei Huang[1], Richard Gardner[3] and Vladimir Jiranek[1]

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Abstract ID: 161
Type: e-poster, Session 4

Colour development in red wines is a dynamic process, involving extraction of grape-skin anthocyanins and conversion into stable pigmented polymers. Yeast play a role, producing metabolites, hydrolytic enzymes which have been implicated in colour stabilisation and decolourisation, via pigment adsorption onto the yeast surface. We investigated the genetic basis of colour modulation during fermentation, using a chemically defined grape juice medium (CDGJM) with added grape skin anthocyanins. Two quantitative trait loci (QTLs) were identified in a population of 96 F2 segregants resulting from a cross between two selfed-diploid strains derived from Enoferm M2 and Zymaflore F15. *OYE2* (*YHR179W*), *ROF1* (*YHR177W*) and *HPF1* (*YOL155C*) from chromosomes 8 and 15 respectively, were deleted in the selfed-diploids, M2 ho::Hph and F15 ho::Nat haploids, and the re-constructed F1 hybrid (M2 ho::Hph x F15 ho::Nat). The strains were fermented in 100 mL CDGJM and the colour of the model wines and yeast biomass visually measured. We show that yeast mutants lacking Oye2p (F15Δoye2 and M2Δoye2) produce significantly lighter coloured wines; the degree to which varied between spore progeny. The observed phenotype was independent of pigment adsorption onto yeast cells. The data related to Δrof1 and Δhpf1 is less clear, highlighting the genetic complexity associated with colour development. Further research is needed investigate the mechanism behind wine decolourisation in Δoye2, and whether over-expression would improve colour stability, an attribute associated with wine quality.

LARGE SCALE SULFITE RESPONSE STUDY REVEALS SPECIFIC ADAPTATION OF B. BRUXELLENSIS POPULATIONS THROUGH HUMAN-RELATED SELECTION

Marta Avramova[1,2], Amélie Vallet-Courbin[3], Julie Maupeu[3], Paul Grbin[2], Anthony Borneman[4], Cristian Varela[4], Warren Albertin[1,5], Isabelle Masneuf-Pomarede[1,6].

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Type: e-Poster, Session 1

The yeast species *Brettanomyces bruxellensis* is associated with important economic losses due to red wine spoilage. The most common method to prevent and/or control *B. bruxellensis* spoilage in winemaking is the addition of sulfur dioxide into must and wine. However, recently, it was reported that some *B. bruxellensis* strains could be tolerant to commonly used doses of SO_2. In this work, *B. bruxellensis* response to increasing SO_2 concentrations (from 0 to 0.6 mg.L-1of molecular SO_2 was assessed on 145 isolates representative of the genetic diversity of the species, and from different fermentation niches (roughly 70 % from grape wine fermentation environment, and 30% from beer, ethanol, tequila, kombucha, etc.). Three behaviours in terms of lag phase, maximal growth rate and maximal population were defined: sensitive, tolerant and resistant strains. The relationship between SO_2 tolerance/resistance and genotyping using microsatellite markers was confirmed with high reliability (90%). The detection of both resistant and tolerant growth profiles suggests that *B. bruxellensis* strains have using genetic transformants of sensible and tolerant *B. bruxellensis* strains were performed in presence of sulfites. A contrasted relative fitness of the tolerant strain at high SO_2 concentration (0.4 and 0.6 mg/L) confirmed the previously stated hypothesis that tolerant strains could have a relative selective advantage in the presence of SO_2 compared to sulfite sensitive ones, thus suggesting a specific adaptation to the main antimicrobial used in winemaking. This work contributes to a deeper understanding of this wine spoilage microorganism in means of genetic and phenotypic diversity and sheds light on putative evolutionary strategies for adaptation to human related environment of this non-conventional model yeast species.

OENOLOGICAL PHENOMES OF *LACHANCEA THERMOTOLERANS* REFLECT PATTERNS OF DOMESTICATION AND ALLOPATRIC DIFFERENTAITION

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The yeast *Lachancea thermotolerans* (ex. *Kluyveromyces thermotolerans*) can positively contribute to the acidity and aroma profile of fermented beverages such as wine. This common constituent of the grape/wine microbiota also occupies a range of natural habitats worldwide. The differentiation in the *L. thermotolerans* population is driven by geographic determination and ecological niche, as revealed by our recently-developed 14-microsatellite method. The natural isolates are grouped based on their geographic origin, whereas clustering of anthropic (in particular oenological) isolates suggests domestication events within the species. To determine whether, and to what extent, the strains differ in oenological traits and harbour signatures of domestication and/or local divergence, 94 previously genotyped strains were characterised in *Vitis vinifera* cv. Chardonnay fermentations. The extensive dataset comprised microbial growth and fermentation kinetics parameters, production of primary and secondary metabolites and the resultant (de)acidification, i.e. 114 measured/derived parameters for triplicate fermentations. The common oenological features of *L. thermotolerans* strains were their glucophilicity, relatively extensive fermentation ability (7.3% v/v ethanol), low production of acetic acid and formation of lactic acid. A seven-fold variation was observed in concentration of lactate, significantly affecting the pH of the wines, which ranged between 3.16 - 3.81. An untargeted analysis of volatile compounds revealed that 58 out of 90 volatiles were affected at an *L. thermotolerans* strain level. Linear discriminant analysis performed using the obtained metabolic dataset showed the separation of *L. thermotolerans* genetic groups driven by distinct fermentation performance and production of (non)-volatile metabolites. Together, these results provided a population-wide insight into the extent of phenotypic variability in oenologically-relevant traits in *L. thermotolerans*, whilst adding support for the occurrence of domestication events and allopatric differentiation within this remarkable yeast species.

Appendix 3: Map of Brewery and Distillery Tour



Conference venue: Hotel NH Edelweiss, Conference Opening Ceremony: Hotel Llao Llao, Bus tour: Cerro Campanario, Punto Panoramico, Blest, Wesley, Berlina and Manush Breweries and Madoc Distillery