Attendance and presentation at the 14th International Congress on Yeasts in Awaji Island, Japan, 11-15 September 2016 (“Yeasts for Global Happiness”)

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Abstract

An AGWA travel award enabled the attendance of Dr Michelle Walker at the 14th International Congress on Yeasts held at Awaji Yumebutai, Hyogo Prefecture in Japan (11-15 September 2016). This congress, held every four years, attracted some 440 academic and industry delegates from about 40 countries, and included several reputable international speakers. The conference explored different yeast(s) and their genetics, synthetic biology and their application in the production of alcoholic beverages, and biotechnology (biofuel/high value products). The conference provided opportunities to meet informally with other researchers in related areas of yeast research. Student participation was actively encouraged through sponsorship by the conference organisers and The Carl Singer Foundation. Students awarded scholarships gave short talks (as well as poster presentations) on their research.

An oral presentation (and poster) entitled ‘Mapping of genes responsible for yeast-derived modulation of colour in model red wine’ was given. A visit was undertaken to the research laboratories of Professor Hiroshiri Takagi (Nara Institute of Science and Technology). His research on how proline protects yeast from alcoholic stress during fermentation is of particular interest, as improved use of this nitrogen source in juice has been a long standing interest of our group.

Executive Summary

- Dr Michelle Walker travelled to Awaji Yumebutai in Hyogo Prefecture in Japan to attend the 14th International Congress on Yeast (ICY14) with Mr Chen-Wei (Max) Huang and Ms Eelin Tek (PhD students in the Jiranek group)
- A 12 minute oral presentation was given on ‘The mapping of genes responsible for yeast colour modulation of a model red wine” (see Appendix 1 - Presentation abstract)
- A poster presentation was given on the same research topic as the oral presentation
- The meeting consisted of 10 concurrent sessions, five workshops incorporating 11 plenary lectures, one keynote lecture and five short talk sessions. Topics included medically relevant yeast, industrial yeast and their applications (including Asian alcoholic beverages, and wine), systems/synthetic biology, yeast biology, stress response and adaption, ecology and taxonomy of non-conventional yeast, as well as lab automation and imaging technology.
- Twelve reputable international speakers presented on topics of academic interest,
some of which were relevant to wine microbiology: genome engineering to decipher genome function and use in breeding, genomics of stress adaptation and tolerance, yeast as a model for ecology and evolution, application of metabolic engineering in bio-refineries. Those of relevance to wine fermentation are discussed.

- Discussions were held with several prominent yeast researchers on topics related to activities of Jiranek group, including new paradigms and opportunities for further strain optimisation.
- Attendance at a satellite workshop on non-conventional yeast, during a one day visit to the Nara Institute of Science and Technology (NAIST). Potential future research collaborations with Professor Hiroshi Tagaki (NAIST) were initiated.

**Key activities and outcomes**

Dr Walker’s attendance at the ICY 14 conference was beneficial in gaining insights into current research related to the production of alcoholic beverages including wine. Topics of particular interest included ecology, looking at the role of insects in yeast(s) population diversity, how spontaneous fermentations, while dynamic in their population diversity, may include yeast which are endemic to a particular wine region; the use of gene and genome editing using the CRISPR Cas9 system in deciphering gen(e)ome function and their potential application in breeding and use in construction of microbial cell factories (for bio-refineries), as well as flavour/aroma modulation through use of non-conventional yeast and new Saccharomyces cerevisiae strains generated by chemical mutagenesis.

Applied research related to Sake production, was featured extensively in the conference. Delegates were tutored on Sake production, different Sake styles and what aroma/flavour descriptors and physical parameters (degree of rice grain polishing) were associated with quality. Delegates were given the opportunity to taste different styles of Sake (a brewed fermentation product) and Awamori (a distilled product, which is unique to Okinawa region of Japan).

**Presentations relevant to yeast and wine fermentation**

Several lectures and posters were devoted to gen(e)ome engineering technologies as a means of deciphering genome function, and their application in future targeted breeding of new and novel strains.

Dr Satoshi Harashima (Sojo University, Kumamoto, Japan) spoke on the development of various genome engineering technologies which allow the simultaneous manipulation of many
genes, rather than traditional breeding and cloning methods which are lengthy, repetitive and require tedious manipulation of many genes to introduce a specific trait in a strain. Emerging methods such as PCR mediated chromosome splitting (PCS), deletion (PCD), duplication (PCDup) incorporating CRISPR Cas9 technology to allow for multiple genome manipulations in a single step, were discussed as means of reorganising the yeast genome to study genome function and construct novel yeast strains which display huge genome and phenotypic diversity. An example was the artificial construction of segmental aneuploids (duplication of chromosomal regions) similar to which naturally occur in industrial yeast, and have been shown to be beneficial to adaptation to stressful environments and therefore useful in biotechnology.

Dr Jef Boeke (NYU Langone Medical Center, New York, USA) and Dr Sakkie Pretorius (Macquarie University, Queensland) gave updates on the synthetic yeast genome, Sc 2.0 project, which aims to replace the endogenous genome with a synthetic one, whereby structural elements such as introns, sub-telomeric regions, and transposons are systematically deleted and strategically placed recombination sites introduced to enable genome scrambling using an ‘inducible evolution system termed SCRaMbLE (Synthetic Chromosome Rearrangement and Modification by Lox P-mediated Evolution)’. This project is being undertaken by a consortium of research groups worldwide including Australia (Macquarie University/AWRI - chr. 14 and 16). The project aims to address fundamental questions such as what is the minimal size of the yeast genome without compromising on viability. Can the yeast genome be reorganised from 16 chromosomes into a single 12MB chromosome without any deleterious effect? To date, 16 chromosomes have been condensed into eight chromosomes, and attempts to reduce the number to four are in progress. In addition to the construction of mosaic genomes (via genome shuffling in heterologous diploids generated between industrial yeasts and strains with single synthetic chromosomes), new ‘neo’ chromosomes, incorporating plant and human pathways, are being constructed in yeast, as models for diseases as well as biotechnology application.
The occurrence of natural interspecific hybrids (and mosaic genomes) was highlighted in several posters, and two talks given by Dr Vivien Measday (University of British Columbia, Canada) at ICY14 and the satellite workshop at NAIST. She spoke on *Saccharomyces cerevisiae* and *S. uvarum* population dynamics and composition in geographically separated vineyards growing Pinot Noir and Pinot Gris (with fermentations conducted in the laboratory), and in spontaneous fermentations conducted in the winery. The studies were undertaken over two vintages in the Okanagan Valley. *MET2* PCR was used to differentiate between the two species (cleavage of *S.c MET2* (by *EcoRI*) and *S.u. MET2* (by *PstI* restriction enzymes) as well as microsatellite typing. The importance of tank sanitation and vessel construction was highlighted. In the case of one winery where concrete egg fermenters were used, Laffort Rosé (a killer strain) was found to dominate several fermentations of Pinot Gris. The strain had not been used in the winery for several years, alluding to the yeast being able to survive in the pore structure of the concrete. The strain was not evident the following vintage, when the fermentations were conducted in stainless steel fermentation vessels which had been properly sanitised.

Overall, winery fermentations were not dominated by commercial strains, but by a diverse number of strains, closely related to them, whereby the strains had undergone ‘loss of heterozygosity’. Commercial and commercial-related yeast were identifiable in vineyards but at a lower frequency (for Pinot Noir). Persistent *S. uvarum* populations were found in the case of Pinot Gris, in both the vineyard and winery (where in the latter, aseptically prepared ‘Pied de cuvè’ starter cultures were also used to inoculate the fermentation). This technique was shown to reduce the commercial and commercially related *S. cerevisiae* populations. Both studies identified distinct indigenous *S. cerevisiae* strains unique to the Okanagan District, which did not identify with 100 ‘typed’ commercial strains, and are of particular interest in terms of marketing the wine as typical of this wine producing region, similar to the case in France and Italy.

The role of insects as vectors was the subject of study of several posters, whereby non-*Saccharomyces* species were identified from insect frass. Dr Duccio Cavalieri (University of Florence, Italy) presented work on yeast-wasp ecology as a model for Evolutionary Systems Biology. When the paper wasp *Polistes dominula* was used as a model, yeast were shown to multiply in the wasp’s gut, and were transferable within the colony from queen to larvae and workers. Furthermore, the wasp gut was conducive to outbreeding of yeast, as Dr Cavalieri was able to show that when *S. paradoxis* and *S. cerevisiae* yeast were fed to wasps, the yeasts
could sporulate and undergo interspecific hybridisation. This research reflects the identification of hybrids between *S. cerevisiae* and *S. bayanus* found in naturally occurring in wasps. Dr Cavalieri alluded to the role of individual wasp species in the geographical separation of distinct populations within the *S. cerevisiae* clade. The importance of native wasps as potential sources of new yeast strains, better suited to undergoing fermentations using grapes with high sugar content, should not be ignored.

Several presentations were given on understanding the mechanisms by which yeast are able to adapt to environmental stress. Dr Hiroshi Tagaki (NAIST) spoke on his group’s research into understanding how cellular proline and arginine content were important in protecting yeast from oxidative stress during bread making. His group has identified the key gene players in yeast, in nitric oxide signalling in reducing reactive oxygen species (ROS) resulting from oxidative stress. Several proline accumulating mutants of baker’s yeast have been constructed as part of this project, using ethyl methane sulfonate and screening on Azetidine-2-carboxylic acid. Similarly, mutants producing leucine, (isolated on 5,5,5-trifluoro-DL-leucine) are in use in the manufacture of Sake, as these mutants produce high quantities of isoamyl acetate (banana aroma) or ‘ginjoko’. Delegates were able to taste Awamori produced from the new strain Hyper Yeast 101, produced by Shinzato Shuzo Brewing Co. EMS mutants are regarded as non-GM yeast, and acceptable by consumers for use in the food/beverage industry.

Dr Shingo Izawa (Kyoto Institute of Technology, Kyoto, Japan) discussed how small heat-shock proteins (*HSP26* and *30*) required for stress adaption were preferentially translated during glucose deprivation and under ethanol stress, when protein synthesis was generally repressed. The gene *BTN2* was shown to be needed only under ethanol stress, whilst *HSP26* was expressed under glucose depletion. *BTN2* is thought to play a role in potentially enhancing cellular activity at the end of fermentation. Both *HSP26* and *BTN2* promoters were strongly expressed under these conditions, and were of potential value for new expression plasmids.

Hiroyuki Yoshimoto (Kirin Company Ltd, Kanagawa, Japan) reported on the phenomenon sugar-induced cell death (SICD) in bottom fermenting yeasts (*S. pastoris, S. bayanus* and *S. cerevisiae/bayanus* naturally occurring hybrids). He demonstrated that when there was insufficient non-sugar nutrients compared to sugar, SICD resulted in cell death due to ROS species generated via impaired glycolysis. Sugar consumption was improved through addition of amino acids. This phenomenon is of particular relevance given that in Japan, beer is not always produced exclusively from malt, but may be substituted with other sugars, because of consumer preference and taxes imposed on beer production. For example, a sparkling beer
made with wine yeast to provide citrus flavour via β-citronellol production, whilst sweetness is derived from unfermented maltose.

Other examples of applied research relevant to the wine industry, included the use of non-*Saccharomyces* yeast in new mixed fruit-grape wines, as healthier alternatives to traditional wine (reduced alcohol with antioxidant properties), and confirmation that in brewing, repeated re-pitching of yeast (after 15 times) quickly led to the generation of small nucleotide polymorphism repeats, rather than large genomic rearrangements, which could have adverse effects on fermentation performance.

**Areas of potential benefit to research funded by AGWA (UA 1302)**

From attending the conference, it is evident that strain improvement requires a holistic approach which combines the knowledge gained from understanding how yeast adapt to different stressful conditions, with various improvement strategies. EMS mutagenesis provides a means to obtain new yeast with improved phenotypes, as was successfully demonstrated in the case of Sake yeast. Increased genetic heterogeneity of the initial yeast strains used in the improvement strategies can be gained from conventional breeding and segregation of resulting progeny, but also through alternative strategies which confine the recombination events in the original F1 hybrid and not the resultant F2 progeny via sporulation (Robine et al. (2007). Genome-Wide Redistribution of Meiotic Double-Strand Breaks in *Saccharomyces cerevisiae*, Mol. Cell. Biol. 27 (5) 1868-1880). The construction of segmental aneuploids in industrial yeast similar to those described by Dr Davis Ng (National University of Singapore) may also be useful, given that yeast aneuploidy is associated with improved fitness in fermentation. The construction of F1 hybrids from meaningful crosses and subsequent tetrad dissection, *cf* the 96 F2 (F15 x M2) sequenced hybrids used to map the yeast genes related to colour (UA1405), similarly would be useful for high throughput screening of fermentation traits such as vigour, sugar utilisation, nitrogen utilisation etc and the identification of useful genetic markers.

Yeast isolates derived from spontaneous fermentations (differentiated by geographical region), as well as yeasts isolated from a variety of ecological niches, including native insects, provide a valuable and under-used resource in the evaluation of potentially useful ‘industry ready’ strains with novel oenological properties. Together, with adaptive evolution strategies, they are valuable in generating ‘tailored’ yeast with multiple desirable oenological traits for specific winemaking needs.
**Conference presentation**

The conference consisted of 10 sessions and five workshops held over five days (11-15 September). A talk was given in one of four concurrent sessions on ‘short talks from poster abstracts’ held on the morning of the final day, which consisted of a 12 minute presentation and three minutes of question time.

**Outputs as outlined in the original application PPA 001659**

The conference proceedings have been provided to the Jiranek research group. Details on presentations related to our current research on a number of topics including adaption to stress during wine and Sake production, yeast dynamics/interactions during spontaneous and co-fermentations as well as yeast ecology (insect/yeast), and use of CRISPR cas9 gene editing will be discussed with individual members working in related fields. In addition to individual reports provided to Wine Australia, an industry publication is to be co-authored by the attendees, identifying the key learning points from the conference program of interest to the Jiranek research group and Australian wine industry. The article will summarise the key points of the individual final reports written for the GWRDC in a 1200-2000 word article to be submitted for publication in the Australian and New Zealand Grapegrower and Winemaker magazine.
Mapping of genes responsible for yeast-derived modulation of colour in model red wine

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Introduction: Colour is the first sensorial attribute perceived by wine consumers and together with flavour and aroma, influences their overall impression of wine quality. Colour development in red wines is a dynamic process, involving the extraction of grape skin anthocyanins and their transformation to stable pigmented polymers; a process influenced by chemical and microbial factors. Yeast metabolites, biomass and hydrolytic enzymes are implicated as modulators of colour development. This study reports on the mapping of genes that affect pigment adsorption to the yeast biomass and subsequent colour of model red wine.

Methodology:
96 F2 progeny, from a genetic cross between two homosporic derivatives of wine yeast Zymaflore F15 and Enoferm M2, were fermented in triplicate using a chemically defined grape juice containing added grape skin polyphenols. Fermentation performance and colour data were quantified and correlated to the annotated 96 genomes, to identify which allelic combinations contributed to fermentation related traits.

Results and Discussion: Two quantitative trait loci (QTLs) located on chromosomes 8 and 15 were identified through correlation analysis of the visual colour differences (by CIElab) observed between the ‘wines’. The same region in chromosome 15 and a minor QTL on chromosome 6 was also correlated with colour differences in pigmented yeast biomass. Work is in progress to further characterise these QTLs, as well as genetically map other measurable fermentation parameters to specific DNA sequences or alleles.

KEYWORDS: Saccharomyces cerevisiae, QTL mapping, wine colour, fermentation