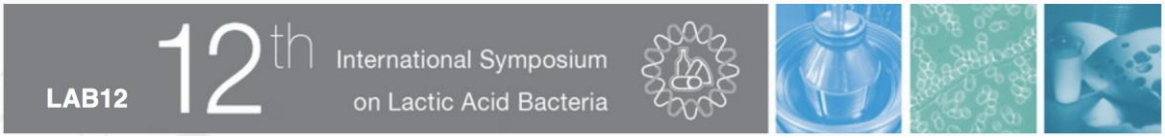
Attendance at the 12th International Symposium on Lactic Acid Bacteria



FINAL REPORT to
Australian Grape and Wine Authority

Project Number: AGT 1611

Principal Investigator: Prof Vladimir Jiranek

Research Organisation: The University of Adelaide

Date: 27 September 2017

Project Title:
Attendance at the 12th International Symposium on Lactic Acid Bacteria.

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Abstract

The 12th International Symposium on Lactic Acid Bacteria was held in Egmond aan Zee, the Netherlands from 27 - 31 August 2017. The conference attracted around 660 participants. The AGWA Travel Grant supported Jiao Jiang to attend this conference to present her current wine lactic acid bacteria (LAB) research through a poster presentation and network with other international researchers working in the food and beverage fields.

Executive Summary

Jiao Jiang is a PhD candidate in the Wine Microbiology and Microbial Biotechnology group at The University of Adelaide, supervised by Professor Vladimir Jiranek. During the conference, Jiao Jiang presented a poster on her current research titled 'Directed evolution of *Oenococcus oeni* for enhanced malolactic fermentation'. The abstract for the poster is included in the Appendix.

The talks and posters during the conference provided overviews of the current development in five scientific fields; microbial communities, genetics and metabolism, fermentation and industrial application, host-microbe interaction, and bacteriophage and antimicrobials.

Seminar presentations of interest

There were three talks about the use of directed evolution (DE) or adaptive evolution (AE) to generate novel strains. This method involves cultivating microbes in the presence of increasing amounts of inhibitory compounds over a period of time, during which stable phenotypes accumulate gradually, making it possible for the selection of improved strains. This type of breeding method is practical and as the strains isolated are not genetically modified, they are more acceptable to the consumers.

Associate Professor Kevin Verstrepen talked about DE of beer yeast strains for improved ethanol tolerance using a continuous culture approach. The procedure was very similar to what has been used in our group; however, his group used microdroplets to do fermentation assays, where fermentation and analysis were scaled down to a droplet, and could be conducted using a robot. This method is less laborious and high-throughput, therefore it would be worthwhile to study if it can be used in the wine industry to evaluate novel yeast or LAB. His group also employed fruit flies to study aromas generated from yeast strains simply

by measuring the density of flies in each ferment. This method is easy to set up and is less expensive than analysis by a sensory panel, and might be worthwhile trying with wine in the future to help select yeast/LAB strains that are able to produce pleasant aromas.

Associate Professor Herwig Bachmann introduced basic knowledge about DE and he also presented several DE examples with LAB. In this talk, he pointed out that the way in which glucose is metabolised could be a constraint for the progress of DE. Slow fermentation of glucose generates acetate and three units of ATP whereas fast fermentation produces lactate but with only two units of ATP. Also, organic acid produced during DE leads to a change in the living environment of the microorganisms, thereby limiting DE. His group showed that one of the ways to break the evolutionary constraints is to evolve microbes in a dynamic environment, which is the approach I have been using in my PhD studies.

Professor Richard Lenski introduced his well-known DE study on *E. coli*, which has been going on for more than 30 years. His group mainly focuses on the dynamics of adaptation by natural selection (e.g. is improvement slow and gradual, or are there periods of rapid change? How long can fitness increase?); repeatability of evolution; integration of phenotypic and genomic evolution. His group set up 12 populations from the same strain, and used a serial transfer approach (1:100 transfer into media with limited glucose daily). However, each population had different mutations, which highlights that reproducibility of DE is low. Therefore, it might be good to set up several DE experiments with the same wine LAB strain to study the important genes in response to stress, as each DE experiment can generate novel strains with different mutations.

Apart from these talks, Professor Bas Teusink gave examples showing that *E. coli* and yeast produced just the right amount of proteins for growth. He also highlighted that for *Lactococcus lactis* generated during DE, the proteome of the evolved strain was the same as the parent. This indicated that the stress response of the DE strains generated in my PhD project cannot be studied using proteome analysis. However, the reason why the evolved strains maintained the same proteome of the parent during DE is unknown and needs further investigation.

Professor Marie-Pierre Chapot-Chartier and Miss Ana Rute Neves gave talks about polysaccharide biosynthesis. Prof Marie-Pierre Chapot-Chartier introduced two new cell wall polysaccharides discovered by her group and the roles of several genes that are involved in the biosynthesis process. CWPS might be involved in cell division and play a role to protect against phage infection. Miss Ana Rute Neves's talk was mainly focused on the biosynthesis of EPS, which could impact the quality of wines. Therefore, knowledge of genes involved in EPS synthesis could help selecting LAB strains with no EPS production simply by sequencing-based method.

The rest of the talks were mainly about bacteriophages and bioinformatics, which was not quite relevant to the wine industry. However, the application of CRISPR/cas 9 in *Lactococcus lactis* introduced by Prof Simon Vanderels could provide a clue on how to use this genome modification tool to study gene functions with *Lactobacillus plantarum*, the genetic profile of which is close to that of *O. oeni*.

Networking

Attendance at the LAB symposium allowed Jiao Jiang to discuss her work with other leading international LAB scientists and representatives from the food and beverage industry. The use of DE to produce multi-stress tolerant *O. oeni* strains from her work attracted much interest from French and Spanish researchers. Senior scientists also gave valuable advice on future science career and fellowship opportunities.

Heineken Experience

Jiao Jiang joined the social program that was organised by the conference committee, which was the visit to the Heineken Experience. Jiao Jiang learned about the history of Heineken (one of the three biggest breweries in the world), beer brewing through a 3D tour, and also tasted beers. This place attracts lots of international tourists, and sells not only beers but also beer-related products, like specially designed beer bottles and backpacks and beers with the visitor's name printed on it. The most fascinating part was the 3D tour where spectators were asked to feel like malts and therefore could experience beer making from fermentation to bottling and consuming. In addition, the display of old brass tanks for beer brewing was also impressive, and attracted a lot of visitors' attention. From touring the Heineken Experience, and experiencing how beer was traditionally made, it is suggested that a similar display could

be made at the National Wine Centre of Australia, to further enhance its attraction to tourists. A similar 3D wine tour would be attractive and knowledge of general winemaking processes could be transferred to visitors in a novel but simple way, which they may never forget.

Laboratory visit

Jiao Jiang also took the opportunity to visit Professor André Uitterlinden's lab in the Erasmus University Medical Centre, Rotterdam. Research in his group focuses on genetic causes of diseases like osteoporosis, diabetes, and affiliated characteristics such as obesity, height and age-at-menopause. This has been studied by genetic analyses in two major cohort studies (with over 12,000 subjects in each). He is also collaborating with many international epidemiological study populations, and has published 680 papers in peer-reviewed journals. From the laboratory visit, Jiao Jiang learned about the usefulness of population genetics. This tool could be used to identify the loci or even the genes involved in grapevine disease resistance or environmental stresses such as drought, and eventually assist the breeding of optimised grapevines.

Conclusion

By attending the 12th International Symposium on LAB, Jiao Jiang showcased the research funded by Wine Australia, at a prestigious international conference, which provided opportunities to potentially attract leading international researchers to collaborate with the Australian wine sector. The new LAB research ideas and skills gained from attending the conference, brewery visit and lab visit will help Jiao Jiang to contribute to the Australian wine industry in the future.

Dissemination of knowledge gained from attendance at the conference

Jiao Jiang has copied the abstract book with abstracts of all the posters for group members to read. She has also shared the interesting talks and posters with her supervisors and colleagues working on either LAB or CRISPR/cas9.

Appendix: Poster Abstract

Directed evolution of *Oenococcus oeni* for enhanced malolactic fermentation in wines

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Oenococcus oeni is the most commonly used lactic acid bacteria to conduct malolactic fermentation (MLF) during winemaking. However, the growth and fermentation of this bacteria is often inhibited by harsh physio-chemical conditions commonly found in wines, such as high ethanol, low pH, sulfur dioxide (SO_2) and medium chain fatty acids. Previous work in our group using directed evolution (DE) with increasing ethanol in a continuous culture generated an ethanol tolerant strain A90 using MRS medium with 20% (v/v) apple juice (MRSAJ). Preliminary characterisation showed A90 was more ethanol tolerant than the parent in MRSAJ but did not show superiority when tested in wine-like conditions. Therefore, DE was used to further enhance multi-stressor tolerance of *O. oeni* to ensure a more efficient MLF. Strain A90 was cultivated in wine-like/wine media with increasing levels of ethanol, acidity, and SO_2 concentration for approximately 350 generations in a continuous culture. Phenotypic changes were observed after approximately 150 generations with continual improvement up to 350 generations. Three superior strains that completed fermentation in the shortest time in wine-like media were selected via high-throughput screens. These strains possessed higher tolerance than A90 to low pH, ethanol, SO_2 and medium chain fatty acids in wine-like media. This highlights the improved multi-stressor tolerance that the evolved strains developed during the course of DE. Additionally, the superior strains fermented faster in various wines. The genomes of the best two strains were sequenced and single nucleotide polymorphisms between these strains and A90 were determined. Gene expression and passaging experiments are ongoing, to gain an insight into the mechanism of improved MLF performance by these *O. oeni* strains in wine and their stability.