Stream 2.1: Optimising fermentation performance to maximise wine production efficiency

1. Abstract:

A major concern for winemakers is the risk of fermentations struggling to start, running very slowly or failing to finish. Hence a key objective of this stream was to address the issue of suboptimal fermentations. Through this research, significant improvements in reliability and efficiency of primary and secondary fermentations can be achieved which bring no additional costs to winemaking. For example, judicious choice of wine yeast strains is critical in low pH juice, and the essential information regarding which wine yeast strains to use in certain problem musts is now available.

Malolactic fermentation (MLF) has been made more robust by the development of co-inoculation strategies where yeast and MLF bacteria are active simultaneously. It was demonstrated that overall fermentation time can be reduced by up to 12 weeks through co-inoculation strategies and that resultant wines have enhanced fruity characters.

The genomes of six wine yeasts, three *Brettanomyces* yeasts, and twelve malolactic bacteria were sequenced. This has provided improved knowledge of the genetics of wine yeast and bacteria. The 'Brett' yeast genomes revealed mechanisms for the evolution of increased sulfite tolerance and will be a critical resource to future-proof the industry against a resurgence of 'Brett'. In addition, a wine yeast gene deletion library (WYGDL) comprising more than 2,500 yeast strains, each with a different gene deleted was created. This resource enables identification of genes that impact on yeast robustness and wine sensory properties; information that is required for rapid screening and breeding of improved strains. Central to the above, the AWRI Culture Collection more than doubled in size to 2,800 strains (not including the WYGDL), and a database was created which enables rapid searching for individual strains and their pertinent features.

2. Executive summary:

A major cost and perennial concern for winemakers is the risk of fermentations struggling to start, running very slowly or failing to finish without remedial intervention. A key objective of this stream was to address the issue of suboptimal fermentations. An initial focus on improving yeast stress tolerance pointed to the importance of finding the right match between grape juice composition and choice of wine yeast. This led to generating a collection of over 100 different Chardonnay juices, which were used to identify factors that are critical for fermentation. It was discovered that different wine yeast strains have different levels of tolerance to limited potassium availability and low pH, and when SO_2 is also considered, some commonly used wine yeast strains are severely compromised.

In addition, it was found that there was little benefit in the use of rehydration nutrients for the mitigation of pH-related fermentation performance issues. These results indicate that choice of yeast strain, and not the addition of nutrient supplements, is more likely to provide a practical solution to problems associated with fermentation of low pH must; there are some wine yeasts that should be avoided when the pH of a juice is very acidic. This knowledge will make a significant contribution to reducing the risk of suboptimal fermentations and provide a strong foundation for future research on optimising fermentation performance and reliability.

Secondary, malolactic, fermentation (MLF) can also be problematic for winemakers; sluggish MLF can extend overall fermentation time by months, increasing operation costs and the risk of spoilage (e.g. by *Brettanomyces* yeast ('Brett'), with associated product downgrade. An inoculation regime was developed in which the malolactic bacterium is added very early, so that MLF and primary fermentation are simultaneous. This strategy, known as co-inoculation, delivers substantial increases in fermentation efficiency; overall malolactic fermentation times were reduced by up to 12 weeks. In addition, co-inoculation produced wines with enhanced fruity volatile composition, thus enabling the modulation of wine style.

Winemakers know that some strains of wine yeast create challenging environments for MLF bacteria; the wrong combination of yeast and bacterial strains will almost certainly compromise the progress of MLF. This issue was addressed by screening, at laboratory-scale, a selection of wine yeast/MLF bacteria combinations. Included in this screen were bacterial strains isolated from Australian wineries, which proved to be particularly robust when grown alongside even the most 'difficult' wine yeasts.

Taking advantage of new technologies in DNA sequencing, the whole genomes of six wine yeasts, three 'Brett' yeasts and twelve malolactic bacteria were sequenced. This has led to a much greater understanding of what is special in these microorganisms. For example, it is now known that there is substantial genetic variation among isolates of the 'Brett' yeast, (*Dekkera bruxellensis)*, from different Australian wineries and some of this is likely to determine variation in levels of sulfite tolerance. The DNA sequence data points to mechanisms involved in the evolution of increased sulfite tolerance and will be a critical resource to future-proof the industry against a resurgence of Brett-derived wine spoilage.

A wine yeast gene deletion library (WYGDL) was generated comprising over 2,500 yeast strains, each with a different gene deleted in the same parental background. This resource is enabling the identification of genes that are important for robustness in wine yeast and genes that impact on wine sensory properties.

Data from screening the WYGDL and wine yeast and bacteria genomic sequencing are now being used to generate genetic markers for wine-relevant traits (e.g. tolerance to acidic pH). This enables rapid screening of the culture collection for candidate strains with desirable properties and will enhance targeted breeding programs to improve on currently available germplasm.

At the core of any microbiological laboratory is the Culture (or germplasm) Collection. The AWRI Culture Collection has more than doubled in size to 2,800 strains (not including the WYGDL) over the past seven years. To help cope with the expanding collection, a database is now in place, with fields that enable rapid searching and access to data and information on strain ID, provenance and other pertinent features. This enables efficient and effective tracking of, and access to the collection. Stringent, internationally recognised quality assurance measures are in place to ensure the identity and reliability of strains in the collection.

Affiliation	**Area of support/contribution**
Australian Genome Research Facility Ltd	Genomic and transcriptomic sequencing support through Wine Yeast Systems biology initiative
Australian Proteome Analysis Facility	Proteomics support through Wine Yeast Systems Biology initiative
Bioplatforms Australia Ltd	Funding for Wine Yeast Systems Biology initiative
Lallemand	MLF trials, comparing strains of *O. Oeni* for impacts on wine sensory
Macquarie University	Proteomics support through Wine Yeast Systems Biology initiative
Queensland University	Fluxomics and mathematical modelling of wine fermentations through Wine Yeast Systems Biology initiative
Stanford University,USA	Comparative genomics of wine yeast
The Yalumba Wine Company	Support through industry trials, viticultural expertise, and provision of samples for Chardonnay juice bank and metagenomics projects
University of Melbourne	Metabolomics support through Wine Yeast Systems Biology initiative
University of New South Wales	Infomatics support through Wine Yeast Systems Biology initiative

Affiliation	Area of support/contribution
University of Toronto, Canada	Provison of knock-out cassettes for construction of Wine Yeast Gene Deletion Library
Victoria University	PhD scholarship for work on yeast stress tolerance
454 Life Sciences, A Roche Company, USA	Support for sequencing seven strains of *Saccharomyces*

3. Background:

A perennial problem for winemakers is the risk of suboptimal fermentations requiring remedial intervention and leading to product downgrade or loss. Costs incurred as a result of this can be considerable, thereby compromising the international competitiveness of Australian wines in the international market. Thus, it is critical to develop strategies, protocols and microbial strains to reduce the incidence of suboptimal wine fermentations.

The reliability and efficiency of wine fermentations depend on grape must quality and composition, and on the robustness and handling of microorganisms used in vinification; suboptimal fermentations are likely to result if any of these are wanting. This stream addressed the problems of suboptimal ethanolic and malolactic fermentations, from a microbiological perspective.

Methods available to wine researchers at the commencement of work in this stream were largely micro and molecular biological, neither of which is well suited to research on complex systems. New methodologies utilitising systems-based approaches were developed due to the high level of biological complexity in a wine fermentation. This required: the generation of wine yeast gene deletion library (WYGDL) which can be screened to identify yeast genes that are important for wine-relevant traits; establishment of genomic sequencing approaches to better understand the genetics (including genetic variation) of wine yeast and bacteria; and a systematic approach to analysing grape juice chemical composition, including the development of a Chardonnay juice bank.

In 2006, there was only rudimentary knowledge of genetic variation in yeast and bacteria that are used in primary and secondary fermentations. Little was known about how this variation interacts with variations in juice composition to determine fermentation outcomes. In addition, there was little known about the impacts of interactions between yeast and bacterial strains on outcomes in MLFs.

This stream addressed all of these matters, and had the following expected outcomes:

- WYGDL, comprising over 2,500 yeast strains each with a different gene deleted in the same parental background, established and methodology available for rapid screening to identify genes that confer desirable attributes.
- Knowledge and strategies for better control of MLF to ensure efficient successful and cost-effective fermentations.
- Enhanced ability to exploit bacteria to promote development of wine sensory properties.
- Availability of robust ethanol stress-tolerant wine yeast strains that will reduce the risk of stuck and/or sluggish ferments.
- A world-class microorganism culture collection that provides a range of strains (some commercially unavailable) that enables industry flexibility to produce wines that respond to market demands.

4. Stream objectives:

The objectives for this stream, as listed in the AWRI 7-year R,D&E Plan were:

- Establishment of a WYGDL and methodology for rapid screening to identify genes that contribute to increased robustness and other desirable attributes.

- Development of bacterial inoculation strategies that will ensure rapid and successful MLFs. This research will also assess the capacity of MLF to impart desirable sensory attributes to wine and thus increase wine value.
- Novel, non-GM wine yeasts will be developed with increased robustness and more resilience when confronted with 'inhospitable' grape must and, therefore, less likely to generate suboptimal fermentations.
- Expansion, provision and maintenance of a world class microorganism culture collection for the Australian wine industry that will enable the rapid identification, storage and recovery of wine microorganisms with a range of defined oenological characteristics.

Changes made to Outputs and Milestones:

- On the completion of the WYGDL, the opportunity existed to extend the scope of yeast genomics activities. Taking advantage of new technologies in DNA sequencing, the genomes of six wine yeasts, three 'Brett' yeasts and twelve malolactic bacteria were fully sequenced; a synthetic biology platform was developed to introduce novel flavor pathways in yeast (this is reported for Stream 1.3); and metagenomics approaches were explored to characterise heterogenous yeast populations in spontaneous fermentations.
- Novel wine yeasts with significantly increased ethanol robustness could not be generated (see Results and Discussion below); because of this, the focus shifted towards identifying factors in grape juice composition that impact negatively on yeast performance. The main objective of reducing the risk of suboptimal fermentation was achieved through the generation of new knowledge about risk factors in combination with benchmarking of yeast performance over a wide range of conditions.

5. Methodology:

To construct the WYGDL, a collection of gene knock-out cassettes (provided by Prof. Charles Boone, University of Toronto) was used to delete each of the non-essential genes in a wine yeast background, creating a collection of yeast strains, each with a different gene deleted in the same genetic background. Standard molecular biology techniques were used and high-throughput technologies were developed to achieve this. All knock-out cassettes were amplified using Polymerase Chain Reaction (PCR), and the cassettes transformed into the parent wine yeast strain using a roboticised liquid handling workstation. Quality assurance measures included a PCR-based approach with primers targeting the inserted knock-out cassette and its flanking region. Confirmed transformants were prepared for storage and placed into the AWRI Culture Collection.

High-throughput, roboticised, analytical techniques were used to screen the WYGDL and other sub-collections of yeast and bacteria in the AWRI Culture Collection. Growth inhibition assays were used to determine, amongst other parameters, yeast ethanol tolerance. High-throughput methods were also developed for biochemical assays such as sugar utilisation in high-throughput, microscale (200 µL) fermentations.

A novel competitive fitness assay of the WYGDL was adopted to identify genes that are important for tolerance to low pH stress. A pooled inoculum carrying all strains in the WYGDL was grown in low pH juice over many generations. Survivors were identified by DNA sequencing of a sample of the population at the end of the experiment; because each strain in the WYGDL has a unique DNA that enables it to be readily identified and quantified by sequencing.

Genome sequencing technologies were used to sequence the genomes of wine bacteria and yeasts. Bioinformatics tools were used for genome assemblies and to perform comparative genomics. A range of systems biology methodologies (e.g. transcriptomic, proteomic and metabolomic analyses) were employed across Streams 1.3 (particularly in development of a low-alcohol wine yeast) and 2.1.

Non-GM, adaptive evolution, was used for the generation of novel yeast strains with increased robustness. This involved growing populations of chemically mutagenised yeast cells in a selective

medium (e.g. a medium with increasing levels of ethanol) over tens or hundreds of generations. Surviving yeast cells were then characterised for phenotypic changes (e.g. increased ethanol tolerance).

Juices collected for the Chardonnay juice bank were analysed using enzymatic and spectral methods to determine common compositional traits (sugar, YAN, TA, pH etc.). Inductively coupled plasma optical emission spectroscopy (ICP-OES) was used to determine elemental composition.

Standard microbiological and oenological methodologies were used to assess MLF performance of bacteria following different inoculation regimes. Growth of bacteria was determined by optical density and viable counting. Chemical (e.g. fermentation product analysis and diacetyl determinations), and sensory analyses, were performed on the wines produced.

Microtitre plate, high throughput assays were used to screen *O. oeni* strains from the AWRI Culture Collection to identify novel robust bacteria that grow in hostile conditions (e.g. with yeast strains that can be inhibitory to MLF) and metabolise malic acid.

Characterisation of flavour-determining enzymes from *O. oeni* was performed using deuterated substrates and standard enzyme assays in cell-free extracts.

Standard microbiological (e.g. biochemical assays) and molecular biology (e.g. DNA fingerprinting) techniques were used for identification of yeast and bacterial strains in the culture collection. All yeast and bacterial strains were prepared for storage at -80ºC, in accordance with standards recommended by the World Federation for Culture Collections.

A purpose-built database with extensive search capabilities was developed to record a broad range of data (e.g. photographs, records of DNA fingerprint gels, descriptions of provenance, including winery or wine region) on strains in the Collection.

6. Results and discussion:

Establishment of a wine yeast gene deletion library (and systems biology platform)

A wine yeast gene deletion library (WYGDL) comprising over 2,500 mutant yeast strains, all derived from the same parent and each with a different gene knocked out, was generated and made available to researchers (Borneman et al. 2007b). This collection is providing a foundational resource for systems biology-based approaches to wine research and is now being applied to investigating the genetic basis of important wine yeast traits including robustness (fitness) and the identification of genes involved in flavour production.

To further develop a systems biology platform for wine yeast research (Borneman 2008c, 2008d), and in parallel with the development of the WYGDL, a wine yeast genome was sequenced; the yeast chosen was the same strain as was used as the parent for construction of the WYGDL. This work was a ‘world first’ and revealed the presence of novel genetic material that is absent from the genome of the laboratory strain of *S. cerevisiae* (Borneman et al. 2008a and 2008b). To better understand what is unique about wine yeasts (compared to non-wine strains of *S. cerevisiae*) comparative genomic techniques were developed and used to map variation among several industrial strains of *S. cerevisiae* from wine, brewing and biofuel industries (Borneman, et al. 2011b and 2012a). Large reservoirs of genetic variation among strains was uncovered. This variation is now being investigated for its influence on wine-relevant traits, and will be important for future development of novel, improved wine yeast strains.

To extend systems-based approaches and enable access to state-of-the-art technologies and methods for AWRI biosciences research, techniques, including genomics, transcriptomics, proteomics and metabolomics approaches were developed (Schmidt et al. 2012b, Borneman, et al. 2011b and 2012a, Herderich et al., 2012) and are now applied across many AWRI projects (see, for example ‘Low-

Ethanol' research in Stream 1.3.). The relevance and importance of this work has been described in a number of research and review papers (Borneman et al. 2007a; Borneman et al. 2012d and 2013a), a book chapter (Borneman et al. 2009) and industry journals (Chambers et al. 2009).

One area of research to benefit from access to systems-based approaches was sequencing the genome of the wine spoilage yeast *Dekkera bruxellensis* (Curtin et al. 2012b). The strain sequenced was found to be an allotriploid hybrid strain (it has three copies of a *Dekkera* genome, two of which are very similar; the other originating from a more distant source) that contains many genes and metabolic pathways not found in *S. cerevisiae*. These pathways provide the metabolic flexibility for this problem species to actively grow in the winery environment and spoil wine. A subsequent multi-strain comparative genomics investigation of *D. bruxellensis* genetic diversity indicated that there are significant differences in the genetic make-up of different *Dekkera* strains, and these differences correlate with wine relevant phenotypes such as sulfite tolerance. A publication describing this has been submitted to a peer reviewed journal (Borneman et al. 2013b), building on the AWRI discovery that some Australian *Dekkera* strains are more sulfite tolerant than others (Curtin et al. 2012a).

Improving MLF: increasing knowledge through systems-based approaches

From a molecular biology perspective, the MLF bacterium *Oenococcus oeni* is one of the most intractable of microorganisms, largely because it is not amenable to many routine protocols used by modern gene technologies. Comparitive genomics provides a means of getting around this limitation; sequencing the genomes of many, diverse strains of this bacterium is enabling its genetic complexity to be unravelled, linking genetic elements to wine-relevant traits.

Genomic analysis of the *Oenococcus* genus, performed at the AWRI (Borneman et al., 2012b), revealed aspects of how *O. oeni* has adapted to its ecological wine niche. For example, it has genes for the metabolism of pentoses and arabinose; sugars that are found in wine at the end of primary fermentation. In contrast *O. kitaharae*, the only other identified *Oenococcus* species, has adapted to its ecological niche, distilled Shoshu residue, carrying genes for example, for maltose utilisation but lacking the genes necessary to grow in wine.

Genetic variation across the *O. oeni* species was revealed, first using a microarray approach (Borneman et al. 2010 and Bartowsky and Borneman 2011c) and then through genomic sequencing of twelve *O. oeni* strains (Borneman et al. 2012c). By having the DNA sequences of such a large number of strains available, it was possible to scope the genetic variation within *O. oeni* (Borneman and Bartowsky 2011a). It was discovered that there is a conserved genomic core common across all strains, and a much larger than previously envisaged pan genome (the pan genome is the whole set of genes across a species). Of particular interest, there was a clear group of Australian *O. oeni* isolates distinct from strains isolated from other parts of the world.

The very large pan genome relative to the core means that there is considerable genetic variation (approximately 30%) across *O. oeni* strains. This large pool of genetic variation has the potential to impact on MLF performance and wine quality parameters. For example, it was observed that there are distinct sub-groups of *O. oeni* with different cell wall polysaccharide, sugar utilisation and β-glycosidase genes indicating the potential for choosing different *O. oeni* strains to modulate wine sensory properties (Borneman et al. 2012c).

Improving MLF: impact of inoculation regime on MLF efficiency and wine sensory characteristics determined

Relative to traditional MLF, which is inoculated post-alcoholic fermentation (AF), co-inoculation of MLF bacteria and yeast was found to reduce overall vinification time and positively influence wine composition, which is reflected in wine sensory characteristics (Bartowsky 2008a; Abrahamse and Bartowsky 2011 and 2012). MLF inoculation regime in Shiraz (winemaking at a laboratory-scale of 1.5 kg and industry-scale with 9 kL ferments) demonstrated that early MLF inoculation leads to more robust MLF and reduces the overall time for vinification by up to 12 weeks (Abrahamse and

Bartowsky, 2012). Concentrations of volatile compounds differed with MLF inoculation regime; co-inoculated or early MLF inoculated Shiraz wines had higher concentrations of fruity volatile compounds, resulting in fruitier wines **(**Abrahamse and Bartowsky, 2011 and 2012; Bartowsky and Abrahamse 2012b**)**. A co-inoculation trial undertaken in Chardonnay had similar outcomes; again the overall vinification time was reduced relative to a sequential inoculation (Bartowsky et al. 2008d).

Compatibility among yeast and bacterial strains is a critical factor impacting on the reliability of MLFs. For example, if a yeast strain produces high concentrations of SO_2 during alcoholic fermentation, the efficiency of MLF might be compromised depending on the robustness of the MLF bacterium (Bartowsky 2011a). Yeast and bacterial compatability screening for MLF robustness and efficiency was performed on a selection of strains from the culture collection. This included several Australian *O. oeni* isolates, which proved to be particularly robust, even in the presence of yeast that had a substantial negative impact on other *O. oeni strains* (Abrahamse and Bartowsky, 2013).

Choice of bacterial strain was shown in many MLF experiments to significantly influence sensory properties of wines (Bartowsky et al. 2008d; Costello et al. 2012a; Cozzolino et al. 2012). Several bacterial strains that enhance production of volatile compounds and impact on wine fruity characters were identified (Bartowsky et. al 2011a). This work demonstrated that choice of bacterial strain has the potential to tailor wines by, for example, modulating buttery (diacetyl) and berry-fruity (ethyl esters) attributes (Bartowsky and Pretorius 2009b).

As an extension of the above, MLF trials were undertaken with several bacterial strains in Cabernet Sauvignon over four vintages. This work demonstrated that MLF can have significant effects on fruity sensory properties. Furthermore, several factors influenced these MLF-induced effects, including choice of bacterial strain, wine composition and grape variety (Bartowsky et al. 2011a, 2011b; 2012a).

In subsequent research aimed at identifying bacterial enzymes involved in flavour development during MLF, two enzyme activities were uncovered (Costello et al. 2012b). One was an acyl co-A: alcohol acyltransferase, the first enzyme of this type to be identified in MLF bacteria. This enzyme, together with a reverse esterase, was found to be responsible for the production of fruity ethyl esters (e.g. ethyl hexanoate).

An alternative MLF bacterium, *Lactobacillus plantarum*, which is already available to winemakers in Europe, was trialed in a Cabernet Sauvignon vintage trial to explore its potential use in Australian red wine production. MLF was completed in a timely fashion and the resultant wine was sound and had appropriate varietal fruity characters (Bartowsky et al. 2012a). This bacterium is now available to Australian winemakers.

It has been known, at least anecdotally, for some time that oak characters can be enhanced by conducting MLF in barrels. This was shown to be the case in controlled experiments where, relative to yeast, MLF bacteria released significantly higher levels of oak-lactone from glycosidic precursors (Bartowsky and Hayasaka 2009a).

Addressing the issue of suboptimal primary fermentations

As ethanol accumulates during fermentation it causes substantial stress for yeast cells and this is thought to be a causative factor in suboptimal fermentations. Thus attempts were made to generate wine yeast strains with high levels of ethanol tolerance (Schmidt et al. 2006). A non-GM, adaptive evolution strategy was trialed, requiring the development of a high throughput screen for the large numbers of yeast isolated from the evolving population (Tran et al. 2012). Ethanol-tolerant wine yeast strains were generated, but increases in tolerance relative to the parental strain were marginal and did not translate into measurable improvements in performance in grape juice. This highlighted the limitation of adaptive evolution as a strategy for yeast strain development and required a change of direction to address the issue of suboptimal fermentations.

The work on ethanol tolerance, together with information from various sources, including data from

the AWRI helpdesk, highlighted a gap in our understanding of the relationship between juice composition, choice of wine yeast and the impacts of these variables on fermentation efficiency and reliability. A study of juice composition and yeast strain response to compositional variation was initiated to fill this gap, as described in the following.

A Chardonnay juice bank, comprising over 100 different juices, was screened for impacts on fermentation performance. From this, and a detailed chemical analysis of the juices, it was found that potassium concentration and pH are critical factors impacting on yeast performance. In addition, different wine yeast strains exhibited different levels of tolerance to limiting potassium and low pH (Schmidt et al. 2011b). These findings have led to improved fermentation management strategies to minimise the risk of suboptimal ferments (Schmidt et al. 2011a). Follow-up work in which 50 commercial wine yeast from the AWRI Culture Collection were screened for tolerance to low pH showed a sizable proportion of strains (greater than 15%) performed less well in low pH juice fermentations (Schmidt 2012a). It has subsequently been shown that pH can interact with SO_2 such that even moderate pH and SO_2 conditions can, in combination, promote a sluggish start to fermentation (Schmidt 2012a).

Screening and performance profiling of juices with varying composition necessitated the development of a small-scale, high-thoughput fermentation platform. Comparing outcomes of fermentation at different volumes, it was shown that growth and relative performance profiles of wine yeast were the same in 0.2 mL, 200 mL and 3000 mL fermentation vessels. Improvements in methodology led to the establishment of 0.2 mL ferments as a standard procedure for screening of yeast and media at the AWRI (Liccioli et al. 2011).

To better understand why different wine yeast strains exhibit different levels of tolerance to low pH requires knowledge of which genes contribute to this trait. To this end, a pooled inoculum carrying all strains in the WYGDL was put through competitive fitness assays (i.e. it was grown in low pH juice over many generations) after which the survivors were identified by DNA sequencing (each strain in the WYGDL has a unique DNA tag that enables it to be identified and quantified). Candidate wine yeast genes contributing to tolerance of low pH were identified (Schmidt et al., 2013a).

Fermentation additives, sold by yeast suppliers, are claimed to reduce the risk of suboptimal fermentations, but little is known about their impacts at low pH. Six products from three suppliers were trialled, individually and in combination, to determine their efficacy in promoting fermentation efficiency in low pH grape juice. It was found that there was little benefit in the use of rehydration nutrients for the mitigation of pH-related fermentation performance issues. As a practical outcome, these data suggest that choice of yeast strain, and not the addition of nutrient supplements, is more likely to provide a practical solution to problems associated with the fermentation of low pH must (Schmidt et al., 2013b).

Experimental work on the impacts of oxygen additions to fermentation efficiencies complement the above work and are reported in Stream 1.3.

Expansion, provision and maintenance of a world class microorganism culture collection

The AWRI manages a yeast and bacterial culture collection for the Australian wine industry and AWRI research projects. Over the past seven years, this collection has grown from 700 to 2000 yeast and from 300 to 800 bacterial strains. These numbers do not include the yeast gene deletion library. The collection serves as a repository of yeast and bacteria for wine companies across Australia and the AWRI is a long standing member of the World Federation for Culture Collections. In addition, it is now listed as a source of microbial strains in the Atlas of Living Australia (www.ala.org.au) (Bartowsky 2008a).

To facilitate efficient and effective management of the collection (e.g. processing the deposition and supply of strains, and managing intellectual property), a database (http://www.awri.com.au/research_and_development/wine-microorganism/) has been developed which lists all strains in the collection and, where available, gives information on their provenance. All newly deposited yeast strains are now identified to genus and species level. All yeast and bacterial strain details are recorded in the database, along with known oenological characteristics. This process, with associated microbiological quality assurance, is essential because yeast and bacterial strains in the collection are available for distribution to the Australian wine industry, oenology teaching courses, and Australian and international research institutions.

The Culture Collection database was designed to include links to data including: (i) microscope images of yeast and bacteria (ii) molecular identification gels (e.g. ITS profiles of the yeast strains), and (iii) publications which use the strain (peer-reviewed and industry journal publications).

The collection is instrumental as a resource for a range of current and future AWRI research projects including characterisation of spoilage microbes such as *D. bruxellensis* and acetic acid bacteria collected from across the Australian wine industry (Bartowsky 2008a). The collection has also been screened to identify yeast other than *Saccharomyces* species which produce wines with low-ethanol content (see report on Stream 1.3).

Non-*O. oeni* wine bacteria, including *Lactobacillus* sp. were screened for growth and MLF efficiency, in synthetic wine media and red wine, using the robotic liquid handling station. Several candidate strains were identified for further evaluation in red and white wine (Moncalvo et al. 2013).

7. Outcome and Conclusion:

Establishment of a wine yeast gene deletion library and systems biology platform

A Wine Yeast Gene Deletion Library (WYGDL) comprising over 2,500 wine yeast strains, each with a different gene knocked out in the same parental background is available to wine researchers across Australia to screen for wine relevant phenotypes. This provides a means of fast tracking research aimed at identifying genes that are important in vinification (fermentation efficiency and reliability) and for wine quality.

High-throughput assays for rapid screening of large collections of microbes are available to wine scientists. These assays are of particular importance in screening phenotypes (traits) for genetic mapping. Two assays that are of particular relevance in the context of wine research include a microvinification assay and a yeast stress tolerance assay.

Data from sequencing the genomes of six wine yeast strains, commonly used in Australian winemaking, was made available, assembled and annotated. This provides a rich resource of information on genetic variation across these strains. The peer reviewed manuscripts describing this work identified and gave insight into several previously unknown genetic elements found in wine yeast but not in laboratory strains. Scientists working on any of these strains are now in a position to utilise these data to inform their experimental work, interpret their data and develop new strains with improved, wine-relevant phenotypes.

The genome sequences of three wine-isolates of *D. bruxellensis* were made available. Data from this work indicate that there is substantial genetic variation among strains which has specific implications to understanding the drivers behind characteristics such as variations in levels of tolerance to sulfite treatment. Evidence suggests *D. bruxellensis* is evolving increased resistance to current treatment strategies (as demonstrated in previous AWRI research). Data generated from this project has already improved our understanding of the mechanisms involved in the evolution of sulfite tolerance and will be a critical resource in the development of future strategies to control ‘Brett’ in the winery.

Improving MLF: increasing knowledge through genetics approaches

Whole-genome sequence data for 12 *O. onei* strains was made available. This rich source of data can be mined to determine the metabolic potential of this bacterium, and to identify strain to strain variation that impacts on wine-relevant traits. As more strains are sequenced, and their wine-relevant traits mapped to their genomes, it will be possible to rapidly screen *O. oeni* strains to isolate variants with combinations of traits that are suited to Australian winemaking conditions and to shape wine style in a targeted fashion.

Improving MLF: impact of inoculation regime on MLF efficiency and wine sensory characteristics determined

The efficiency of MLF has been greatly enhanced by the development of co-inoculation strategies in this stream, which, relative to traditional (sequential) inoculations, can reduce overall fermentation time by up to 12 weeks. In addition, co-inoculation or early-MLF inoculation can enhance fruity volatile composition in finished wines, thus providing the opportunity for winemakers to modulate wine style. Further, from yeast/bacterial compatability studies, several robust Australian isolates of *O. oeni* have been identified.

An AWRI fact sheet titled 'Using malolactic fermentation (MLF) to modulate wine style', has been developed and is distributed on an ongoing basis (http://www.awri.com.au/wp-content/uploads/mlf_modulation_AWRI_fact_sheet.pdf). This fact sheet describes to winemakers the benefits of alcoholic fermentation/MLF co-inoculation; it also explains how the choice of bacterial strain impacts on wine sensory properties such as enhanced buttery and/or fruity characters. The feedback has been very positive and approximately 50 wineries (small, medium and large) have adopted AF/MLF co-inoculation.

Addressing the issue of suboptimal primary fermentations

One of the expected outcomes of this stream was to provide wine yeast strains with increased ethanol tolerance. After numerous attempts, however, this goal was not realised: strains with increased tolerance were generated, but improvements were too marginal to be of value in industrial fermentations. Nonetheless, significant progress was made on the main objective of reducing the risk of stuck and/or sluggish fermentations (which was the driver for developing more robust wine yeast).

Improved knowledge of grape juice composition and its impacts on wine yeast performance led to a revision of the composition for defined medium used in model grape juice fermentations. This important development for wine yeast researchers has enabled improved modelling of problem ferments and is expected to lead to improved strategies to maximise fermentation efficiency and reliability.

Work in this stream also highlighted the potential negative impact of SO_2 at low pH on wine yeast performance. Importantly, the level of sensitivity to such challenging conditions was yeast strain dependent; some commercially available yeast strains proved to be considerably more robust than others. In addition, commercially available nutrient supplements did not enhance yeast tolerance to low pH. Thus, choice of yeast strain, and not the addition of nutrient supplements, is more likely to provide a practical solution to suboptimal fermentation problems associated with low pH must fermentation.

An AWRI webinar was prepared and presented that aims to raise awareness of winemakers to the potential for pH-related fermentation performance issues, and viable solutions (Schmidt 2012).

Data on Chardonnay juice composition for a broad range of Australian Chardonnay, arising from analysis of the Chardonnay juice bank, is available to Australian winemakers and wine scientists (Schmidt et al., 2011b). Through an improved understanding of what constitutes a 'normal' juice composition, this knowledge has been proven very valuable to help winemakers troubleshoot

fermentation performance problems as they arise during vintage.

Expansion, provision and maintenance of a world class microorganism culture collection

The AWRI Culture Collection has more than doubled in size over the past seven years. A database, with fields that enable rapid searching and access to data and information on strain ID, provenance and other pertinent features, is now in place (http://www.awri.com.au/wp-content/uploads/microorganism_culture_collection.pdf). This enables efficient and effective tracking of strains and access to the collection. Stringent quality assurance measures are in place to ensure identity and reliability of strains in the collection.

To date, 594 yeast strains have been identified to species level, with DNA fingerprints that enable strains to be distinguished; in addition approximately 100 bacteria have been identified. This leaves over 1400 yeast and about 800 bacteria to be characterised.

Flyers detailing the AWRI Culture Collection have been provided to winemakers and scientists at various conferences and distributed with industry journals. The flyers explain practical aspects, how to access yeast or bacterial strains in the collection, and how to deposit strains and/or collections of strains for safekeeping. This has led to an increasing number of wineries storing their yeast and bacterial strains in the AWRI Culture Collection.

8. Recommendations:

This stream has delivered world leading research in wine yeast and bacterial genomics, providing a strong foundation to develop genomic maps of wine-relevant traits in strains of *Saccharomyces* and *Oenococcus*. These maps will enable improved and rapid screening of new isolates and strains already available in the AWRI Culture Collection or from other sources, to identify and/or develop (via informed breeding programs), improved wine microorganisms. This will make it possible to deliver robust yeast and bacterial strains that produce predictable, desirable sensory characters in wine. This outcome contributes significantly to the competitiveness of the Australian wine industry by reducing the risk of suboptimal fermentations (see below) and enabling winemakers to more effectively tailor their wines to market segments. To capture the full value of the germplasm held in the Culture Collection, it will be necessary to sequence the genomes of large numbers of wine yeast and bacteria and systematically collect data on their phenotypic traits. Given the reduced cost and enhanced capacity available through next generation sequencing and high through-put phenotyping, this foundational data set could be generated with reasonable resources and in a short time-frame (to be addressed in Projects 3.2.1-3.2.5 in the AWRI's 2013-2018 R,D&E plan).

Work in this stream contributed significantly to improving the reliability and efficiency of primary fermentations. Whilst the importance of pH to fermentation efficiency has long been appreciated, it was not realised how much varation in tolerance to low pH exists across wine yeast strains, or the degree to which pH and SO_2 interact negatively on progress of fermentation. This presents an opportunity for future research into robustness of wine yeast strains with the aim of making improved strains available to winemakers. While it is unlikey that this can be achieved by adaptive evolution strategies, combining whole genome sequencing with characterising 'robust' phenotypes provides a promising strategy. Based on methodolgies developed in this stream, genetic markers for tolerance of low pH (along with other stressors such as SO_2 and ethanol) can be identified. These markers would provide the means to screen yeast collections for 'fittest' candidates that can be trialled immediately and used as breeding stock. Ideally, this approach would be complemented by research into the genetics of flavour production, enabling the generation of robust and 'flavour-active' yeast strains which are tailored to distinctive juice composition and/or products (to be addressed in Project 3.2.1 in the AWRI's 2013-2018 R,D&E plan).

It is also important to note that whilst there has been much communication and interaction with winemakers (via AWITC, webinars, roadshows and publications) about the important findings on

variation in wine yeast robustness, much of the work leading to these discoveries was performed over the last two to three years of the project. Thus, there are still opportunities and unlocked potential to be realised from on-going dissemination of the outcomes to the industry. In particular, it will be important to inform winemakers about which available wine yeast strains are most robust in the face of low pH must; judicious choice of yeast strain is a cost neutral way of decreasing the risk of a suboptimal fermentation (to be addressed in Projects 4.1.1, 4.1.3 and 4.1.4 in the AWRI's 2013-2018 R,D&E plan).

The AWRI research and development on secondary fermentation regimes, which demonstrated the efficacy and robustness of AF/MLF co-inoculation, has led to significant adoption of this strategy by Australian producers. However, most research and uptake has been in the area of red wine production, leaving MLF strategies in white wine vinification as an opportunity for the future. There are many challenges in white wine progressing through MLF and early indications suggest that co-inoculation will prove to be particularly beneficial (to be addressed in Project 3.2.4 in the AWRI's 2013-2018 R,D&E plan).

An area that remains relatively unexplored is the reliability and impact of MLF in sparkling base wine production, because of the efficacy in co-inoculation in 'difficult' juices there is clearly an opportunity to extend studies from this Stream to sparkling wines (to be addressed in Project 3.2.4 of the AWRI's 2013-2018 R,D&E plan).

With three strains of *D. bruxellensis* now sequenced and knowledge gained of phenotypic variation in wine-relevant traits (e.g. SO_2 tolerance), there is a solid foundation to develop new knowledge that will enable future-proofing of the wine industry against a resurgence of Brett in Australian wine production. To realise this potential, further work is required to determine the molecular basis of tolerance and sensitivity of *D. bruxellensis* to SO_2. One approach to this is to evolve new strains of this yeast in a 'high' SO_2 environment, then to sequence the genome of strains with increased tolerance to determine how they have adapted to this stress. This will enable the development of markers to identify problem strains as they arise in the industry and provide a tool for developing alternative strategies to combat Brett (to be addressed in Project 3.5.2 of the AWRI's 2013-2018 R,D&E plan).

9. Budget reconciliation:

Financial Year	Receipts / Income ❶	Outgoings / Expenditure ❷
Year 1: 2006/2007	$899,815	$899,815
Year 2: 2007/2008	$887,736	$887,736
Year 3: 2008/2009	$1,044,342	$1,044,342
Year 4: 2009/2010	$1,206,355	$1,206,355
Year 5: 2010/2011	$1,090,540	$1,090,540
Year 6: 2011/2012	$870,127	$870,127
Year 7: 2012/2013	$808,908	$808,908
TOTAL	$6,807,823	$6,807,823

❶ Note that the GWRDC – AWRI Investment Agreement budget was established and approved at an aggregate level, with variances to budget (i.e. annual overspends and underspends) reported and considered at that same aggregate (i.e. whole of agreement) level. The receipts / income relating to a Stream for any year therefore equate to the outgoings / expenditure within that Stream for that year, as any variances between total Investment Agreement funding received and total funds expended were considered at the whole of Agreement rather than individual Stream level.

❷ Includes a pro-rated share of Theme 5 *Executive management and administration*.

I hereby certify that this statement is true and accurate.

Signature of duly authorised representative.....

Chris Day — Group Manager – Corporate Services — 29/11/2013

Name: *Title:* *Date:*

10. References:

Abrahamse, C.E., Bartowsky, E.J. (2011) Timing of malolactic fermentation inoculation in Shiraz grape must and wine: Influence on chemical composition. World Journal of Microbiology & Biotechnology 28: 255-265.

Abrahamase, C.E., Bartowsky, E.J. (2012) Inoculation for MLF reduces overall vinification time: New research based on laboratory and winery trials in Shiraz delivers potential time and cost benefits. The Australian & New Zealand Grapegrower & Winemaker 578: 41-46.

Abrahamse, C.E., Bartowsky, E.J. (2013) Development of a micro-scale microbiological screen for compatibility of yeast and bacterial strains in MLF. The 15th Australian Wine Industry Technical Conference, Sydney, New South Wales. The Australian Wine Industry Technical Conference Inc., Adelaide, South Australia (see appendix 1). *Unpublished material –information available on request.*

AWRI's new R,D&E plan 2013-2018, http://www.awri.com.au/about_the_awri/rde-plan/

Bartowsky, E.J. (2008a) The AWRI wine microorganism culture collection: a treasure trove of yeast and bacteria strains for the future. The Australian & New Zealand Grapegrower & Winemaker 537: 101-103.

Bartowsky, E.J. (2011a) Chapter 16: Malolactic Fermentation. Joshi, V.K. (ed) Hand Book of Enology: Principles, Practices and Recent Innovations. Haworth Press Inc., New York. pp 526-563.

Bartowsky, E., Abrahamse, C. (2012b) Early inoculation for MLF can reduce overall vinification time: laboratory trials in Shiraz. Technical Review 197: 15-20.

Bartowsky, E.J., Borneman, A.R. (2011c) Genomic variations of *Oenococcus oeni* strains and the potential to impact on malolactic fermentation and aroma compounds in wine. Applied Microbiology and Biotechnology 92: 441-447.

Bartowsky, E., Costello, P., McCarthy, J. (2008a) MLF – Adding an 'extra dimension' to wine flavour and quality. The Australian & New Zealand Grapegrower & Winemaker, Annual Technical Issue 533a, 60-65.

Bartowsky, E., Costello, P., Krieger-Weber, S., Markides, A., Francis, L.,d Travis, B. (2011b) Influence of MLF on the fruity characters of red wine: bringing chemistry and sensory science together. Wine & Viticulture Journal 26: 27-33.

Bartowsky, E.J., Costello, P.J., Francis, L., Travis, B., Krieger-Weber, S., Markides, A. (2012a) Influence of *Oenococcus oeni* and *Lactobacillus plantarum* on the ester composition of Australian Cabernet Sauvignon. Practical Winery & Vineyard Journal. Spring, 57-59.

Bartowsky, E.J., Pretorius, I.S. (2009b) Chapter 11. Microbial formation and modification of flavour and off-flavor compounds in wine. König, H., Unden, G., Fröhlich, G. (eds) Biology of microorganisms on grapes, in must and wine, Springer, Heidelberg. pp 211-233.

Bartowsky, E.J., Hayasaka, Y. (2009a) Bacteria's role in shaping oak character. Wine Business Magazine December/January, 56-59.

Borneman, A., Forgan, A., Bellon, J.R., Ryan, O., Boone, C., Chambers, P.J. (2007b) Creation of a wine yeast genome deletion library for the discovery of yeast strains with desirable winemaking characteristics. Blair, R.J., Williams, P., Pretorius, I.S. (eds.) The 13th Australian Wine Industry Technical Conference, Adelaide, South Australia. The Australian Wine Industry Technical Conference Inc., Adelaide, South Australia: 269.

Borneman, A.R. (2008c) The potential of systems biology in winemaking. Technical Review 173:13-21.

Borneman, A.R. (2008d) The potential of systems biology for winemaking. Blair, R.J., Williams, P.J. and Pretorius, I.S. (eds) Proceedings of the thirteenth Australian Wine Industry Technical Conference, Adelaide, South Australia. Australian Wine Industry Technical Conference Inc., Adelaide, South Australia: 227-230.

Borneman, A.R., Bartowsky, E.J. (2011a) Comparative genomics in the wine bacterium *Oenococcus oeni*. Microbiology Australia 32: 174-176.

Borneman, A.R., Chambers, P.J., Pretorius, I.S. (2007a) Yeast systems biology: modelling the winemaker's art. Trends in Biotechnology 25: 349-355.

Borneman, A.R., Forgan, A.H., Pretorius, I.S., Chambers, P.J. (2008a) Comparative genome analysis of a *Saccharomyces cerevisiae* wine strain. FEMS Yeast Research 8: 1185-1195.

Borneman, A.R., Forgan, A.H., Pretorius, I.S., Chambers, P.J. (2008b) Unraveling the genetic blueprint of wine yeast. *Aust. New Zealand Wine Industry Journal* 23: 26-28

Borneman, A.R., Chambers, P.J., Pretorius, I.S. (2009) Systems biology as a platform for wine yeast strain development. König, H., Unden, G., Fröhlich, J. (eds) Biology of microorganisms on grapes, in must and wine. Springer, Heidelberg. pp 399-418.

Borneman, A.R., Bartowsky, E.J., McCarthy, J.M., Chambers, P.J. (2010) Genotypic diversity in *Oenococcus oeni* by high-density microarray comparative genome hybridization and whole genome sequencing. Applied Microbiology and Biotechnology 86 (2): 681-691

Borneman, A.R., Desany B.A., Riches D., Affourtit J.P., Forgan A.H., Pretorius I.S., Egholm M., Chambers P.J. (2011b) Whole-genome comparison reveals novel genetic elements that characterize the genome of industrial strains of *Saccharomyces cerevisiae*. PLoS Genetics 7: 1-10 e1001287.

Borneman, A.R., Desany, B.A., Riches, D., Affourtit, J.P., Forgan, A.H., Pretorius, I.S., Egholm, M., Chambers, P.J. (2012a). The genome sequence of the wine yeast VIN7 reveals an allotriploid hybrid genome with *Saccharomyces cerevisiae* and *Saccharomyces kudriavzevii* origins. FEMS Yeast Research 12: 88-96.

Borneman, A.R., McCarthy, J.M., Chambers, P.J., Bartowsky E.J. (2012b) Functional divergence in the genus *Oenococcus* as predicted by genome sequencing of the newly-described species, *Oenococcus kitaharae*. PLoS ONE 7: 1-10 e29626.

Borneman, A.R., McCarthy, J.M., Chambers, P.J., Bartowsky, E.J. (2012c) Comparative analysis of the *Oenococcus oeni* pan genome reveals genetic diversity in industrially-relevant pathways. BMC Genomics, 13: 373.

Borneman, A.R., Pretorius, I.S., Chambers, P.J. (2012d) Comparative genomics: a revolutionary tool for wine yeast strain development. Current Opinion in Biotechnology DOI: 10.1016/j.copbio.2012.08.006

Borneman, A.R., Schmidt, S.A., Pretorius, I.S. (2013a) At the cutting-edge of grape and wine biotechnology. Trends in Genetics DOI: 10.1016/j.tig.2012.10.014.

Borneman, A.R., Zeppel, R., Chambers, P.J., Curtin, C.D. (2013b) Insights into the *Dekkera bruxellensis* genomic landscape: Comparative genomics reveals variation in ploidy and nutrient utilization potential amongst wine isolates. PLOS Genetics. *Manuscript submitted for publication –information available on request.*

Chambers, P.J., Borneman, A.R., Schmidt, S., Hack, J.C., Varela, C., Mercurio, M., Curtin, C.D., Cozzolino, D., Ugliano, M., Herderich, M.J., Pretorius I.S. (2009) The dawn of a new paradigm for wine yeast strain development. Australian & New Zealand Wine Industry Journal 24: 16-18.

Costello, P., Francis, I.L., Bartowsky, E. (2012a) Variations in the effects of malolactic fermentation on the chemical and sensory properties of Cabernet Sauvignon wine: interactive influences of *Oenococcus oeni* strain and wine matrix composition. Australian Journal of Grape and Wine Research 18: 287-301.

Costello, P.J,, Siebert, T., Solomon, M., Bartowsky, E.J. (2012b) Synthesis of fruity ethyl esters by acyl coenzyme A: alcohol acyltransferase and reverse esterase activities in *Oenococcus oeni* and *Lactobacillus plantarum*. Journal of Applied Microbiology 114: 797-806.

Cozzolino, D., McCarthy, J.M., Bartowsky, E.J. (2012) Comparison of near infrared and mid infrared spectroscopy to discriminate between different *Oenococcus oeni* strains following malolactic fermentation in red and white wines. Food Control 26: 81-87.

Curtin, C.D., Borneman, A.R., Chambers, P.J., Pretorius, I.S. (2012b) De-novo assembly and analysis of the heterozygous triploid genome of the wine spoilage yeast *Dekkera bruxellensis* AWRI1499. PLoS ONE 7: 1-10 e33840.

Curtin, C.D., Kennedy, E., Henschke, P.A. (2012a) Genotype-dependent sulphite tolerance of Australian *Dekkera* (*Brettanomyces*) *bruxellensis* wine isolates. Letters in Applied Microbiology 55: 56–61.

Herderich, M.J., Curtin, C., Hayasaka, Y., Mercurio, M. (2012) Metabolomics and the quest for understanding wine quality. Oeno2011- Actes de colloques du 9e symposium international d'oenologie de Bordeaux, Dunod, Paris, 609-614.

Liccioli, T., Tran, T.M.T., Cozzolino, D., Jiranek, V., Chambers, P.J., Schmidt, S.A. (2011) Microvinification—how small can we go? Applied Microbiology and Biotechnology 89: 1621–1628.

Moncalvo, A., Abrahamse, C., Bartowsky, E. (2013) Screening of Australian *Lactobacillus* strains for wine stress tolerance and MLF performance. The 15th Australian Wine Industry Technical Conference, Sydney, New South Wales. The Australian Wine Industry Technical Conference Inc., Adelaide, South Australia *Unpublished material –information available on request.*

Schmidt, S.A., Tran, T.M.T., Chambers, P.J., Herderich, M.J., Pretorius, I.S. (2006) Developing indicators of wine yeast performance: an overview of the impact of ethanol stress. Australian & New Zealand Wine Industry Journal 21: 24–30.

Schmidt, S.A., Dillon, S., Kolouchova, R., Henschke, P.A., Borneman, A.R., Forgan, A., Chambers, P.J., Pretorius, I.S. (2011a) Grape juice and wine yeast: happy marriages and how to avoid getting stuck with the wrong partner. Wine & Viticulture Journal July/Aug: 30–34.

Schmidt, S.A., Dillon, S., Kolouchova, R., Henschke, P.A., Chambers, P.J. (2011b) Impacts of variations in elemental nutrient concentration of Chardonnay musts on *Saccharomyces cerevisiae* fermentation kinetics and wine composition. Applied Microbiology and Biotechnology 91: 365-375.

Schmidt, S.A., Jacob, S.S., Ahn, S.B., Rupasinghe, T., Krömer, J.O., Khan, A., Varela, C., (2012b) Two strings to the systems biology bow: co-extracting the metabolome and proteome of yeast. Metabolomics DOI:10.1007/s11306-012-0437-1: 1-16.

Schmidt, S.A. (2012a) Winemaking at low pH: avoiding stuck fermentations in whites and sparkling wines [Webinar]. Retrieved from https://attendee.gotowebinar.com/recording/210872382867568128.

Schmidt, S.A., Li, J., Kolouchova, R., Borneman, A.R., Henschke, P.A., Chambers, P.J. (2013a) Understanding the genetic basis of tolerance and sensitivity to low pH in wine yeast. The 15th Australian Wine Industry Technical Conference, Sydney, New South Wales. The Australian Wine Industry Technical Conference Inc., Adelaide, South Australia. *Unpublished material –information available on request.*

Schmidt, S.A., Kolouchova, R., Henschke, P.A., Chambers, P.J. (2013b) Management of fermentation performance in low pH juices – Can fermentation nutrient additives help? The 15th Australian Wine Industry Technical Conference, Sydney, New South Wales. The Australian Wine Industry Technical Conference Inc., Adelaide, South Australia. *Unpublished material –information available on request.*

Tran, T.M.T., Stanley, G.A., Chambers, P.J., Schmidt, S.A. (2012) A rapid, high-throughput method for quantitative determination of ethanol tolerance in *Saccharomyces cerevisiae*. Annals of Microbiology 10.1007/s13213-012-0518-4: 1-8

Contact:
Communication Manager
Tel: (08) 8313 6600