Technical notes

Case study: development of *Saccharomyces cerevisiae* × *Saccharomyces mikatae* wine yeast hybrids and their potential to deliver alternative wine styles

Introduction

Winemakers wanting to differentiate their wines have a number of options available, one of which is choice of yeast. Many wineries that use large-scale fermentation vessels for wine production rely on inoculation of a proven robust, industrial yeast strain to provide reliability and consistency to their winemaking. While this process minimises the risks both of stuck/sluggish ferments and the proliferation of spoilage microorganisms, the wines produced can have a relatively simple flavour and aroma profile. Conversely, terms such as 'complexity' and 'rounded palate structure' are more often attributed to wines produced by wild or spontaneous fermentations, where a wide range of microorganisms all contribute metabolites to form a diverse matrix of flavour-active components.

One approach to mimic the benefits of spontaneous fermentations is to inoculate with multiple *Saccharomyces cerevisiae* and non-*Saccharomyces* strains. However, this tactic can often be unpredictable due to variability in strain growth and fitness level in the inhospitable environment of wine fermentations. In an endeavour to widen the range of flavour-active metabolites produced by a single industrial yeast strain, new breeds of yeast have been developed that incorporate the genomes of multiple species. These are known as interspecific hybrid yeast.

Hybrid yeast are not genetically modified organisms, instead they are generated using natural yeast mating techniques. The mating events are rare but make use of the innate capacity of different groups of yeast to recognise each other through hormones that they produce. Using these methods, hybrids have been produced between a number of different genetically divergent *Saccharomyces* species and a robust industrial *S. cerevisiae* wine yeast. The resultant hybrid yeasts introduce a much greater genetic diversity into winemaking practice. They produce a wider range of flavour-active metabolites than their *S. cerevisiae* wine yeast parent and consequently add complexity to wine flavour and aroma profiles.

In a bid to produce wines with novel flavour-active metabolites, hybrids were generated between a robust industrial *S. cerevisiae* strain (AWRI 838) and a distant *Saccharomyces* species, *Saccharomyces mikatae*, that has not previously been associated with fermentation industries. With a 75% genome sequence match, the evolutionary distance between *S. cerevisiae* and *S. mikatae* is considerable, similar to that between a human and a mouse!

Screening fermentation properties of hybrid strains

Five hybrid progeny were assessed for fermentation proficiency in Chardonnay juice (250 g/L reducing sugars, 227 mg/L yeast assimilable nitrogen, pH 3.0). The resultant wines were characterised by analysis of basic wine chemistry and flavour-active fermentation compounds. All five hybrid strains completed fermentation within 15 days, with little difference in overall duration of fermentation between the S. *cerevisiae* parent and the hybrid strains (Figure 1).

However, growth profiles within the first four days following inoculation differentiated some of the hybrids (Figure 2). The wine yeast parent strain and two hybrid strains (CXM1 and CXM4) showed strong growth within 48 hours of inoculation. Three of the five hybrids found the stresses challenging and showed an extended lag-phase after inoculation, with no discernible growth until the third (CXM2 and CXM3) or fourth (CXM5) day. No

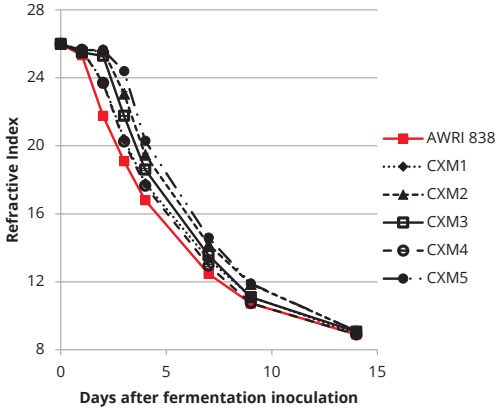

Figure 1. Sugar utilisation by wine yeast parent (AWRI 838) and hybrid strains during Chardonnay juice fermentation

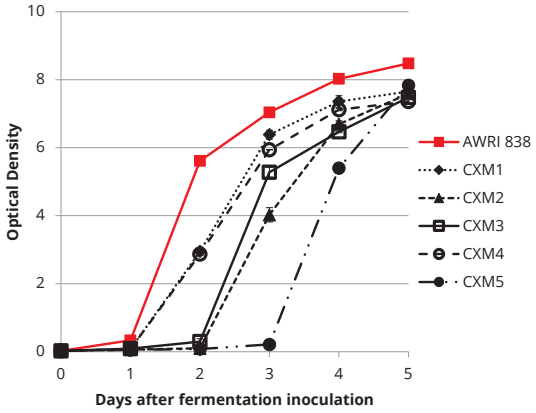

Figure 2. Cell growth by wine yeast parent (AWRI 838) and hybrid strains during Chardonnay juice fermentation

fermentation data are shown for the *S. mikatae* parent as this yeast was unable to grow in Chardonnay juice.

Wine chemistry

Analysis of basic wine chemical composition showed that while the hybrid strains produced similar concentrations of ethanol to the wine yeast parent, they produced higher concentrations of glycerol and lower concentration of acetic acid (Table 1). The 'vinegar' aroma of acetic acid is not desirable for winemakers or wine consumers. Wine yeast strains capable of producing wines with higher levels of glycerol and lower levels of acetic acid can therefore contribute to an improvement in wine quality. Chemical analysis also revealed that two hybrid strains were unable to metabolise all sugars in the wine within the duration of the experiment, leaving residual fructose (CXM2 with 4.5 g/L and CXM3 with 2.2 g/L).

Flavour and aroma analysis

Industrial wine yeasts are required to both quickly increase cell numbers to out-compete indigenous (potentially spoilage) microorganisms and completely metabolise all grape sugars. For this reason, only the two hybrid strains with the strongest fermentation properties (CXM1 and CXM4) were targeted for in-depth wine fermentation analyses. Chardonnay wines produced by these two hybrids and the parent wine yeast were analysed for 17 targeted yeast-derived volatile flavour-active metabolites previously determined to be important contributors to wine flavour and aroma. Both hybrid strains showed differences in a number of the target compounds analysed relative to the wine yeast parent.

Hybrid strain CXM1 produced higher concentrations of almost all target analytes relative to the wine yeast parent, specifically 'fruity' and 'banana' flavour compounds (ethyl propanoate, ethyl 2-methyl butanoate and 2-methyl butyl acetate). Conversely, hybrid CXM4 produced lower concentrations of almost all target analytes (Figure 3). However, both hybrids produced much higher concentrations of 2-phenyl ethyl acetate ('floral') and much lower concentrations of ethyl acetate ('nail polish') than the parent wine yeast. Increasing the concentration of a

Table 1. Basic wine chemistry of Chardonnay wines produced by wine yeast parent (AWRI 838) and hybrid strains

	AWRI 838	CXM1	CXM2	CXM3	CXM4	CXM5
Glucose (g/L)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Fructose (g/L)	<0.1	<0.1	4.5	2.2	<0.1	<0.1
Ethanol (%)	16.3	16.4	16.1	15.8	16.1	16.4
Glycerol (g/L)	9.6	11.1	11.6	12.1	11.4	12.1
Acetic Acid (g/L)	0.4	<0.1	<0.1	<0.1	0.22	<0.1

flavour or aroma compound can increase the sensory impact of that particular compound, but may also lead to the masking of other flavours or aromas. Conversely, lowering the concentration of a compound, particularly one with a negative sensory attribute such as ethyl acetate, affects not only the compound concerned, but may also un-mask other flavours and aromas.

In addition to targeted analysis of aroma compounds, exploratory analysis of volatiles produced by the hybrids was undertaken. This approach permits the discovery of potentially volatile metabolites resulting from the introduction of non-*cerevisiae* genomic elements.

A total of 30 compounds were identified in the solvent-extractable portion of the Chardonnay wines including ethyl esters, acids, phenols and alcohols. Differences in 18 of these compounds were observed in wines made with the hybrid yeast, such as 2-phenyl ethanol ('rose'), 9-deconoic acid ('fruity'), 3-hydroxy-4-phenyl-2-butanone ('caramel'), 3-methyl thiol propenol ('meat', 'potato') and ethyl-2-hydroxy-3-phenyl propanoate ('smoky') (Figure 4). Interestingly, two compounds produced at higher concentrations by the hybrid strains have previously been shown to be generated in wine in high levels by non-*Saccharomyces cerevisiae* species: isobutyric acid (*Torulaspora delbrueckii)* and 2-phenyl ethyl alcohol (*Kluyveromyces lactis)*. Two compounds produced at higher levels by the hybrid strains remain unidentified.

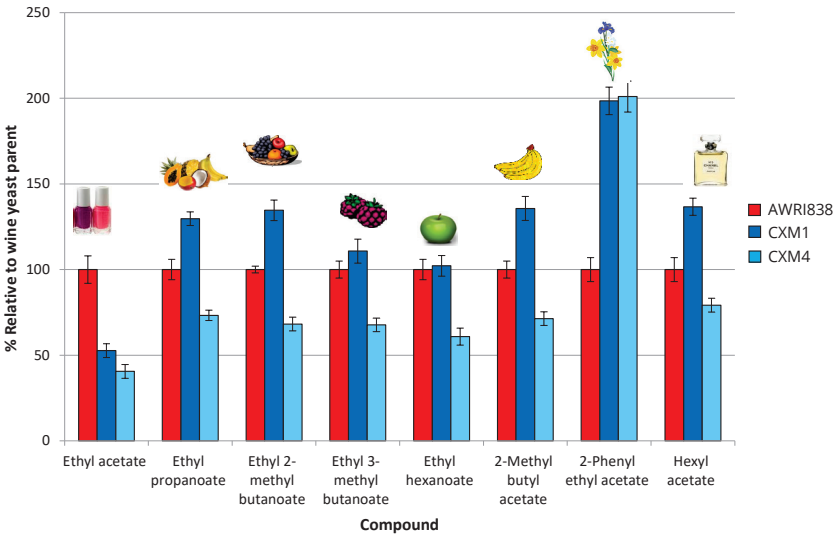

Figure 3. Selected results from targeted analysis of yeast-derived volatile flavour-active metabolites produced by wine yeast parent and hybrid strains

The two unidentified compounds may indicate that the *S. mikatae* parent is contributing novel metabolites, not previously recognised, to wines.

Industry trial

The success of this work led to the inclusion of hybrid strain CXM1 (AWRI 2526) in a tirage trial designed to bring novel flavours and aromas to sparkling wines. Sparkling wines produced using the hybrid at Yalumba, Australia and Gusbourne Estate, Bolney Wine Estate and Plumpton College, UK were showcased at the 2016 International Cool Climate Wine Symposium held in Brighton, UK. Sensory evaluation of Pinot Noir sparkling wines made by AWRI 2526 notes 'attractive rose petal and strawberry nose', 'baked strawberry fruits', biscuit and brioche' and 'rich mouthfeel'. An account by one of the delegates attending the workshop (Sally Easton, Master of Wine) was published online (Easton 2016).

Conclusions

Interspecific hybridisation by natural yeast mating is an important non-genetically modified strategy for developing improved wine yeast for industrial purposes. This work showed that the presence of a *S. mikatae* genome within an existing commercial wine yeast had a favourable impact on the production of flavour-active fermentation metabolites, potentially

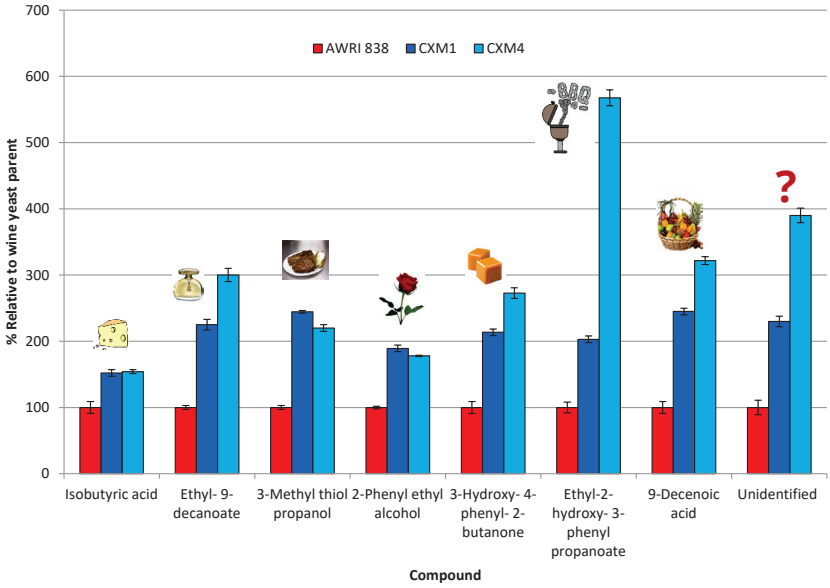

Figure 4. Selected results from exploratory analysis of solvent-extractable volatile compounds produced by wine yeast parent (AWRI 838) and hybrid strains

producing complex wines with characters akin to those of spontaneous ferments. New yeasts that offer the safeguard of an inoculated ferment while providing complexity to wines assist winemakers by delivering additional tools to develop new wine styles.

Yeast manufacturer AB Biotek has commenced exploration of commercial production for AWRI 2526 (a hybrid strain initially created by the AWRI in 2002) and it is anticipated that an active dried yeast product will be available for winemaking trials by the 2020 vintage. Winemakers interested in undertaking a trial in collaboration with the AWRI and AB Biotek can contact Dr Jenny Bellon for further information.

Additional information on the development of *S. cerevisiae* × *S. mikatae* interspecific wine yeast was published in the journal *PLoS ONE* (Bellon et al. 2013).

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