Wine yeast: where are they from and where are we taking them?

By Anthony Borneman, Paul Chambers, Simon Schmidt, Angus Forgan,
Radka Kolouchova, Markus Herderich and Dan Johnson
The Australian Wine Research Institute, PO Box 197, Glen Osmond, South Australia 5064


Managing director
Dan Johnson

Advances in DNA sequencing have allowed analysis of the genomes of large numbers of wine yeast strains for the first time. Results show that wine yeast have low genetic variation compared with the overall genetic diversity of the species Saccharomyces cerevisiae (which also encompasses brewing, baking, sake and palm wine yeasts). This new understanding will be harnessed in future yeast breeding programs.

AT A GLANCE:

- *Saccharomyces cerevisiae* is a species of yeast used by humans in baking, brewing, and winemaking for thousands of years
- AWRI scientists have sequenced the genomes of 212 strains of *Saccharomyces* yeast, including 106 commercially available wine yeasts and 13 brewing strains
- The wine yeast strains were found to have low genetic diversity and high levels of inbreeding
- A number of commercially available yeast strains were found to be genetically identical
- Yeast breeding programs will now be better informed when choosing strains from outside the wine yeast group to introduce greater diversity and novel winemaking characteristics into new wine yeast strains.

WINE YEAST – FROM SPONTANEOUS FERMENTS TO PURE STARTER STRAINS

Humans have been producing and consuming wine for more than 7000 years, making it one of the first processed agricultural products. Until the middle of the 20th century, wine fermentation relied on naturally occurring yeasts. However, spontaneous fermentations often had inconsistent results and, due to their long fermentation times, were vulnerable to spoilage. One of the most significant technological advances in winemaking was the introduction of pure starter strains of *Saccharomyces cerevisiae* in the 1950s and 1960s. Many commercial wine fermentations are now inoculated with these commercial strains to ensure consistent, reliable and reproducible fermentations and there are now hundreds of commercial wine yeast starter cultures available.

Using the latest DNA sequencing technologies, it is now possible to sequence the entire genome of an organism and because yeast genomes are relatively small (1/250th of a human genome), the task can be achieved in a reasonable timeframe. This has allowed scientists at the AWRI and in other laboratories around the world to sequence a large number of yeast strains and start to understand how genetic differences can translate into practical winemaking effects.

Genome sequencing has shown that, in general, strains of *S. cerevisiae* isolated from vineyards and those used in winemaking form a closely related 'family' group (Borneman *et al.* 2011, Fay and Benavides 2005, Liti *et al.* 2009), which also contains yeast isolated from Mediterranean oaks. These latter yeasts are thought to represent the historical ancestor of the 'domesticated' wine yeasts used by winemakers around the world (Almeida *et al.* 2015). If oak trees were indeed the original habitat of the organism that has evolved into *S. cerevisiae* wine yeasts, the part played by this tree in the history of winemaking is even more important than previously thought and began long before oak barrels were used to store wine.

Interestingly, research has shown that strains of *S. cerevisiae* isolated from wineries or vineyards in countries outside of Europe are not related to those countries' indigenous yeast strains, except those isolated from sites close to wineries (Hyma and Fay 2013). This suggests that European wine strains have accompanied the migration of winemaking around the globe and are now maintained as distinct populations (Clowers *et al.* 2015, Fay *et al.* 2004, Warringer *et al.* 2011). Intriguingly, despite their common geographic origins and roles in the production of alcoholic beverages, wine strains are genetically distinct from the *S. cerevisiae* strains used for brewing or sake production (Borneman *et al.* 2011, Dunn *et al.* 2012).

INVESTIGATING THE DIVERSITY OF WINE YEAST

In order to investigate the genetic diversity that has been captured by more than 50 years of commercial wine yeast development, AWRI scientists sequenced the genomes of 212 strains of *Saccharomyces* yeast from the AWRI culture collection (Borneman *et al.* 2016). The strains included 106 commercially available wine yeasts from nine different yeast suppliers and 13 commercially available brewing strains. Despite sourcing a wide variety of wine yeast strains, nearly all were found to reside within a highly inbred group with relatively little genetic variation compared with the global pool of *S. cerevisiae* genetic diversity (Figure 1). The wine yeast group (shown in blue) was found to include a prominent sub-

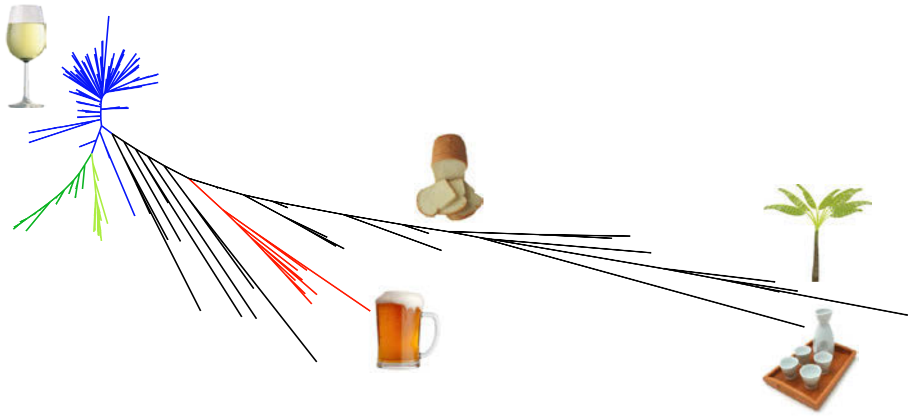
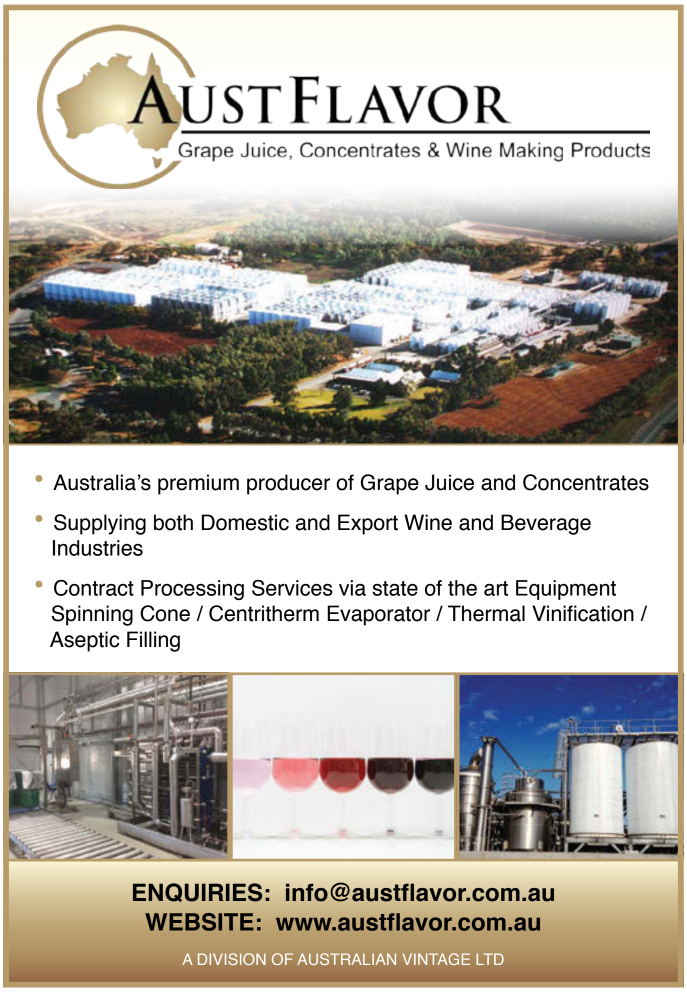


Figure 1. A yeast 'family tree'. A relatedness tree for the 236 yeast strains sequenced, representing the genomic differences between strains by the distance between them in the tree. For example, the wine yeast strains (blue and green) are shown quite close together, representing their low genomic variation. In contrast, the sake and palm wine strains are shown a long way from the wine yeast strains, indicating that they are genetically quite different.

group that contains over a third of all wine strains and can be further divided into two distinct lines of descent. The largest of these is dominated by a family of champagne yeasts (shown in dark green) and the second is dominated by fructose-fermenting strains and naturally occurring interspecific hybrids (light green).

Data analysis revealed that many of the yeast strains sequenced, including some offered by different commercial suppliers, appeared genetically identical. In total, 69 strains could be divided into 23 distinct equivalence groups (with the two largest carrying six strains each) and 13 groups contained at least two independent commercial isolates. A further key finding was that the genetic variation in wine yeast strains of *S. cerevisiae* is very limited. This means that breeding programs that draw only upon existing wine yeast strains are likely to be constrained in their attempts to develop new strains with novel and/or improved traits. Strain development efforts should be looking to introduce new variation from outside of the wine yeast grouping, to increase the genetic (and, therefore, winemaking) diversity that can be employed by the wine industry.

An 'extreme' example of this approach has been the development of interspecific non-genetically modified hybrid wine yeasts. These are developed by mating a *S. cerevisiae* wine yeast with an alternative *Saccharomyces* yeast such as *Saccharomyces kudriavzevii*. This brings into the wine yeast a genome that is very different from that of *S. cerevisiae* strains. As might be expected, the progeny from these crosses can introduce some interesting traits into the wines they



produce, including novel aromas and positive mouthfeel characters.

A drawback of interspecific hybrids is that they are sterile and, therefore, cannot be used in subsequent breeding programs. Analysing the genomes of *S. cerevisiae* strains from sources outside of the wine industry will allow the identification of strains from within the same species that are genetically distant from wine yeast. These strains will have the potential to bring new traits into the wine yeast gene pool when mated with existing wine yeast. The progeny from these crosses should be fertile and, therefore, able to be used in subsequent breeding programs to optimise the combination of traits in a single yeast strain.

WHERE TO NEXT?

Thanks to developments in DNA sequencing technologies, there is an exciting future in the field of wine yeast research and development. New yeast breeding programs will be developed based on in-depth knowledge of the *S. cerevisiae* gene pool. This knowledge will enable the generation of wine yeast strains that are robust, reliable, and capable of imparting novel, desirable sensory attributes, without necessitating the use of genetic modification techniques.

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