Spoilt for choice: picking the right yeast in a vibrant market

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Winemakers have access to a wide array of different yeasts, but it can be difficult to choose the best yeast for a particular must. The yeast research program at the AWRI has employed new techniques to assess large numbers of commercially available wine yeast, to understand their performance under different conditions and the genetic basis for their behaviour. Results will give winemakers more information on which to base yeast choice and also help guide the development of future yeast strains.

SO MANY YEASTS, SO LITTLE TIME

When selecting a wine yeast to inoculate a particular must, the key points considered by a winemaker, in order, might be:

- the ferment needs to finish – yeast must be robust under the conditions of the must
- the wine should be free of negative attributes – for example, it should have low volatile acidity (VA) and hydrogen sulfide (H_2S)
- the wine should have positive stylistic attributes – such as a defined mix of aroma attributes or high levels of polysaccharides.

While working through these steps seems simple enough, there are more than 200 commercially available yeasts from which to choose (Figure 1). To make matters worse, these are spread across many different manufacturers, which provide varying amounts of detail on product specification sheets, making direct comparisons challenging. In some years, it can be hard enough to get past the first point, that is, ensuring a ferment finishes in a timely fashion. Even though it is possible to optimise must conditions through nutrition additions, water additions (to reduce high sugar levels, in accordance with regulatory limits) and consideration of fruit quality and transport prior to processing, fermentation does not always progress as well as winemakers would like. The degree to which must conditions are stressful for yeast can vary because of factors outside of winemakers' control (weather conditions during vintage, juice composition) and due to winemaking interventions (SO_2 during processing,

AT A GLANCE

- Wine yeasts are highly diverse in their winemaking performance, and there are more than 200 available commercially.
- No single yeast is ideal for all conditions.
- Research at the AWRI has identified strains that perform well under different types of challenging conditions.
- Genetic factors that contribute to improved performance are being identified and this knowledge will be useful in future yeast development programs.

Figure 1. Some of the more than 200 commercially available wine yeasts.

harvest timing decisions, must temperature).

Several recent projects at the AWRI have been working towards a greater understanding of the environmental variables that can inhibit yeast, and better matching of musts with yeast strains, through understanding the diversity of commercially-available yeasts. A key challenge faced when this work commenced was to determine how to evaluate the performance of large numbers of yeasts in large numbers of environments without requiring many thousands of individual ferments to be performed. A way forward became possible by combining yeast molecular biology, advancements in DNA sequencing technology (Smith *et al.* 2010) and statistical approaches for the evaluation of this type of information (Anders *et al.* 2013). These advancements meant that rather than having to evaluate each yeast strain separately, many different yeast strains could be assessed at once.

TESTING WHICH YEASTS THRIVE UNDER DIFFERENT CONDITIONS

If all the yeast strains can be pooled together into a single fermentation vessel, then the focus shifts to the different environments in which they will be trialled. To enable the performance of individual yeast strains from within a pool to be assessed, it is necessary to be able to tell them apart. This was achieved through introducing a molecular barcode into each of 87 different yeast strains which, as a group, represent the broader spectrum of available yeasts (Figure 2). The barcoded pool of yeasts was then inoculated into different juices or media with different base compositions or additives, and the strains that benefitted most from those specific conditions were seen to dominate in a 'survival of the fittest' type contest. The proportions of strains within the different conditions were determined after the experiment by sequencing the barcodes and counting the number of times a specific barcode (strain) was detected.

Using this approach, the performance of 87 different yeasts was evaluated in conditions including: low pH, low temperature, high SO_2, low vitamins, high sugar, and low yeast assimilable nitrogen (YAN). A representation of the performance of these yeasts in those different conditions is provided in the heat map of Figure 2 (right hand side). While some conditions had little effect, or appeared to affect all yeast similarly, other conditions were highly discriminatory. What also stood out is that while there were some exceptionally high performing strains, these were generally restricted to specific conditions. No single yeast strain was optimal in all conditions. Even among a group of highly related strains (Figure 2, boxed in grey) there was diversity in their capability to perform well in different conditions.

One example of a performance difference that could be useful in several winemaking scenarios is the ability to initiate growth and fermentation at reduced temperature. ▶

Phylogenetic relationships between strains

Environments in which strains were evaluated

Barcoded

Unused

Fitness

Improved

Reduced

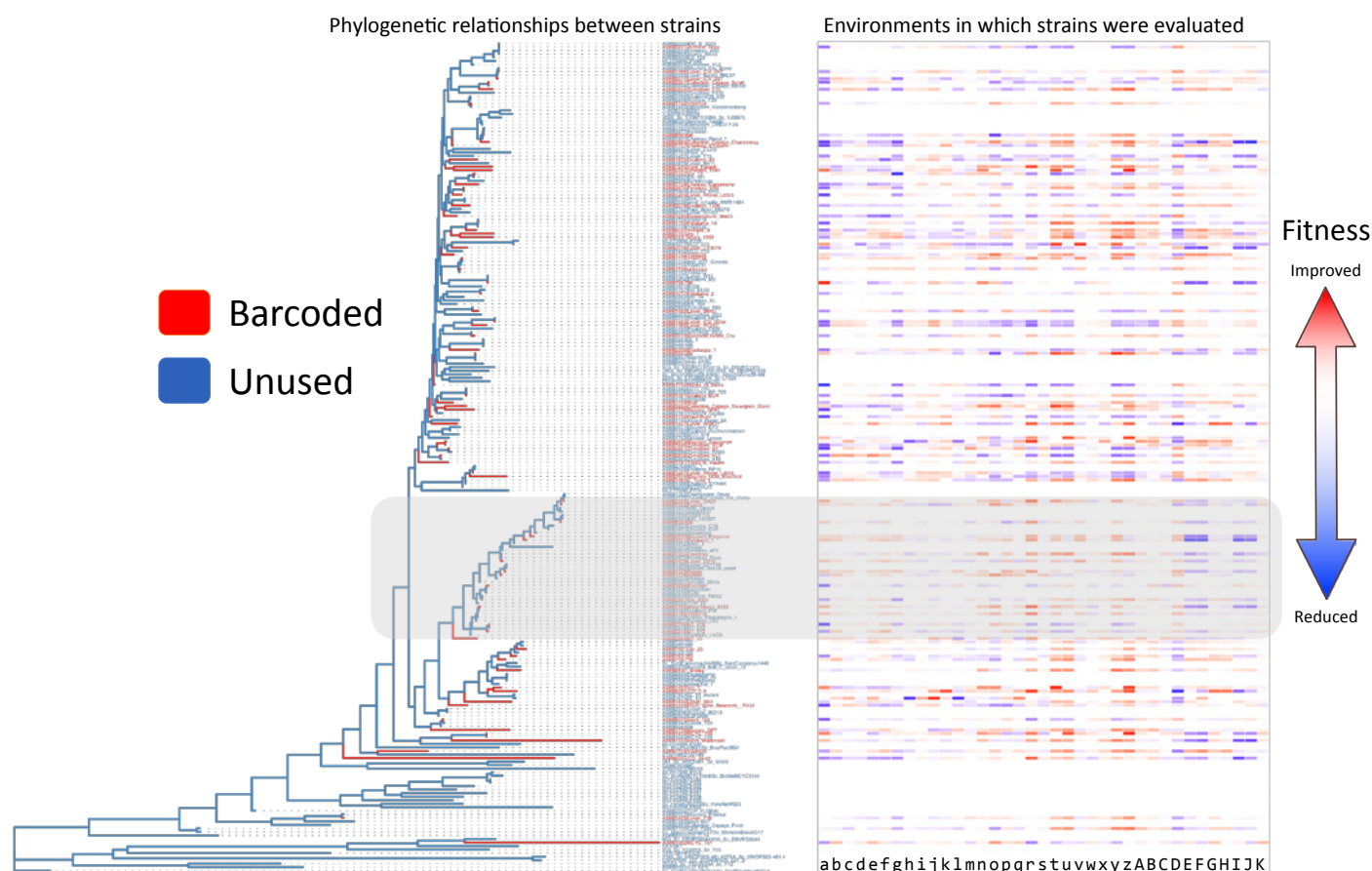abcdefghijklmnopqrstuvwxyzABCDEFGHIJK

Figure 2. Wine yeast relationships and their stress tolerance profiles. Wine yeast relationships are based on whole genome sequencing of wine yeast strains, displayed on the left. Strains coloured red have been barcoded for the purpose of fitness profiling. The heat map on the right represents fitness profiles of strains in different wine-relevant conditions. Red indicates increased fitness and blue indicates reduced fitness.

During later vintages, such as the one that was experienced in 2017, the much cooler harvest temperatures of late autumn were responsible for more difficult fermentation conditions. In other years it might be that fruit has been chilled prior to processing and the must is unusually cool. In these cases, it may be desirable to initiate fermentation at the cooler temperature to ensure some level of protection is provided while the must warms, or even for the entire fermentation to be performed cold (12°C or less).

Most wine yeasts are very slow or have no capacity to perform in very cold conditions. However, there are a small number of yeasts, including some hybrids of *S. cerevisiae* with *S. kudriavzevii* (Sc x Sk), that can substantially outcompete most other yeasts in the cold (Pérez-Torrado et al. 2017). These yeasts can ferment to dryness when others will fail to complete the ferment (Figure 3). Much like the broader collection of strains, not all Sc x Sk hybrids have this property, so knowing that a strain is a *kudriavzevii* hybrid does not necessarily mean that strain is cold tolerant. Figure 3 shows fermentation profiles of three *kudriavzevii* hybrids, only one of which can complete fermentation at 12°C. This demonstrates the importance of profiling the performance of individual strains of yeast.

IDENTIFYING THE GENETIC DRIVERS OF PERFORMANCE

By combining knowledge of the genetic and performance characteristics of yeast strains, it is possible to gain insight into why individual strains behave the way they do. Understanding why is important, not only because it is intellectually satisfying to finally solve an unanswered puzzle, but also because those answers generate tools that can be used to breed the next generation of yeasts. In the food and beverage industry, genetic modification is not currently an option for generating new yeast, and as such, traditional breeding approaches are relied on to deliver improvements in 'workhorse' yeasts. A key tool in these traditional approaches is the use of molecular markers that can guide the development process. These markers are obtained by building
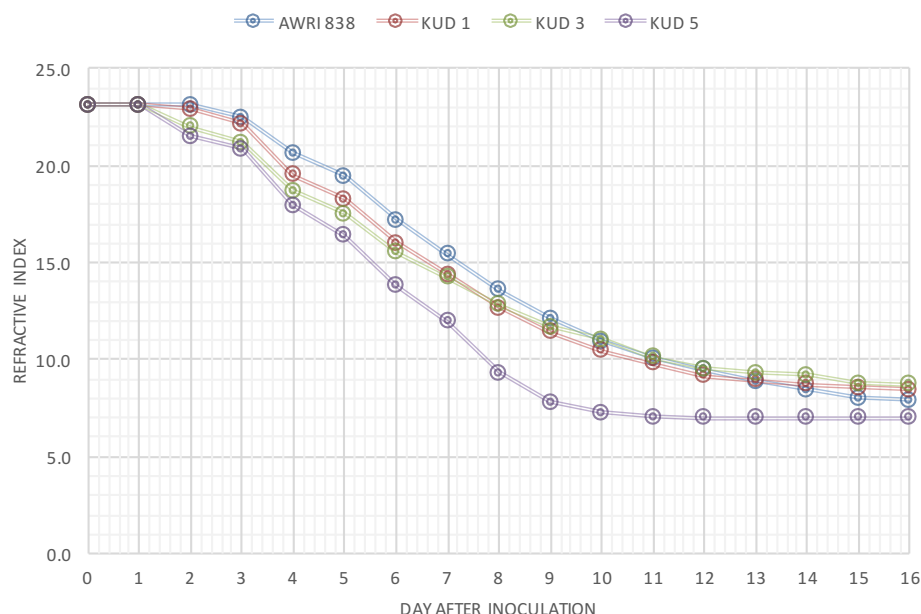

Figure 3. Fermentation kinetics of hybrid yeast strains at 12°C, comparing siblings from a cross between S. cerevisiae with *S. kudriavzevii* (KUD1, KUD2, KUD5) with parental strain *S. cerevisiae* (838) during fermentation of Chardonnay juice.

associations between performance-related properties and specific genetic elements that can easily be detected. Markers can be things like the number of copies of a specific gene, such as the factor that contributes to copper tolerance or the arrangements of chromosomes that contribute to sulfite tolerance.

As different sets of markers become available it may become possible to ascertain some of the properties of a strain without having to perform fermentation experiments. When new strains are isolated from environmental samples or generated through breeding experiments, much about them can be learned before they find their way into a fermentation. Then, when the fermentation experiments or pilot-scale trials are performed, they can be more targeted, focussing the sometimes costly and time-consuming analytical work on strains that already meet a range of previously identified criteria.

WHERE TO NEXT?

The aim of this work is to compile the information about strain performance properties into integrated reference charts that can be used to assist with yeast strain selection across a wide range of must types and winemaking priorities. By bringing together performance and genetic information

about the many yeast strains available, it will also be possible to produce an expanded and objective foundation for future strain development programs.

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