Technical notes

Understanding differences among wine yeast strains in their ability to release 'tropical' thiols

When it comes to wine flavour and aroma, yeast can take a lot of credit. During fermentation, wine yeasts not only produce ethanol and carbon dioxide, but also a range of volatile compounds such as esters, higher alcohols, fatty acids and volatile sulfur compounds. Together, these compounds make a major contribution to overall flavour, even though they are only produced in small amounts (Cordente et al. 2012).

Different strains of wine yeast produce varying amounts of different flavour compounds, which means that wine yeast can be used as a tool by winemakers to creatively modulate wine style (Figure 1). Understanding how and to what degree strains differ is key in making an informed yeast strain choice. Commercial yeast manufacturers offer a wide range of yeast strains and there are thousands of strains in culture collections around the world (including at the AWRI) where little is known about their winemaking characteristics. The AWRI's yeast research program is working to characterise the contributions of different yeast strains to wine aroma, and thereby help winemakers choose the best strain to suit their wine style.

One area of recent focus has been the differences among yeast strains in the production of two volatile thiols, 3-mercaptohexan-1-ol (3-MH) and 4-mercapto-4-methylpentan-2-one (4-MMP). These compounds impart desirable 'tropical fruit' characters to wine and are important contributors to the flavour of Sauvignon Blanc and Chardonnay wines (King et al. 2010, Capone 2016). In grapes they are found in their non-volatile precursor form, attached to cysteine or glutathione. During fermentation, wine yeasts can take up these precursors

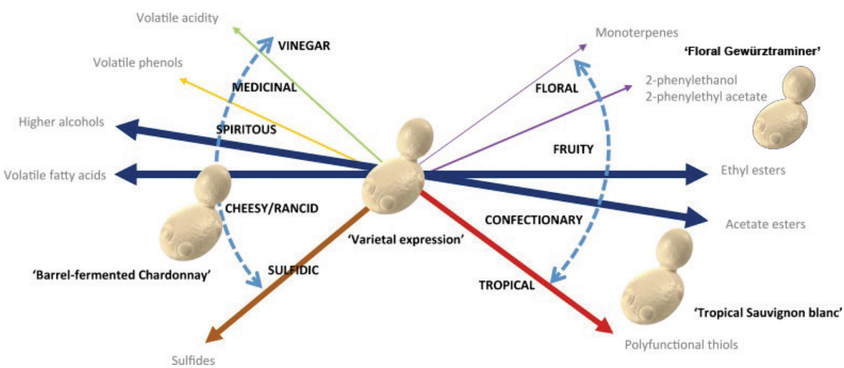

Figure 1. Examples of 'flavour phenotypes' of different wine yeast strains and the groups of compounds that drive the flavours.

and break them apart to release the corresponding free thiols (Figure 2) (Winter et al. 2011) but their ability to do so varies.

To assess this variability among different strains, a series of small-scale fermentations were carried out in a chemically defined grape juice media supplemented with grape-like concentrations of the thiol precursors. The fermentations were conducted with 82 different wine yeast strains, approximately half commercially available strains and half natural isolates from the AWRI Wine Microorganism Culture Collection.

Results showed a 20-fold difference among the yeasts in their ability to release 3-MH and a 35-fold difference in their ability to release 4-MMP. Interestingly, about 70% of the strains released low amounts of both thiols from their precursors; about 20% were classified as moderate releasers; and only 10% of the strains produced high amounts of these volatile thiols. No trends were observed between commercial wine strains and natural isolates in their ability to release 4-MMP and 3-MH, suggesting that there has not been an active selection from yeast manufacturers for strains with a higher thiol-releasing capacity. However, strains recommended by manufacturers for white winemaking tended to produce higher amounts of both 4-MMP and 3-MH than strains recommended either for red wine only, or both red and white wine (Figure 3).

Several genes involved in the release of 3-MH and 4-MMP from their precursors have previously been identified (Holt et al. 2011, Cordente et al. 2015, Roncorini et al. 2011). Genomic sequence data was reviewed for most of the strains in this study, and this analysis showed a natural variability among the different strains in one of these genes, *IRC7*, which largely explained the observed difference in volatile thiol release. Most of the strains (70%) possessed an inactive *IRC7* gene, which limited their ability to release volatile thiols (low

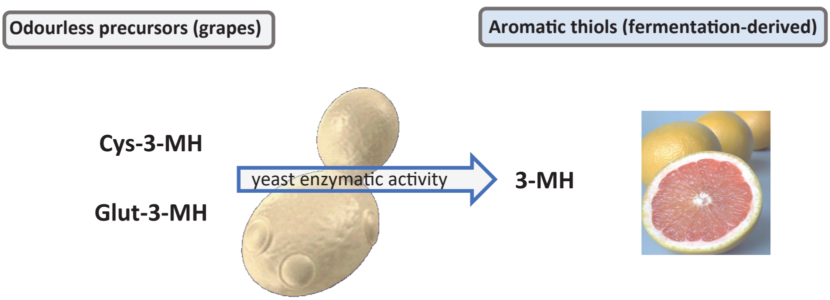

Figure 2. The odourless precursors of 3-MH, cysteine-3-MH (cys-3-MH) and glutathione-3-MH (glut-3-MH) are found in grapes. During alcoholic fermentation, the yeast converts glut-3-MH, in a series of enzymatic steps, into cys-3-MH. Then cys-3-MH is cleaved by different yeast enzymes (*STR3, IRC7*) to release the aromatic thiol 3-MH. The aroma descriptors of 3-MH are 'grapefruit' and/or 'passion fruit'.

releasers); while only a small percentage of strains (30%) had an active or partially active *IRC7* gene (high and moderate releasers, respectively), which allowed them to produce higher amounts of these thiols. Therefore, the DNA sequence of *IRC7* can be used as a molecular marker to determine the potential of any given strain to release volatile thiols. Investigations are currently under way as to why the inactivation of *IRC7* is such a common feature amongst wine yeast, and whether there is any advantage related to its inactivation.

It was also important to explore whether the information obtained from fermenting synthetic media could be used to predict how a particular wine strain would perform in winemaking conditions. To do this, small-volume fermentations of Chardonnay juice were conducted with a subset of 23 wine strains, and volatile thiols were analysed at the end of fermentation. A strong correlation was found between the concentrations of 3-MH produced in the

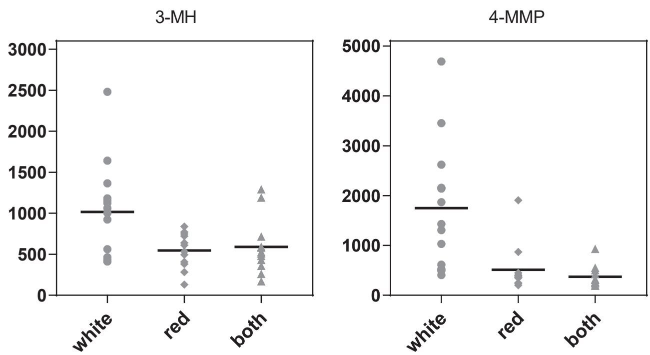

Figure 3. Distribution of 3-MH and 4-MMP (ng/L) concentrations in synthetic wines made with 39 different commercial wine strains. Strains are classified into three groups according to their recommended use by yeast manufacturers (white: used in the production of white wines; red: used in red wines; both: used both for white and red wines). The levels of 3-MH and 4-MMP in the individual wines are shown by grey symbols, and the average values in each of the groups is indicated by a black horizontal line.

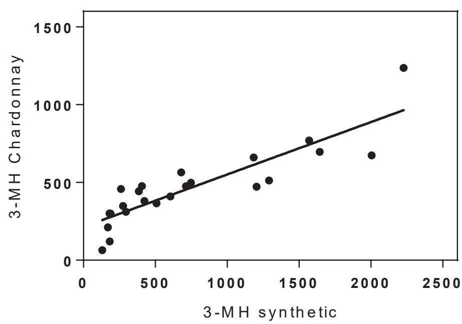

Figure 4. Relationship between 3-MH concentrations (ng/L) in fermentations of a synthetic media and a Chardonnay juice with 23 different wine strains. The regression line is shown, and the goodness of the fit between both data sets is expressed as $R^2 = 0.765$ ($P<0.0001$).

Chardonnay wine and those previously found in the synthetic media (Figure 4). These results confirmed that model ferments using synthetic media were good predictors of a strain's capacity to release varietal thiols under winemaking conditions.

Conclusion

Wine yeast strains can produce varying profiles of aroma compounds, and the yeast strain chosen can have a big impact on the final flavour and aroma of wine. This work has shown the significant diversity in thiol release capability of wine yeasts and identified a molecular marker that can be used to predict a yeast's potential to release volatile thiols. Identification of this marker opens the door for breeding of wine strains with enhanced thiol-producing capabilities, enabling the release of untapped 'tropical' thiol aroma in different white wine varieties. While it has been known for some time that thiols are dominant aroma compounds in Sauvignon Blanc, a recent survey has shown that thiols are also significant contributors to the aroma of Chardonnay wines and are well accepted by the majority of white wine consumers (Capone 2016). Furthermore, different market segments can be defined depending on preferences for the tropical characters provided by these thiols (Swiegers et al. 2009, King et al. 2010). This suggests that controlling varietal thiol concentration in white wine is one path to targeting specific market segments. The work described here will help winemakers control the degree to which varietal thiols contribute to the aromas of their wines by revealing the thiol-releasing potential of wine yeast and through the generation of new yeast with defined thiol-releasing capabilities.

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