A rose by any other name: novel wine yeast that impart floral aromas

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While there is substantial variation amongst existing wine yeast strains in their capacity to influence wine style, some desirable flavour compounds are not produced at concentrations where they significantly impact wine style. As part of ongoing yeast strain development projects at the Australian Wine Research Institute, novel wine yeast that make 'floral'/'rose' aroma compounds have been generated.

INTRODUCTION

"A rose by any other name would smell as sweet" (William Shakespeare). While varietal 'floral' aromas in wine are typically the product of grape-derived terpenoid flavour compounds, products of yeast metabolism 2-phenylethanol (2-PE) and 2-phenylethyl acetate (2-PEA) are considered important contributors to 'rose' aroma (Vilanova *et al.* 2013). That's not to say that yeast are what make roses smell like roses, but that they can enhance the 'floral' sensory properties of wine and other fermented foods and beverages (Fukuda *et al.* 1990, Dueñas-Sánchez *et al.* 2014).

This is but one of several examples where plant and yeast metabolism overlap and make the same or similar compounds. In this case the metabolic pathway involved is named after German biochemist Felix Ehrlich, who noted that various flavour-active fusel alcohols had similar chemical structures to amino acids, therefore, he proposed they were derived from them (Hazelwood *et al.* 2008). During winemaking, flavour compounds formed by yeast via the Ehrlich pathway confer a range of aromas and flavours that contribute to the overall bouquet of wine (Cordente *et al.* 2012).

Generally, in white varietals the concentrations of 2-PE and 2-PEA in finished wines are below their aroma perception thresholds (Vilanova *et al.* 2013), meaning their contribution to wine style would likely be minimal.

A ROSE AMONGST THE THORNS

To generate a novel yeast that imparts floral aromas, a well-known and widely utilised wine yeast strain, AWRI796, was exposed to a chemical selection process previously applied to saké and baking yeast (Fukuda *et al.* 1990, Dueñas-Sánchez *et al.* 2014). A toxic analogue of the amino acid phenylalanine was incorporated into solid agar plates that a large number of AWRI796 cells were spread onto, and a separate set of plates were used for AWRI796 cells that had been previously exposed to a mutagenic agent. Only cells that carry mutations in key phenylalanine biosynthetic pathway genes (that enable the cell to make more of its own supply of this essential amino acid) can grow in the presence of this toxic analogue. The mutagenic agent increases the frequency of isolating cells that carry such a mutation. While it may be harsh to refer to AWRI796 as a thorn, cells carrying

AT A GLANCE

- Substantial variation is present amongst existing wine yeast strains in their capacity to influence wine style.
- Some desirable flavour compounds are not present in wine at concentrations where they significantly contributed to wine style.
- Novel wine yeast that make 'floral'/'rose' aroma compounds have been generated.
- This will enable winemakers to 'dial up' these aromas as desired.

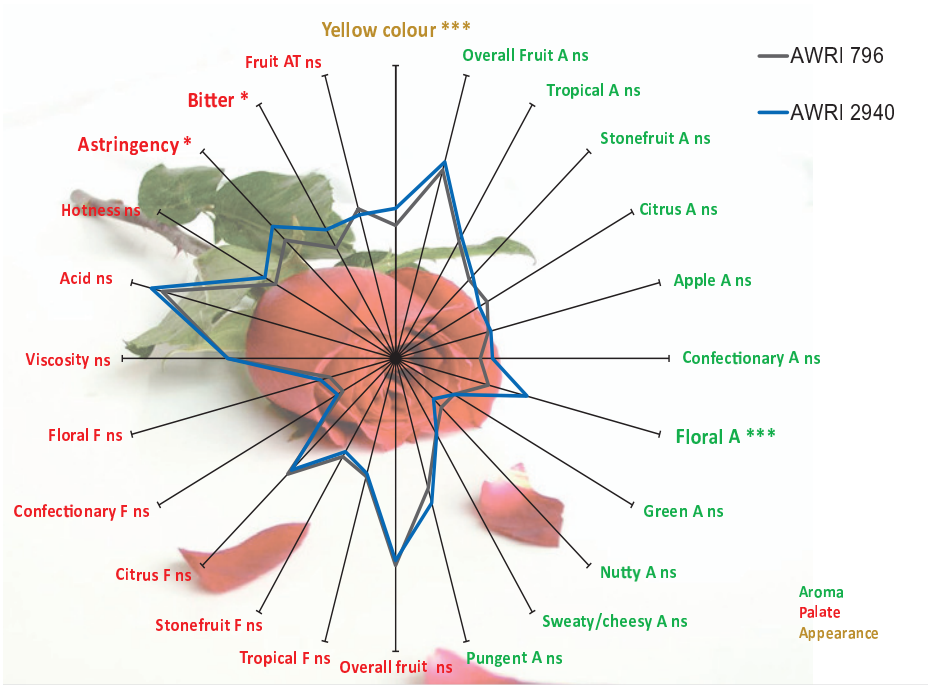

Figure 1. Mean ratings for aroma and flavour attributes for Chardonnay wines (20L scale) produced using AWRI 796 and the 'rose' strain AWRI 2940 (n = three fermentation replicates × 10 judges). ns, not significant; *p < 0.05; ***p < 0.001 Aroma and flavour attributes are shown in green, and red, respectively.

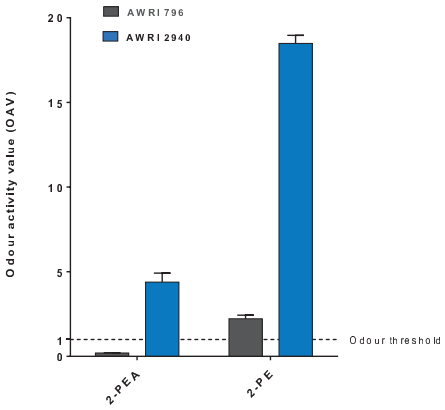

Figure 2. Odour activity values (OAV) for the 'rose' aroma compounds 2-phenylethanol (2-PE) and 2-phenylethyl acetate (2-PEA) in Chardonnay wines (20L), produced using AWRI 796 (black) and the 'rose' strain AWRI 2940 (blue). Data is expressed as the mean and standard deviations of three fermentation replicates. An OAV higher than one (odour threshold) indicates a possible contribution of the compound to the final aroma of wine.

mutations that allow them to overproduce phenylalanine do indeed smell like roses. A by-product of cells making large amounts of phenylalanine is that more is available for biosynthesis of the flavour-active compounds 2-PE and 2-PEA. All yeast derived using this and related protocols are not genetically-modified (GM) and are suitable for immediate application by the wine industry.

The AWRI screened 153 AWRI796 mutants for floral aromas, and 17 of those were used to conduct fermentations in the laboratory. The resultant wines were analysed for a suite of volatile aroma compounds. Ten mutants that produced significantly higher concentrations of 2-PE and 2-PEA had their whole genomes sequenced and, importantly, six of these were 'spontaneous mutants' that had been derived without use of the mutagenic agent, which means they carried a small number of changes (<10 nucleotides out of 12 million) in their genomes. This enabled identification of two genes in the aromatic amino acid biosynthetic pathway that harboured possible causative mutations.

A pilot-scale winemaking trial was performed with one of the 'spontaneous' mutants (AWRI2940) using a high quality Adelaide Hills Chardonnay juice. There was no difference observed between the mutant and parent strain AWRI796 with regard to fermentation kinetics and overall wine chemistry. Sensory assessment revealed that the wines made with AWRI2940 were rated significantly higher in the floral aroma attribute (Figure 1), and contained 7- and 37-fold higher concentrations of 2-PE and 2-PEA (Figure 2), respectively. Less clear are the drivers for perceived differences in palate terms 'astringency' and 'bitter', or the apparent slight increase in 'yellow colour'; the flow-on impact of altered amino acid metabolism during wine fermentation is currently being investigated.

CONCLUSIONS

By harnessing classical yeast strain development techniques, yeast that impart floral aroma during wine fermentation have been isolated and characterised. These strains have the potential to provide a tool for winemakers to produce more floral wine styles, and tailor new products to appeal to consumers.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Petaluma winery for the supply of high quality Chardonnay grapes for the winemaking trial. This work was financially supported by Australia's grapegrowers and winemakers through their investment body, Wine Australia, with matching funds from the Australian Government. The AWRI is part of the Wine Innovation Cluster.

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