Development of novel wine yeast strains using adaptive evolution

By

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A thesis submitted for the degree of Doctor of Philosophy, in the Faculty of Sciences

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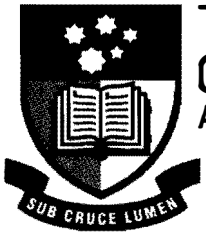
November 2006



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Thesis Summary

Throughout the course of the wine fermentation, yeast are exposed to several simultaneous and sequential stresses, including hyperosmotic shock, nutrient limitation and ethanol accumulation, which are thought to contribute to the development of stuck or sluggish fermentations. Apart from the extended processing time, fermentations in this state can also produce unfavourable aromatic and flavour compounds, collectively leading to increased manufacturing costs and reduced quality. As a consequence there is demand from winemakers for yeast which can overcome these problems, by assuring the rapid completion of fermentation with minimal production of undesirable metabolites. The aim of this study was to produce and characterise more robust strains and address this demand.

Utilising a sequential batch fermentation system we have used adaptive evolution to generate variants of the commercial *Saccharomyces cerevisiae* wine strain L-2056 and a haploid derivative thereof, C911D, which have superior fermentative performance and/or altered metabolite yields. Thus, both evolved cultures produce more glycerol than the respective parent, with the evolved haploid (FM5) also producing a reduced final ethanol concentration and the evolved diploid (FM16) being able to complete fermentation within a shorter duration. Ten clonal isolates were randomly chosen from each of the haploid and diploid evolved cultures and examined in order to determine the degree of heterogeneity of the evolved cultures. In both cases, 9 of the 10 clonal isolates produced similar fermentation phenotypes to the mixed culture from which they were derived.

Transcriptome analysis of mixed cultures and clonal cultures revealed large changes in transcript abundance (magnitude and number of genes) at several time points across fermentation in reference to the parent strains. A commonality noted in a high proportion of genes found to be differentially expressed was the occurrence of a *GCR1* binding site in their promoter regions. Sequencing of the *GCR1* promoter region in the evolved clone FM16-7

revealed several base pair differences compared to the parent L-2056. We propose that these changes may have led to an altered pattern of expression of *GCR1*-regulated genes and in turn the improved fermentation phenotype evident in the evolved diploid cultures.

To further investigate the significance of alterations in the expression of a specific selection of these genes, deletion strains were constructed in the appropriate parental strain background. Fermentation trials conducted with these deletants revealed fermentation phenotypes which approximated those of the evolved cultures. That is the deletion of either *HXT1*, *HXT3*, *GLC3*, *ALD6* and *INO1* in C911D or *ALD6*, *GPH1* and *RCK1* in L-2056 yielded phenotypes similar to the evolved haploid and diploid respectively. No one deletant strain completely matched the magnitude of the improvement in fermentation of the respective evolved culture. As such it is postulated that a number of mutations are responsible for the acquired phenotypes in the evolved cultures.

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