Identification of genes affecting glucose catabolism in nitrogen-limited fermentation

By

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Thesis Summary

When assimilable nitrogen becomes limiting in fermentation processes such as winemaking, sugar transport systems of the inoculated yeast are inactivated and biomass formation is restricted. As a consequence such fermentations may fail to catabolise all available sugar leaving the product out of specification, at greater risk of spoilage and deterioration and needing greater input to rectify. In recognition of this critical importance of assimilable nitrogen in the successful completion of several fermentation processes, this study has sought to develop yeast strains that utilise this typically limited nutrient group more efficiently. Wine strains, usually of *Saccharomyces cerevisiae*, are known to differ in the efficiency with which they exploit nitrogen. As a consequence, so-called 'nitrogen efficient' strains may offer greater prospects for reliable completion of fermentation.

With the aid of transposon mutagenesis together with a high throughput method for analysis of multiple micro-fermentations, nitrogen efficient mutants were identified that were able to catabolise more sugar for a given amount of utilised nitrogen. Mutants displaying improved nitrogen efficiency were further characterised in shake-flask fermentations and the affected genes were identified with the assistance of Inverse-PCR.

As wine and laboratory yeast strains can be pheno-typically different, especially in terms of their ability to affect enological fermentations, a haploid derivative of the wine yeast strain L-2056 was developed, such that it could be easily genetically manipulated.

Of the identified genes, disruption of *NGRI* and *GID7*, lead to an enhanced catabolism of sugar in both a laboratory strain and a haploid derivative of a wine strain of *Saccharomyces cerevisiae*, during growth in a chemically defined grape juice medium with limiting nitrogen. Deletion of *NGRI* or *GID7* also resulted in minor changes to the amounts in which selected metabolites
were produced (determined by HPLC). Biomass yield (measured as dry weight) was also decreased in \textit{NGR1} mutants.

Previous studies have demonstrated a strong link between assimilable nitrogen and fermentation rate, when other nutrients are not limiting. The total nitrogen utilised and the timing of nitrogen uptake of \textit{ngs1Δ} and \textit{gid7Δ} strains was found to be very similar to the parent strain. Thus it was hypothesised that \textit{ngs1Δ} and \textit{gid7Δ} strains could be using the available nitrogen differently to enable enhanced glucose catabolism.

Deletion of either \textit{NGR1} or \textit{GID7} was found to affect the expression of genes involved in the core pathway for the utilisation of non-preferred nitrogen sources, known as Central Nitrogen Metabolism (CNM). The transcriptional abundance, measured by Real-Time PCR, of \textit{GDH1}, \textit{GDH2}, \textit{GLT1} and \textit{GLN1} was altered in these mutants. This distorted expression of CNM genes could translate to a re-modelling of enzyme quantities and thus re-distribution of the core nitrogen-containing compounds, and thereby the cellular response under nitrogen-limiting conditions.
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