Enhancing ethanol tolerance of the yeast *Saccharomyces cerevisiae* through modification of growth medium composition

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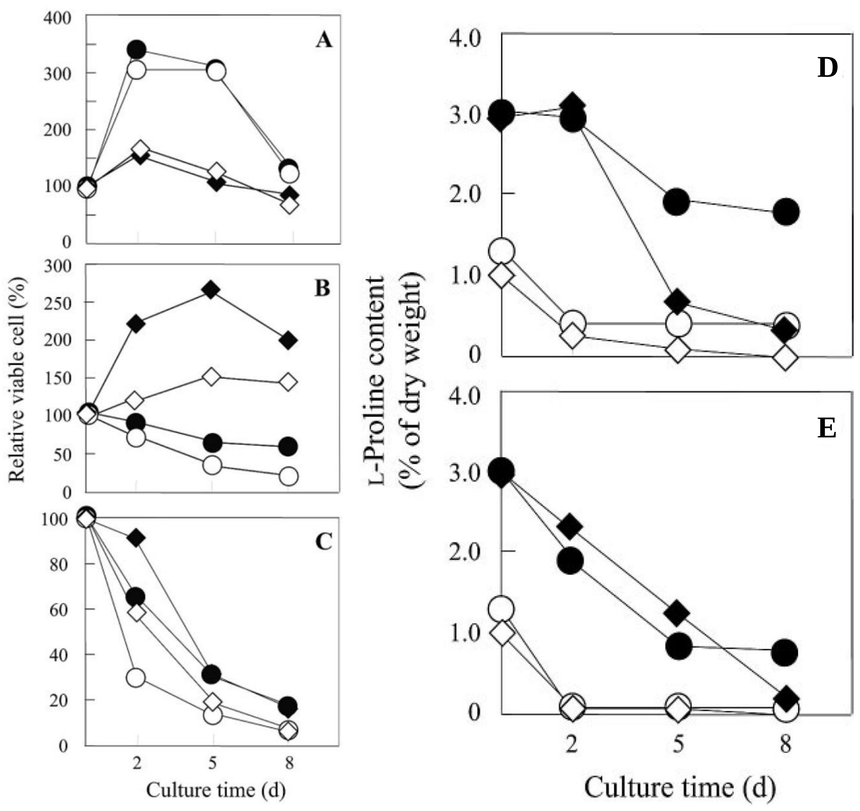
Introduction

The decrease of fossil fuel availability has created a high demand for alternative fuels, including bioethanol produced by yeast fermentation of carbohydrate. Relatively low yields of ethanol can be a major problem in this bioconversion, as yeasts can not tolerate high ethanol concentrations. Approaches to increasing efficiency in the fuel ethanol industry include improving yeast metabolic flux and fermentation rate and selection of yeast with higher ethanol tolerance. This study has an alternative approach, aiming to enhance ethanol tolerance of the yeast *Saccharomyces cerevisiae* through modification of growth medium composition. It focuses specifically on two important components which have been shown to positively affect stress tolerance of yeast; the sugar inositol (Krause *et al.* 2007; Ji *et al.* 2008) and the amino acid L-proline (Takagi *et al.* 2005). Despite this positive role, excess of these substances in the growth medium may be detrimental to the yeast. Therefore, inositol and L-proline content of growth media will be varied in order to define concentrations that potentiate the highest ethanol tolerance and productivity of the yeast.

Methodology

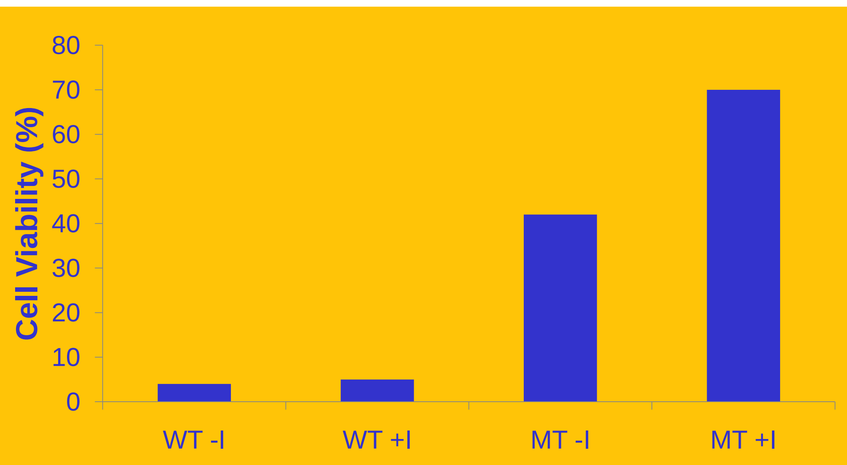
Five yeast strains will be studied under aerobic and anaerobic conditions. Cultures will be supplemented with 10 concentrations of either inositol or L-proline. Cultures will be sampled at beginning and end and at appropriate time intervals and analysed for the following parameters: fermentation rate (by sugar and ethanol concentrations); yeast cell count, budding (cell division) rate and viability; vitality by assessing membrane fluidity (fluorescence spectroscopy); and ethanol tolerance (exposure to 20% ethanol, determining viable cell counts by methylene violet, fluorescent viability probes and plate counts). From this matrix of results, the optimal levels of inositol and L-proline will be identified for (a) maximal ethanol tolerance and (b) maximal cell productivity and fermentation rate.

Proline as an Ethanol Stress Protectant

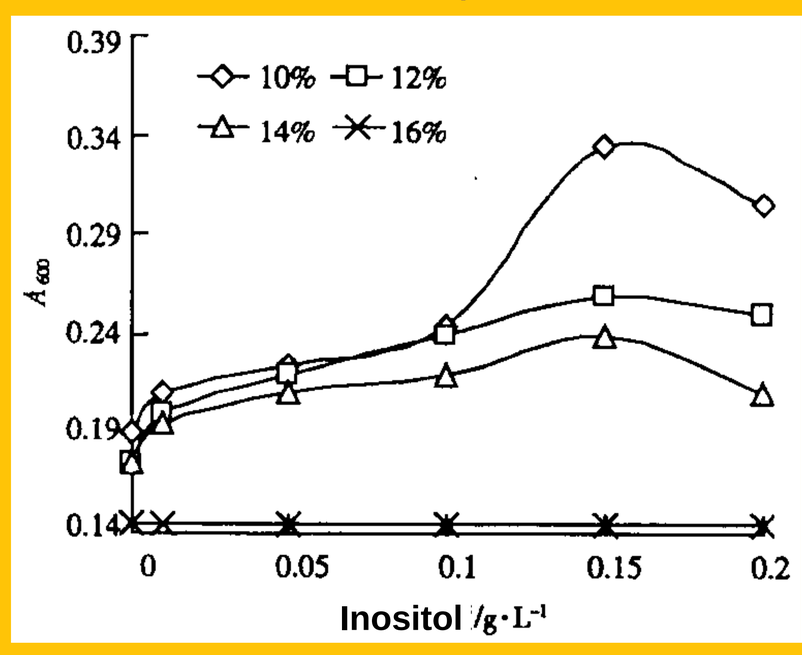


Relative viable cell numbers of laboratory and sake yeast strains grown in SD medium without (A) or with 9% (B) and 18% (C) ethanol, and intracellular L-proline content of laboratory and sake strains grown in SD medium without (D) and with 9% (E) ethanol. The *S. cerevisiae* strains used were the parent laboratory strain (○) and L-proline accumulating laboratory mutant strain (●) and control strain (◇) and L-proline accumulating sake strain (◆) (Takagi *et al.* 2005).

Inositol as an Ethanol Stress Protectant



Cell viability of wild type (WT) and mutant capable of inositol synthesis (MT) yeast strains after growth without (-I) and with (+I) 75 µM inositol and 1 hour exposure to 15% ethanol (data summarised from Krause *et al.* 2007)



Effect of inositol supplementation on cell density of the yeast *Pachysolen tannophilus* with initial ethanol concentrations from 10 to 14% (Ji *et al*. 2008)

References

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