Effects of supplementation with L-proline or inositol on yeast membrane fluidity and ethanol tolerance

Safri Ishmayana[1,2], Ursula Kennedy[1] & Robert Learmonth[1*]

[1]Centre for Systems Biology, University of Southern Queensland, Toowoomba 4350 (Australia)
[2]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Bandung (Indonesia)

The decrease of fossil fuel availability has created a high demand for alternative fuels, including bioethanol produced by yeast fermentation of carbohydrate. Relatively low ethanol yields can be problematic, and approaches to increase efficiency have included genetic modification of yeast to improve metabolic flux, fermentation rate and ethanol tolerance. We investigated an alternative approach, aiming to evaluate novel yeasts for bioethanol production, and further to enhance their ethanol tolerance through modification of growth medium composition. We focused specifically on two important components which have been reported to positively affect yeast stress tolerance; the sugar inositol[1,2] and the amino acid L-proline[3]. Three yeast strains (a bakers' yeast, a wine yeast and a sake yeast), known tolerate 17% ethanol, were studied under aerobic conditions. Cultures were supplemented with various concentrations of either inositol or L-proline, and sampled during respiro-fermentative growth (fermentation of glucose to ethanol) and respiratory growth on ethanol. Physiological parameters, viability, membrane fluidity (by laurdan GP) and tolerance to 18% ethanol were determined. Differences were noted in the growth, ethanol productivity and ethanol tolerance of the yeasts, with significant differences evident in laurdan GP in respiro-fermentative, but not respiratory phase cells. As expected, tolerance to ethanol was significantly higher in respiratory phase cells, also correlating with significantly higher laurdan GP which indicated lower membrane fluidity. However, on trialling L-proline levels from 0.1 to 3 g/L and inositol levels from 0.002 to 0.2 g/L, no significant differences were found in laurdan GP or ethanol tolerance. Thus we could not confirm previous reports of positive effects of L-proline or inositol, due largely to high variability in the data from triplicate experiments. It is possible that the effects of these supplements may be strain dependant; the wine yeast tended towards lower membrane fluidity with 0.5 g/L proline, and in the sake yeast there was a trend towards improved ethanol tolerance with inositol levels above 0.005 g/L. Future experiments will investigate a wider variety of yeast strains and increase repetition to minimise data variability.

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*Corresponding author: e-mail: Robert.Learmonth@usq.edu.au