

CHARACTERIZATION OF OSMO TOLERANCE AND ETHANOL TOLERANCE ABILITY OF A THERMOTOLERANT YEAST

**S. Balakumar, Vasanthi Arasaratnam
and K. Balasubramaniam**

**Department of Biochemistry, Faculty of Medicine
University of Jaffna, Sri Lanka**

A combination of high temperature, ethanol and osmo (sugar) tolerances are clearly desirable characteristics in fermentation processes. A relationship between these tolerances has often been suggested while never being well defined. This paper reports on the ethanol and osmo (sugar) tolerances and the effect of heat shock on enhancing these tolerances of a thermotolerant yeast strain isolated from the distillery environment and developed in this laboratory. The yeast cells were grown in sterile PYN medium, which consisted of (g l^{-1}) peptone, 35; yeast extract, 30; KH_2PO_4 , 20; $(\text{NH}_4)_2\text{SO}_4$, 10; and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 10; with glucose 50g l^{-1} at 36°C with reciprocal shaking (150rpm) for 18h for inoculum preparation. The ethanol tolerance of yeast was determined at different concentrations of ethanol ($0-200\text{g l}^{-1}$) added to the PYN and soy flour 3.5g l^{-1} supplemented PYN media separately and incubated at 40°C with shaking (150rpm). Viable count and ethanol were determined. The viability was reduced with the increase of the added ethanol concentration. Complete cell death was observed at 48h with 150g l^{-1} added ethanol. Soy flour supplemented PYN medium was conducive for better ethanol tolerance. At 100g l^{-1} added ethanol level the viability was improved from 70 to 75% with soy flour supplementation at 48h. At 150g l^{-1} added ethanol level complete cell death and 20% viability were found in PYN and soy flour supplemented PYN medium respectively at 48h. Soy flour supplementation was ineffective at 200g l^{-1} added ethanol level. The osmo tolerance was determined at different concentrations of sorbitol ($50 - 400\text{g l}^{-1}$) added to the PYN and soy flour supplemented PYN media separately. The osmotic effect caused by the non metabolizable sugar was well tolerated by yeasts in soy flour supplemented medium than unsupplemented medium. Combined effect of added ethanol and osmotic effect were studied in PYN and soy flour

Section B

supplemented PYN media separately having sorbitol 200gl^{-1} and $0 - 200\text{gl}^{-1}$ ethanol. The combined effects of osmotic stress and ethanol stress were more pronounced than their individual effects. However the soy flour supplementation has protected the yeast cells from the combined stress. At 100 and 150gl^{-1} added ethanol levels total cell death was observed in PYN medium whereas in supplemented medium 10 and 5% viability was observed respectively. Effect of heat shock along with soy flour supplementation was also studied. Results showed that heat shock further enhanced the ethanol tolerance. Inoculum (18h) was subjected to heat shock at 45°C for 15 min and was added to soy flour supplemented PYN medium having 200gl^{-1} ethanol and incubated at 40°C . Cultures grown in PYN medium at 36°C showed 93% viability at 50h . When the inoculum grown in PYN medium at 36°C was transferred to PYN medium having 200gl^{-1} alcohol and incubated at 40°C , 60 , 20 and 0% viabilities were observed at 10 , 20 and 30h respectively. When the inoculum grown at 36°C was subjected to heat shock at 45°C for 15min and was transferred to PYN medium having 200gl^{-1} alcohol and incubated at 40°C , 70 , 40 , 32 , 18 and 10% viabilities were observed at 10 , 20 , 30 , 40 and 50h respectively.