SACCHAROMYCES CEREVISIAE GENOMIC LIBRARY SCREENING IN SEARCH FOR THE GENE RESPONSIBLE FOR INDUCTIVE ACTIVE GLYCEROL UPTAKE

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In Saccharomyces cerevisiae, physiological response to osmotic stress is done, mainly, by increased synthesis and intracellular accumulation of glycerol as compatible solute. Previous studies revealed the existence of a glycerol/H⁺ symport, inducible by growth under gluconeogenic conditions (Lages and Lucas) and independent of the Fps1 channel for glycerol (Sutherland et al.). In order to isolate the gene encoding for glycerol specific carrier, an isogenic strain to W303-1A, strain YSH642, carrying gpd1 gpd2 mutations unable to synthesize glycerol, was studied for further screening of a S. cerevisiae genomic library. Physiological assays consisting on detection of extracellular alcalinization of cell suspensions upon addition of glycerol and determination of intracellular accumulation of \([^{14}\text{C}]\text{glycerol}\), were performed on glucose-grown cells (repressed cells) and on ethanol-grown cells (derepressed cells). No significant differences were found between the results obtained with either YSH642 and W303-1A strains, from which we concluded that disruptions of GPD1 and GPD2 genes do not interfere with regulation of active glycerol uptake. To choose selection conditions, we assumed that the derepressed activity of the glycerol symporter will contribute to increased halotolerance in gpd1 gpd2 genetic background, provided the presence of extracellular glycerol. Thus, selective medium was designed according with previous phenotypic characterization of salt stress tolerance. Screening of a genomic library of S. cerevisiae in the multicopy plasmid YEp13 with inserts of 8-10Kb at BamHI restriction site, is underway by electroporation of strain YSH642. A field strength of 1500V and resistance of 200_ is being employed giving 0.073% viability and an efficiency of 1.1x10^4 trf/µgDNA, using, as selective medium, mineral medium supplemented with convenient auxotrophic
requirements for both yeast strain and plasmid YEp13, glucose 2% (w/v), NaCl 1.4M and glycerol 50mM. Clones able to grow on this medium are being further characterized for osmotic tolerance and, for glycerol transport activity under conditions of repression.