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}C]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 *Bam*HI 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 1.1×10^4 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.