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Construction of Genetic Recombinant Yeasts with High Environmental Tolerance by Accumulation of Trehalose and Detoxication of Aldehyde

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Abstract : Many environmental factors, such as glucose concentration, ethanol, temperature, osmotic pressure and pH, decrease the production rate of ethanol using yeast as a starter. Fermentation starters with high tolerance to various stresses are always demanded for brewing industry. Trehalose, a storage carbohydrate in cell wall of yeast, plays an important role in tolerance of environmental stress by preserving integrity of plasma membrane and stabilizing proteins. Furan aldehydes are toxic to yeast and the growth rate of yeast is significantly reduced if furan aldehydes were present in the fermentation medium. In yeast, aldehyde reductase is involved in the detoxification of reactive aldehydes and consequently the growth of yeast is improved. The aims of this study were to construct a genetic recombinant Saccharomyces cerevisiae or Pichia pastoris with furfural and HMF degrading and high ethanol tolerance capacities. Yeast strains were engineered by genetic recombination for overexpression of trehalose-6-phosphate synthase gene (tps1) and aldehyde reductase gene (ari1). TPS1 gene was cloned from S. cerevisiae by reverse transcription-polymerase chain reaction (RT-PCR) and then ligated with pGAPZαC vector. The constructed vector, pGAPZC-tps1, was transformed to recombinant yeasts strain with overexpression of ari1. The transformants with pGAPZC-tps1-ari1 were generated called STA (S. cerevisiae) and PTA (P. pastoris) with overexpression of tps1, ari1. PCR with tps1-specific primers and western blot with his-tag confirmed the gene insertion and protein expression of tps1 in the transformants, respectively. The neutral trehalase gene (nth1) of STA was successfully deleted and the novel strain STAΔN will be used for further study, including the measurement of trehalose concentration and ethanol, furfural tolerance assay.

Keywords : genetic recombinant, yeast, ethanol tolerance, trehalase, aldehyde reductase
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