

Metabolic and Adaptive Laboratory Evolutionary Engineering (ALE) of *Saccharomyces cerevisiae* for Second Generation Biofuel Production

Authors : Farnaz Yusuf, Naseem A. Gaur

Abstract : The increase in environmental concerns, rapid depletion of fossil fuel reserves and intense interest in achieving energy security has led to a global research effort towards developing renewable sources of fuels. Second generation biofuels have attracted more attention recently as the use of lignocellulosic biomass can reduce fossil fuel dependence and is environment-friendly. Xylose is the main pentose and second most abundant sugar after glucose in lignocelluloses. *Saccharomyces cerevisiae* does not readily uptake and use pentose sugars. For an economically feasible biofuel production, both hexose and pentose sugars must be fermented to ethanol. Therefore, it is important to develop *S. cerevisiae* host platforms with more efficient xylose utilization. This work aims to construct a xylose fermenting yeast strains with engineered oxido-reductive pathway for xylose metabolism. Engineered strain was further improved by adaptive evolutionary engineering approach. The engineered strain is able to grow on xylose as sole carbon source with the maximum ethanol yield of 0.39g/g xylose and productivity of 0.139g/l/h at 96 hours. The further improvement in strain development involves over expression of pentose phosphate pathway and protein engineering of xylose reductase/xylose dehydrogenase to change their cofactor specificity in order to reduce xylitol accumulation.

Keywords : biofuel, lignocellulosic biomass, *saccharomyces cerevisiae*, xylose

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