Characterization of Yeast Used for Bioethanol Production –Isolation and Breeding for Xylose Fermenting Yeast–

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−Isolation and Breeding for Xylose Fermenting Yeast−

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ABSTRACT

The most abundant feedstock for bio-ethanol production is lignocellulosic biomass, including cellulose, hemicellulose, and lignin. Glucose and xylose are the two major sugars after hydrolysis. Efficient fermentation of xylose is presently still a technological hurdle to overcome for ethanol production from biomass. New xylose fermenting yeasts were isolated, and the characteristics of the new yeast stains were studied in the research.

After three enrichment steps using the medium containing xylose as the sole carbon source, and subsequent plating and fermentation, eight yeast strains were isolated from 85 samples obtained from natural environment including rotten wood samples and hot spring samples. Strains 4 and 5 can produce higher ethanol, while other four strains having higher xylitol producing ability. The eight yeasts were identified by 26S rDNA gene sequencing and sugars assimilation abilities. Strains 4 and 5 were identified as *Pichia stipitis*, while other strains identified as *Candida sp*.

A spontaneous mutant isolation procedure was used by 30~60 days enrichment in media containing 2% (v/v) and 6 % (v/v) ethanol alternately. And then the mutants were selected based on colony size on agar plates containing 40 g/L ethanol. The procedure for isolation was designed based on the hypothesis that the ethanol production is correlated with the ethanol tolerance in yeast.

Obviously improvements in ethanol yield and productivity by two mutants derived from *P. stipitis* No.4 were observed. The highest yield (0.49 g/g) and productivity (1.47 g/ /L·h) were achieved by *P. stipitis* mutant 4 in the medium containing 100 g/L xylose. The highest ethanol concentration (66.3 g/L) was also detected in the medium containing 160 g/L xylose. The ethanol fermentation time was shortened from 96~150 hours to 34.5~60 hours.

The research results are valuable both in practical uses and in scientific development. Studies on the breeding and utilizing of the xylose fermenting yeasts on ethanol production from lignocellulosic biomass will be continued in the future.