103 **Expression of Xylose Reductase and Xylitol Dehydrogenase Genes from *Pichia stipitis* in *Saccharomyces cerevisiae***

○Manee Tantrungkij, Noriyuki Nakashima, Shinichi Kinoshita, Tatsuji Seki, and Toshiomi Yoshida (ICBiotech., Faculty of Engineering, Osaka University, Suita-shi, Osaka 565)

Xylose is a major constituent of hemicellulose which comprises up to 30 – 40 % of renewable biomass in nature, and its utilization has been studied intensively for bio-fuel production. *Saccharomyces cerevisiae* commonly used in the industrial alcohol fermentation, however, cannot ferment xylose to ethanol due to the insufficient enzyme levels and imbalance of NAD/NADH redox system involved in xylose metabolism. In this study, the genes for NADH/NADPH dependent xylose reductase and xylitol dehydrogenase originated from *Pichia stipitis* were introduced into *S. cerevisiae* to produce ethanol from xylose.

To introduce genes for xylose reductase and xylitol dehydrogenase in *S. cerevisiae*, several plasmids were constructed based on autonomously replicating plasmids and an expression vector plasmid, and the resultant plasmids were transferred in laboratory and industrial strains by transformation using the lithium acetate method. The cloned genes were well expressed in *S. cerevisiae*. The transformants harboring plasmid(s) carrying the xylose reductase and xylitol reductase genes were able to grow on the medium containing xylose as a carbon source, while the transformants carrying either of genes could not assimilate xylose although high amount of enzyme was produced. The result suggests that both genes are required for the accumulation of xylose. The ethanol production from xylose by the transformants, however, was less than that by *P. stipitis*, and a significant amount of xylitol was accumulated in the culture broth.

104 **Purification and characterization of xylanase I produced by *Aeromonas caviae* ME-1.**

ºK. B. KUBATA, H. HORITSU, K. KAWAI, K. TAKAMIZAWA and T. SUZUKI.

Div. of Bioresources Util., Fac. of Agri., Gifu University, Japan.

β-1,4-Xylanase catalyzes the hydrolysis of β-1,4-xylosidic linkages of xylan. In the previous paper, we reported that *Aeromonas caviae* ME-1, isolated from the gut contents of *Samia cynthia pryeri*, produced high β-1,4-xylanases. This work presents the purification and characterization of xylanase I produced by *Aeromonas caviae* ME-1. The bacterium was cultivated in optimal medium at 30° C, 72 hr. The supernatant of the culture fluid was used as crude xylanase preparation. The purification was done by ammonium sulfate (95% saturation) precipitation and gel filtration chromatographies using Sephadex G-100 and G-75, and HPLC using SHIM-PACK DIOL-300 column. Enzyme reaction was carried out in 10 ml of 1% of oat spelt xylan in sodium acetate buffer pH 5.0 at 40°C, 30 min and released reducing sugars were determined by SOMOGYI-NELSON method with D-xylose as a standard. One unit of enzyme activity was defined as the amount of enzyme that liberated 1 μmol of xylose equivalent in one minute. Protein was measured by the method of Lowry et al. with bovine albumin as a standard. Xylanase I was purified 11-fold in specific activity with a recovery of 9.3%. The purified enzyme gave a single band protein on native polyacrylamide gel disc electrophoresis and on SDS-PAGE. The xylanase had a K_m of 5.6 mg/ml and an apparent V_{max} of 2800 $\mu mol.min^{-1}.mg$ of $protein^{-1}$. The pH and temperature optima for activity were 7.0 and 50° C, respectively and more than 60% of the enzyme activity was retained from pH 6.5 to 8.0. The molecular weight of the purified xylanase was found to be 20,000 by Mass spectroscopy . Other characteristics are under investigation.