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Title: Efficient Conversion of Woody Biomass to Bioethanol

The more efficient use of biomass is demanded to solve the global crisis such as exhaustion of fossil fuel and global warming. Biomass is carbon fixation products of photosynthesis by plant using solar energy. Therefore, the environmental friendly energy system should be able to construct by control of production and utilization of biomass. Woody biomass, including agriculture residues, wood chips, paper wastes, etc., has already been transferred to bio-ethanol and bio-diesel in some cases and used as energy related products, although many issues such as efficiency and productivity still exist to be overcome. Xylose is one of the major fermentable sugars present in woody biomass, the second most abundant carbohydrate polymer in nature to glucose. The efficient fermentation of xylose is required to develop economically viable processes for producing biofuels such as ethanol from woody biomass. In this study, I mainly focused on efficient fermentation process of bio-ethanol production from woody biomass. For this purpose, I tried to improve the efficiency of enzymes involved in bio-ethanol production such as xylose reductase (XR) and xylitol dehydrogenase (XDH).

In this study, I applied the protein engineering to generate enzymes with completely reversed coenzyme specificity and developed recombinant yeasts containing those engineered enzymes for construction of an efficient biomass-ethanol conversion system. I focused on the fungal xylose metabolic pathway. XR and XDH from Pichia stipitis are necessary for Saccharomyces cerevisiae to ferment xylose to ethanol because of a lack of genes encoding these enzymes in native Saccharomyces cerevisiae. S. cerevisiae transformed with the native genes encoding XR, XDH from P. stipitis and the endogenous gene xylulokinage (XK) ferments xylose to ethanol but has not yet been applied to the industrial bio-process due to the unfavorable excretion of xylitol. Intercellular redox imbalance caused by the different coenzyme specificity of XR (using NADPH/NADH with preference for NADPH) and XDH (exclusively using NAD+) has been thought to be one of the main factors of xylitol excretion. Introduction of NADP+-dependent XDH and NADH-preferring XR generated in this study produced efficient ethanol and prevented the xylitol excretion because of maintaining the intercellular redox balance.