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Improvement of ethanol yield from xylose by breeding of industrial yeast

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Xylose is one of main sugars abundant in biomass like switch grass, corn cobs, rice straws and wood chips. A xylose fermenting yeast has been constructed by recombining genes of xylose reductase (XR) and xylitol dehydrogenase (XDH) from *Pichia stipitis*, and a gene of xylulose kinase (XKS) from *Saccharomyces cerevisiae* under the control of strong promoter. When this recombinant yeast ferments xylose to ethanol, substantial quantities of xylitol and glycerol are also produced as by-products, resulting in less efficient ethanol production, compared with that from glucose. It is considered that cofactor imbalances might be the reason for the inefficient ethanol production, because XR requires NADPH as a cofactor, while XDH uses NAD as a cofactor. *S.cerevisiae* uses NAD for the oxidative reactions and NADPH for the reductive reactions in order to synthesis biomass. *S.cerevisiae* reoxidizes NADH to NAD by the reaction to make glycerol under anaerobic condition. *S.cerevisiae* has three NAD(H) kinases, Utr1p and Yef1p in the cytosol, and Pos5p in the mitochondria. These NAD kinases have different preferences for cofactors as substrates. In this study, we tested whether the deletions or overexpressions of these NAD kinase genes change the cofactor balance and improve the ethanol yields from xylose. The strain disrupted *UTR1* and *YEF1* showed the highest ethanol yield and produced the lowest xylitol and glycerol among the bred strains. It indicates that the shortage of NAD is one of the reasons for a high accumulation of xylitol. Moreover, we found that xylitol was excreted by glycerol channel, Fps1p, at some extent, and the disruption of Fps1p decreased xylitol production and resulted in higher ethanol yield. Industrial yeast often has the ability of high ethanol production, or high stress tolerance. However most of them are multiploid and have no spolulation ability. We tried to delete each set of *UTR1, YEF1* and *FPS1* from one industrial diploid strain by using a marker recycling system, and confirmed these breeding for the fermentation from xylose were also available for industrial yeast of diploid.

Keywords
Bioethanol, Xylose, NAD kinase, Glycerol channel