An improvement of ethanol yield from xylose by disruptions of NAD kinase genes in *S.cerevisiae* .

Xylose is one of main sugars are 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 P.stipitis, and a gene of xylulose kinase (XKS) from S.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. In this study, we investigated functions of NAD(H) kinases in order to correct that imbalances. S.cerevisiae has two NAD(H) kinases, Utr1p and Yef1p in the cytosol. It is known that Utr1p phosphorylates NAD higher efficiently than NADH, and Yef1p uses both NAD and NADH. Disruptions of these genes possibly alter the concentrations of four cofactors, NAD, NADH, NADP, and NADPH. We tested whether the deletions of these NAD kinase genes influence the ethanol yields from xylose. The xylose fermenting yeast strain with the disruption of *UTR1* resulted in a higher ethanol yield, and a lower xylitol and glycerol yield, compared to the host strain. It indicates that the shortage of NAD is one of the reasons for a high accumulation of xylitol. The strain with the disruption of *YEF1* had almost no effect on the ethanol yield, but the strain disrupted both of the two genes showed a higher ethanol yield and produced lower xylitol and glycerol than the strain with the disruption of *UTR1.*