Microbiology (2007), **153**, 3044–3054 DOI 10.1099/mic.0.2007/007856-0

Ethanol production from xylose by recombinant *Saccharomyces cerevisiae* expressing protein-engineered NADH-preferring xylose reductase from *Pichia stipitis*

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Received 6 March 2007
Revised 31 May 2007
Accepted 5 June 2007

A recombinant *Saccharomyces cerevisiae* strain transformed with xylose reductase (XR) and xylitol dehydrogenase (XDH) genes from *Pichia stipitis* (*Ps*XR and *Ps*XDH, respectively) has the ability to convert xylose to ethanol together with the unfavourable excretion of xylitol, which may be due to intercellular redox imbalance caused by the different coenzyme specificity between NADPH-preferring XR and NAD^+-dependent XDH. In this study, we focused on the effect(s) of mutated NADH-preferring *Ps*XR in fermentation. The R276H and K270R/N272D mutants were improved 52- and 146-fold, respectively, in the ratio of NADH/NADPH in catalytic efficiency [(k_{cat}/K_m with NADH)/(k_{cat}/K_m with NADPH)] compared with the wild-type (WT), which was due to decrease of k_{cat} with NADPH in the R276H mutant and increase of K_m with NADPH in the K270R/N272D mutant. Furthermore, R276H mutation led to significant thermostabilization in *Ps*XR. The most positive effect on xylose fermentation to ethanol was found by using the Y-R276H strain, expressing *Ps*XR R276H mutant and *Ps*XDH WT: 20 % increase of ethanol production and 52 % decrease of xylitol excretion, compared with the Y-WT strain expressing *Ps*XR WT and *Ps*XDH WT. Measurement of intracellular coenzyme concentrations suggested that maintenance of the of NADPH/$NADP^+$ and NADH/NAD^+ ratios is important for efficient ethanol fermentation from xylose by recombinant *S. cerevisiae*.

INTRODUCTION

Xylose is one of the major fermentable sugars present in lignocellulosic biomass, the second most abundant carbohydrate polymer in nature after glucose. The efficient fermentation of xylose is required to develop economically viable processes for producing biofuels such as ethanol from biomass (Jeffries & Jin, 2004). Although a few xylose-fermenting yeasts are found in nature (Jeffries, 1983; Kurtzman, 1994), *Saccharomyces cerevisiae* is used universally for industrial ethanol production because of its ability to produce high concentrations of ethanol and high inherent ethanol tolerance; however, native *S. cerevisiae* can not ferment xylose, so engineering *S. cerevisiae* for xylose utilization has focused on adapting the xylose metabolic pathway from the xylose-utilizing yeast *Pichia stipitis*. In this organism, xylose is converted to xylulose by two oxidoreductases: xylose is initially reduced to xylitol by NAD(P)H-linked xylose reductase (XR; EC 1.1.1.21) (Verduyn *et al.*, 1985), and then xylitol is oxidized to xylulose by NAD^+-linked xylitol dehydrogenase (XDH; EC 1.1.1.9) (Rizzi *et al.*, 1989). Finally, xylulokinase (EC 2.7.1.17) phosphorylates xylulose into xylulose 5-phosphate, which is metabolized further via the pentose-phosphate pathway.

Although *S. cerevisiae* transformed with native *XYL1* and *XYL2* genes encoding XR and XDH from *P. stipitis*

Abbreviations: AKR, aldo-keto reductase; CD, circular dichroism; PGK, phosphoglycerate kinase; XDH, xylitol dehydrogenase; *Ps*XDH, XDH from *Pichia stipitis*; XI, xylose isomerase; XR, xylose reductase; *Ct*XR, XR from *Candida tenuis*; *Ps*XR, XR from *Pichia stipitis*; T_m, thermal unfolding transition temperature; WT, wild-type.

Two supplementary tables are available with the online version of this paper.

