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CONSTRUCTION OF RECOMBINANT YEAST EXPRESSING THERMO-STABLE ENZYME FOR EFFICIENT BIOETHANOL PRODUCTION FROM WOODY BIOMASS

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ABSTRACT

Xylitol dehydrogenase from *Pichia stipitis* (PsXDH) is one of the key enzymes for the bio-ethanol fermentation system from biomass sugars. Previously, the C4 mutant (S96C/S99C/Y102C) was constructed with enhanced thermostability by introduction of structural zinc. A high thermostability of PsXDH was obtained by subsequent site-directed mutagenesis of the structural zinc-binding loop. In this study, effects of thermostabilization of xylitol dehydrogenase (XDH) from *Pichia stipitis* on fermentation of xylose to ethanol were estimated using a recombinant *Saccharomyces cerevisiae* expressing together with a native xylose reductase (XR) from *P. stipitis*. The mutated XDH performed the similar enzyme properties in *S. cerevisiae* cells, compared those *in vitro.* The significant enhancement was found in Y-C4 strain, 51% decrease of unfavorable xylitol excretion with 19% increased ethanol production, when compared with the reference strain expressing the wild–type XDH.

Key Words: *Woody biomass, Thermostable enzyme, Xylitol dehydrogenase, Xylose-fermentation, Ethanol production*

1.0 INTRODUCTION

A more efficient use of biomass is demanded to solve the global crisis such as depletion of fossil fuel and global warming. Woody biomass, including agriculture residues, wood chips, municipal solid wastes, paper wastes, etc., has already been transformed to bio-ethanol and bio-diesel in some cases and used as energy related products, although many issues such as efficiency and productivity still need 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 biomass [1].

Although the native *S. cerevisiae* cannot ferment xylose, the *S. cerevisiae* transformed with the native genes encoding XR and XDH from *P. stipitis*, a most potent recombinant strain, acquires the ability to ferment xylose to ethanol [2-4]. Though, this important technology has been found as early as 1983, but not yet been applied to industrial bio-process mainly due to the unfavorable excretion of xylitol during ethanol-fermentation. Xylose consuming yeasts such as *Pichia stipitis* can metabolize through expression of xylose reductase (XR) and xylitol dehydrogenase (XDH). XR catalyzes the reduction of xylose to xylitol where as XDH oxidizes xylitol to xylulose (Figure 1). However, *Pichia stipitis* is susceptible to ethanol and obliges stumpy and carefully controlled oxygenation, these disadvantages averts its usage for industrial ethanol production [5-10].

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