Rational design of xylitol dehydrogenase from *Pichia stipitis* for development of efficient xylose-utilizing recombinant *Saccharomyces*

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Xylitol dehydrogenase (XDH) is the enzyme for catalyzing xyiltol-to-xylulose reaction, which is the important step for transforming hemicellulosic material, xylose, to fermentative sugar. Up to now, xylose-utilizing recombinant Saccharomyces cerevisiae has been constructed by genetically expressing XDH together with xylose reductase. The protein engineering of PsXDH functionality has been required because the high amount of xylitol was observed in xylose-fermentation. In this study, to enhance the thermostability of XDH from Pichia stipitis (PsXDH), we constructed the C4 mutant, which is endowed with additional structural zinc. In addition, for further improvement of PsXDH thermostability, we designed the appropriate structural zinc-binding loop via bioinformatical comparison with other polyol dehydrogenase family members. A high thermostability of PsXDH was obtained by subsequent site-directed mutagenesis of the structural zinc-binding loop.