

Kanya Kokaew 2011: Cloning and Characterization of Xylose Reductase Gene (*XYL 1*) from Thermotolerant Methylophilic Yeast *Ogataea siamensis* N22. Master of Science (Microbiology), Major Field: Microbiology, Department of Microbiology. Thesis Advisor: Assistant Professor Nantana Srisuk, Ph.D. 121 pages.

Xylose reductase gene (*XYL 1*) of the thermotolerant methylophilic yeast, *Ogataea siamensis* N22, was cloned from genomic DNA and screened by PCR. The positive clone namely pOSXR2-88 was found to carry 835 bp of partial *XYL 1* with 3' end missing. The 3' end of *XYL 1* was then cloned from cDNA using 3' RACE and sequenced. The two sequences were then contiged and a 960 bp open reading frame encoding 319 amino acids was found. Nucleotide sequence analysis showed a TATA box-like sequence, ⁻¹¹²TATAA⁻¹⁰⁸, upstream from start codon and polyadenylation signal, ⁹⁷⁴AATAAA⁹⁷⁹, downstream from stop codon. The sequence of *O. siamensis* N22 *XYL 1* was submitted to GenBank (accession number FJ763639). Alignment of deduced amino acids sequence showed that xylose reductase of *O. siamensis* N22 is closely related to aldose reductase of *Candida boidinii* with 72% similarity. The deduced amino acid sequence of *O. siamensis* N22 xylose reductase showed 3 different positions, excluded from enzyme active site, from those of other yeasts. However, the conserved sequence of xylose reductase active site, IPKS, was found.

To overexpress the *XYL 1* in *O. siamensis* N22, the recombinant plasmid pPICOSXR and pGAPOSXR were constructed under the *AOX* and *GAP* promoters, respectively and integrated into host chromosome. Study of gene expression via enzyme activity assay was carried out. The original N22 strain and recombinants revealed specific xylose reductase activity of 0.044-0.098 U/mg protein. The recombinant designated N22-pPICOSXR30 possessed 0.098 U/mg protein which is 2.2 folds of the activity found in the original N22 strain. The pGAPOSXR recombinants showed specific enzyme activity of 0.062-0.093 U/mg protein and the recombinant namely N22-pGAPOSXR3 showed 0.093 U/mg protein which is 1.5 folds of that observed in the original N22 strain. Additionally, xylose reductase of *O. siamensis* N22 and transformants studied were found to use NADPH as a preference coenzyme.

Student's signature

Thesis Advisor's signature