

Uraiwan Masud 2011: Functional Analysis of *adhS* Gene Encoding Quinoprotein Alcohol Dehydrogenase Subunit III and Characterization of NAD⁺-*adh* Genes from *Acetobacter pasteurianus* SKU1108. Doctor of Philosophy (Genetic Engineering), Major Field: Genetic Engineering, Interdisciplinary Graduate Program. Thesis Advisor: Associate Professor Gunjana Theeragool, D.Agr. 179 pages.

The role of the *adhS* gene product, alcohol dehydrogenase subunit III, encoded from *adhS* gene, on the function of PQQ-ADH was investigated by construction of the *adhS* gene disruptant and mutants of *Acetobacter pasteurianus* SKU1108. The *adhS* gene disruptant completely lost its PQQ-ADH activity and acetate-producing ability but retained acetic acid toleration. In contrast, this disruptant grew well, even better than the wild type, in the ethanol containing medium even though its ethanol oxidizing ability was completely lost, while the NAD⁺-dependent ADH (NAD⁺-ADH) was induced. The tentative promoter region of *adhS* gene is located at approximately 118-268 bp upstream from an initiation codon. The essential amino acids for functional subunit III are 104Thr and 73, but not the 22 amino acids at the C-terminal. However, the over-expressed *adhS* gene did not enhance acetic acid production. To elucidate the genes expression of cytosolic NAD⁺-ADHs, *adhI* and *adhII* genes were amplified and cloned into pGEM-T[®] Easy Vector. The Open Reading Frame of *adhI* and *adhII* consist of 1,029 and 762 bp, respectively. The deduced amino acids of ADH I and ADH II were 342 and 253 amino acids exhibit 99.71% and 99.60% homology, respectively with the same proteins from *A. pasteurianus* IFO 3283. The ADH I belongs to group I Zn-dependent long chain ADHs, while the ADH II belongs to group II short-chain dehydrogenase/reductase of NAD⁺-ADHs. The reduction of growth was observed when the NAD⁺-*adh* genes disruptants grown in ethanol medium. In *E.coli*, ethanol remarkably induced *adhI* and *adhII* promoter activities about 1.5 and 2.0 times, respectively. The possible promoter region of *adhI* and *adhII* genes are located at around 81-105 and 73-92 bp, in order, from its ATG start codon and the repressor binding regions might be located closed to these promoters.

Student's signature

Thesis Advisor's signature