

Wilawan Sintuprapa 2008: Characterization of Acetic Acid Adapted Strains and Analysis of Alcohol Dehydrogenase Genes from *Acetobacter syzygii* SKU19.

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*Acetobacter* sp. is one of the most attractive bacteria for proteomic analysis of proteins involved in stress response or adaptation. To elucidate an adaptive response to acetic acid, acetic acid adapted strains were isolated from sequential cultivations of acetic acid sensitive strain, *Acetobacter syzygii* SKU19 (wild type), in a medium containing 1.0% acetic acid. The adapted variants could be divided into two groups based on growth and ability to further oxidize acetate. The first group consisted of cells with increased overoxidation or rapid acetate oxidizer, while the second group contained cells with increased stability to acetate or slow acetate oxidizer. The randomly amplified polymorphic DNA (RAPD) profiles of the genomic DNA showed no obvious difference in genetic background among these adapted strains. In contrast, quinoprotein alcohol dehydrogenase (PQQ-ADH) and aldehyde dehydrogenase (ALDH) activities of these adapted strains were higher than those of the wild type. The result corresponded well with the increased amount of protein with molecular mass of 72-80 (subunit I) and 44-54 kDa (subunit II) when the organism was cultivated in acetic acid containing medium. Three *adh* genes, *adhA*, *adhB* and *adhS* encoding for PQQ-ADH subunit I, II and III, from *A. syzygii* SKU19 were cloned and analyzed for nucleotide sequences. These three genes encoded for 743, 472, and 205 amino acids, respectively. Subunit I contained one additional amino acid and subunit III possessed 3 different amino acids compared with subunit III of an acetic acid resistant strain *A. pasteurianus* SKU1108. Surprisingly, subunit III protein of this strain could not be detected in both membrane and soluble fractions by immunoblot analysis although its gene was cloned and sequenced. Transfer of pCMadhS plasmid carrying *adhS* gene from *A. pasteurianus* SKU1108 into *A. syzygii* SKU19 enhanced growth on media containing various concentrations of acetic acid. Expression of *adhS* gene from *A. pasteurianus* SKU1108 in *A. syzygii* SKU19 could be induced by ethanol but it seemed to be that it was unable to bind with subunit I from *A. syzygii* SKU19.

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