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Effect of Gene Inactivation of *adhE* or *pta-ackA* in NADH Dehydrogenases-Deficient *Escherichia coli* on Anaerobic Metabolic Pathway

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Escherichia coli possesses an efficient catabolic system by which it is able to conserve energy under a wide range of redox conditions. During anaerobic growth, the facultative anaerobe *E. coli* produces products such as acetate, ethanol, lactate, succinate, formate, CO_2, and H_2 (1). The relative rates of formation of these products are controlled by the combined action of various enzymes involved in that metabolism, including the demand for redox balance (2). *E. coli* has two NADH dehydrogenases, 14-subunit intrinsic membrane protein NDH-I (*nuoA-N* operon) (3, 4), and an FAD polypeptide with no iron, NDH-II (*ndh*) (5, 6), which are known to be involved in regulation of the energy recovered from NADH oxidation by controlling the relative levels of the two NADH dehydrogenases. Reoxidation of NADH back to NAD+ is important in cell metabolism for adjusting redox balance (7). Recent days, much effort has focused to purposeful alteration of metabolic pathways because it can provide improved outcomes for metabolic engineering goals (8-10).

Here, the global metabolic effect was examined using gene knockout mutants of the redox sensing regulatory NADH dehydrogenase (NDH) system in *E. coli*. Also, mutations of *ack-ptaA* gene, encoding phosphotransacetylase (PTA) and acetate kinase (ACK) and involving in acetate fermentation pathway, or of *adhE* gene encoding alcohol dehydrogenase (ADH) were introduced into the of NDH deficient strains and the additional effects of those gene inactivations were studied. The gene knockout mutants were constructed in *E. coli* MG1655 strain, using the method of Datsenko and Wanner (10). Metabolites were analyzed by HPLC, and the effects of each gene inactivation to the profile of metabolites were examined by comparing with that of wild type under anaerobic conditions with glucose as a carbon source. The effects on expression of genes involving the central anaerobic metabolic system and other key redox regulatory mechanisms were also investigated by quantitative RT-PCR analysis. Cultures of all the mutant strains showed altered distribution of carbon recovery products and changed pattern of gene expression in central anaerobic metabolic genes. This means each genetic perturbation affected the metabolic network and impacted the carbon flow through the metabolic branch to adapt to given conditions. Also it suggests that the carbon flux was redirected due to the combination of gene transcriptional and translational-, metabolite-,

cofactor-level of regulation by the cell itself. As the results, some informations on improvements and decrease of metabolite production were given with gene expression profile in each mutant strain. This provides a basic model system for further understanding of the redirection pattern of carbon flux and for an integrated quantitative framework for the mathematical modeling in the redox/sensing-regulatory genetic system and its cellular responses in NDH deficient system in *E. coli.*

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