

Genetic modification of platform *E. coli* for metabolic pathway study related to carbon elongation of linear carbon chain fatty acid

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Hexanoic acid is linear carbon chain fatty acid and has been used as a food additive, perfume fixative, antimicrobial agent, etc. Recently, hexanoic acid was reported that can be utilized various and useful chemicals such as ester and alcohol through the conversion by chemical catalyst. Hexanoic acid can be produced by several bacteria such as *Clostridium kluyveri* and *Megasphaera* sp.; the metabolic pathways of the strains were not studied, closely. Therefore, in order to find out obvious function of the metabolism for hexanoic acid production, the metabolic pathway of *Megasphaera* sp. was investigated using transformed *E. coli*. The 7 genes from *Megasphaera* sp. was selected by RNA transcriptome analysis. The RNA expression levels of 7 genes were up-regulated in optimal condition for hexanoic acid production. The 7 genes were acetyl-CoA acyl transferase(ACAT), hydroxy-butryl CoA dehydrogenase(HBD), crotonase(CRT), butyryl-CoA dehydrogenase(BCDH), electron transfer flavoprotein subunits(ETF ab) and Acetyl-CoA transferase(ACT). ACAT, HBD, and CRT were inserted into pGS-21 and, BCDH, ETF ab, and ACT were inserted into pBBR MCS-2 vector. The genetically modified *E. coli* BL21 (DE3) was cultivated in LB broth treated anaerobically. The metabolites of cultivated broth were analyzed by GC and the expressed genes were confirmed using SDS-PAGE.