

# Global Gene Expression Profiling of *E. coli* and Its Mutant Unable to Produce Acetate

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## 1 Introduction

DNA microarray has been used extensively to analyze gene expression profiles of microorganisms. Transcriptome profiling can provide important information about cell physiology and has the potential to identify connections between regulatory or metabolic pathways that were not previously known [5]. Physiological meanings of acetate synthetic pathway in *E. coli* have been studied for many years. It has been reported that the accumulation of acetate in the culture media decreases cell growth rate and production of recombinant protein [3]. Also, it has been suggested that acetyl phosphate plays a role as global regulator affects some regulators and the target genes [4, 1]. In this study, we manufactured DNA microarray containing 2,850 genes including all functionally known and putative ones [6]. Changes in transcriptome level between wild type *E. coli* and its mutant unable to produce acetate, were analyzed qualitatively and quantitatively to examine their physiological and metabolic meanings.

## 2 Method and Results

### 2.1 Bacterial Strains

*E. coli* W3110 (derived from K-12, F<sup>-</sup>IN(*rrnD-rrnE*)1) and its *ackA-pta* mutant (W3110  $\Delta$ *ackA-pta* ::Km<sup>R</sup>) were used in this study. The whole *ackA-pta* operon was eliminated from the chromosome of W3110 in order to remove the effects from *ackA-pta* gene and to ensure the strain unable to produce not only acetate but also acetyl phosphate.

### 2.2 Culture Media

Bacterial cultivations were carried out using modified R medium and Luria-Bertani broth (LB). The modified R medium contains per liter: glucose 10 g; (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>, 2 g; KH<sub>2</sub>PO<sub>4</sub>, 6.65 g; citric acid, 0.8 g; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.7 g; trace metal solution, 5 mL. The trace metal solution contained per liter: FeSO<sub>4</sub>·7H<sub>2</sub>O, 10 g; CaCl<sub>2</sub>, 1.35 g; ZnSO<sub>4</sub>·7H<sub>2</sub>O, 2.25 g; MnSO<sub>4</sub>·4H<sub>2</sub>O, 0.5 g; CuSO<sub>4</sub>·5H<sub>2</sub>O, 1 g; (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O, 0.106 g; Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O, 0.23 g; 35% HCl, 10 mL. The LB broth contains per liter: yeast extract, 5g; tryptone, 10g; NaCl, 10g)

### 2.3 Batch Cultures

Batch cultures of *E. coli* W3110 and its *ackA-pta* mutant were carried out (Figure 1). There has been several reports showing that the growth rate of *E. coli pta* mutant was considerably decreased in minimal media [2]. However, in our study, the growth rate of *E. coli ackA-pta* mutant was similar with *E. coli* W3110. The seed cultures were prepared by the cultivation in a 1 L flask containing 200

mL of modified R medium at 37°C and 250 rpm and were used to inoculate into a 6.6 L bioreactor (Bioflo 3000, New Brunswick Scientific Co., Edison, NJ) containing 2 L of modified R medium.

## 2.4 Transcriptome Analysis

The resulting 2,850 gene probes of *E. coli* were arrayed on poly-L-lysine coated slides using a robotic microarrayer developed in our laboratory [6]. Genes were spotted with intervals of 210  $\mu\text{m}$  and each gene probe was spotted in duplicate on the same slide. Total RNA was isolated from  $1.5 \times 10^9$  cells by Qiagen Rneasy columns as manufacturer's protocol. Fluorescence labeled DNAs were made by reverse transcription of total RNA (25  $\mu\text{g}$ ) with a random hexamer (10  $\mu\text{g}$ ). The DNA microarray was scanned by GenePix 4000B (Axon Instruments, Inc. CA). Signal intensities and local background were determined by GenePix Pro 3.0. From the analysis of the scanned image, 25 genes were up-regulated and 81 genes were down-regulated by more than 2-fold. Also, many changes in gene expression level of known genes related to many parts of energy metabolism, transport, and ribonucleotide biosynthesis were detected.

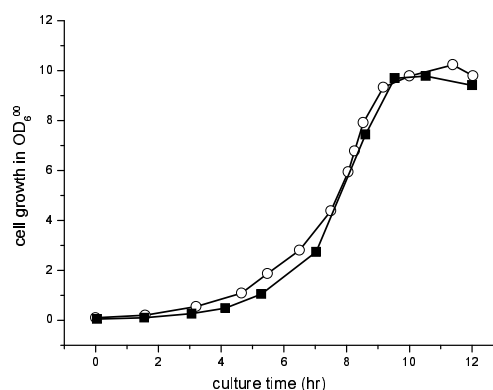


Figure 1: Time profiles of cell growth for *E. coli* W3110 (○) and its *ackA-pta* mutant (■).

## 3 Discussion

In this study, many important changes in cellular metabolism and physiology of *E. coli* between wild type *E. coli* and its mutant were identified by the analysis of transcriptome profiles. The interruption of acetate production caused many metabolic changes and the growth. The detailed results and discussion will be presented along with the possible physiological explanation.

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