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Expression profile analysis of metabolism of *Escherichia coli* during high cell density cultivation using DNA chip

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Abstract

DNA chip containing 207 *E. coli* genes related to important metabolisms such as (TCA cycle, glycolysis, fermentaion and etc) were used to carry out a comprehensive investigation of the change in metabolism and physiology during high cell density culture of *E. coli* by fed-batch cultivation.

Introduction

Biological science is being revolutionized by the availability of much information in regards to a complete genome sequence for many different organisms. Much effort is being devoted to determine the sequence of many other organisms including humans. Having these enormous amount of data with the advanced technology allows us to analyze thousands of genes in parallel, which have recently been developed. With the advanced technology at hand, main trend in biological research is rapidly changing from a structural DNA analysis to understanding cellular function of the DNA sequences. Recently developed DNA chip has emerged as a prime candidate for the performance of such analyses. The study of gene expression on a genomic scale is the most obvious opportunity to be made possible by a complete genome sequences of the model organisms. DNA chip makes it possible to measure the transcription levels of all genes at the same time.

A primary goal of fermentation research is the cost-effective production of desired products using high productivity (i.e. the amount of product formed per unit volume per unit time) techniques. For achieving high cell-density culture (HCDC), many fermentation techniques such as continuous culture, two-stage fermentation, and fed-batch culture have been developed. Among these fed-batch cultures have most often been used to obtain high cell-density. The development of HCDC techniques for *E. coli* has led to efficient, high-level production of various proteins and non-protein products such as amino acids

and poly(3-hydroxybutyrate).

However, with limited understanding of cell physiology and metabolism during high cell density culture, it is hard to develop efficient way for producing desired product by fed batch culture. In this study, we monitored transcriptional level of metabolism related genes during the high cell density culture of E. coli by using DNA chip and analysed expression profile of genes.

Materials and methods

1) Fed-batch cultivation

Escherichia coli W3110 was used in this work.. Frozen glycerol stock (100 μL) at -70 ℃ was used to inoculate 50 mL tube containing 10 mL of modified R medium. After cultivation at 37 ℃ and 250 rpm for 12 h in a shaking incubator, it was transferred into a 1 L flask containing 200 mL of modified R medium. The culture was then grown at 37 ℃ and 250 rpm before it was used to inoculate a bioreactor (6.6 L, Bioflo 3000, New Brunswick Scientific Co., Edison, NJ) containing 2 L of modified R-medium (Lee and Chang, 1993). The composition of the modified R-medium was (per L:) glucose, 10g/L; KH_2PO_4, 6.65g/L; $(NH_4)_2HPO_4$, 2g/L; citric acid, 0.85g/L; $MgSO_4 \cdot 7H_2O$, 0.8g/L; trace metal solution, 5mL/L. The trace metal solution consisted of the following (per L of 5 M HCl): $FeSO_4 \cdot 7H_2O$, 10g; $CaCl_2 \cdot 2H_2O$, 2g; $ZnSO_4 \cdot 7H_2O$, 2.2g; $MnSO_4 \cdot 4H_2O$, 2g; $CuSO_4 \cdot 5H_2O$, 1g; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.1g; $Na_2B_4O_7 \cdot 10H_2O$, 0.02g. Cells were cultured at 37 ℃ and pH 6.8 by automatic feeding of 25% NH_4OH. Dissolved oxygen level was maintained above 40% of air saturation by supplying air (1.5 vvm) and by manually controlling the agitation speed up to 1000 rpm. Pure oxygen was supplemented when required. The composition of the nutrient feed was (per L): glucose, 500g; $MgSO_4 \cdot 7H_2O$, 20g.

2) Preparation of DNA chip

DNA chip containing 207 different genes of *E. coli* was made using microarrayer which was developed by our lab. Genes were spotted with intervals of 500 μm and the spotted *E. coli* genes can be grouped according to their metobolic roles: Glycolysis (42 ea), TCA cycle(29 ea), Fermetaion (22 ea), Chaperones (9 ea), Central intermediary metabolism (32 ea), Pyruvate dissimiliation (5 ea), Secretion-related (10 ea), beta-oxidation (6 ea), Sigma factor (6 ea), Filametaion-related (26 ea), and others (18 ea). Information regarding *E. coli* gene sequence was obtained from *E. coli* genome project home page at Wisconsin University (http://www.genetics.wisc.edu) and the open

reading frames (ORFs) were amplified by the polymerase chain reaction (PCR). Primers for each coding sequence were designed by the primer design program that we developed. For immobilizing DNA on a solid support, a surface of the slide glass is treated with poly-L-lysine (Sigma, St. Louis, U.S.A.). Detailed microarrayer information and experimental procedures are available in our laboratory homepage (http://che.kaist.ac.kr/~apbiot/).

3) Hybridization and Scanning

The cell cutures were collected by centrifugation and resuspended in a 0.3 mL of ice-cold buffer. 30 L of 0.2 M Vanadyl Ribonuclease Complex (Gibco BRL, Gaithersburg, U.S.A.), 0.3 mL of hot lysis buffer, and 3 μL of 20 mg/ml Proteinase K were added and this mixture was incubated in 37 ℃ for 30 min. After phenol was extracted, ethanol precipitated, and dried. The resulting RNA was resuspended in a 200 μL of DEPC-treated water and consecutively treated for 35 min with 12 units RNase-free DNase followed by 15 min with 40 μg of Proteinase K. An additional phenol extraction was performed before ethanol precipitation. Cy5-dUTP (Amersham, Uppsala, Sweden) was incorporated during reverse transcription of RNA by using a primer (Superscript II, Gibco BRL, Gaithersburg, U.S.A.). The reaction mixture was diluted with 470 μL of TE buffer (pH 8.0) and subsequently concentrated to about 20 μL using a Microcentricon-30 (Amicon, Massachusetts, U.S.A.). Purified and labeled cDNA volume was 20 μL at 4×SSC buffer. Before hybridization, the solution was boiled for 2 minutes and then cooled at room temperature. Hybridization was performed for 16 hrs in a 65 ℃ water bath. To prevent drying of the hybridization solution during the hybridization process, 10 μL of 3×SSC was placed on two corners of the hybridization chamber. After hybridization, DNA chips were first washed in a 0.2×SSC with 0.1% (w/v) SDS and subsequently rinsed with 0.2×SSC. Excess liquid on the surface was removed by centrifugation. The DNA chip was scanned by ScanArray 5000.

Analysis of global gene expression data was done by using softwares which were developed by Stanford university (http://cmgm.stanford.edu/pbrown/).

Results and discussion

Bacterial cell growth was shown in Fig. 1. The sampling time points were when the cell density in OD_{600} reached around 1 (S1), 5 (S2), 30 (S3), 60 (S4), 100 (S5), and 140 (S6). The cDNA made from purified mRNA from each sample (S2 to S6) was labeled with the fluorescent dye Cy5 (red) and mixed with a

common reference probe consisting of cDNA made from purified mRNA from cell culture (control, S1) labeled with a second fluorescent dye, Cy3 (green). The image of the subsequent scan (Cy3 and Cy5) shows genes whose mRNAs are more abundant in the control sample as green spots and genes whose mRNAs are more abundant in the S5 (cell growth of 100 in OD_{600}) as red spots. Yellow spots represent genes whose expression does not vary substantially between the two samples. The other images and profile analysis will be presented.

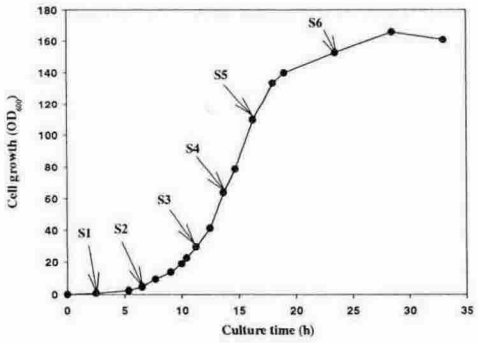

Fig. 1

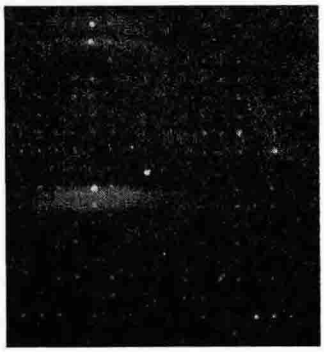

Fig. 2

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