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Combined analysis of transcriptome and proteome for high cell density cultivation of *Escherichia coli*

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Abstract

For understanding physiology and metabolism under various culture conditions, combined analysis of transcriptome and proteome is attractable way. We have manufactured DNA microarray containing 2,850 genes including all functionally known and putative ones. In this study, we report analysis of transcriptome and proteome during the high cell density culture of *E. coli* by using DNA microarray and 2-DE. Fed-batch fermentation of *E. coli* was carried out by exponential feeding of nutrients until the maximum cell density reached 74 g dry cell weight/L (g DCW/L). Changes in transcriptome and proteome during the HCDC are analyzed qualitatively and quantitatively to provide their physiological and metabolic meanings.

Introduction

Recently, techniques to compare the whole cellular transcriptome or proteome in response to various environmental changes have been developed, which are enabling global understanding of cellular physiology and metabolism. Transcriptome analysis allows comparison of global changes in gene expression that occur in response to varying environmental conditions. High-density DNA microarray has been used extensively to analyze gene expression in various prokaryote systems[1]. This type of analysis can provide important information on physiological changes, and consequently can be used to identify connections between regulatory and metabolic pathways that were previously unknown. The proteome analysis using two-dimensional gel electrophoresis (2-DE) in conjunction with MALDI-TOF can also provide valuable information to elucidate the integrated cellular responses when bacterial cells grow under various environments[2].

In this study, we manufactured DNA microarray containing 2,850 genes including all functionally known and putative ones. We report analysis of transcriptome and proteome during the high cell density culture of *E. coli* by using DNA microarray and 2-DE. Fed-batch fermentation of *E. coli* was carried out by exponential feeding of nutrients

until the maximum cell density reached 74 g dry cell weight/L. Changes in transcriptome and proteome during the HCDC were analyzed qualitatively and quantitatively to provide their physiological and metabolic meanings.

Materials and methods

Escherichia coli W3110 (derived from K-12, F-, λ-, prototrophic) was used in this study. Frozen glycerol stock (100 mL) at -70℃ was used to inoculate 50 mL tube containing 10 mL of modified R medium containg 10 g/L of glucose. 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 for 8 h before it was used to inoculate into a 6.6 L bioreactor containing 2 L of modified R medium. The feeding solution contained 700 g/L glucose plus 20 g/L $MgSO_4 \cdot 7H_2O$. During the fed-batch cultures, the feeding solution was added by the exponential feeding strategy[3] to support the specific growth rate (μ) of 0.14 h^{-1}.
Total of 2,850 open reading frames (ORFs) including all functionally known genes were amplified by polymerase chain reaction (PCR). The primers for PCR were purchased from Sigma Genosys (N.S.W., Australia). The resulting 2,850 gene probes were arrayed on poly-L-lysine coated slides using a robotic microarrayer developed previously[4]. Genes were spotted with intervals of 210 μm and each gene probe was spotted in duplicate on the same slide. Total RNA was isolated from 1.5×10^9 cells by Qiagen Rneasy columns as manufacturer's protocol. Fluorescence labeled DNA was made during reverse transcription of total RNA (25 μg) by using a random hexamer (10 μg). The DNA microarray was scanned by ScanArray 5000 (GSI-Lumonics). Signal intensities and local background were determined by Image-Quant (GSI-Lumonics). Following background subtraction, signal intensities were calculated as the percent of total signal as a means of normalization. Hierarchical clustering and self organizing map method was applied to clustering genes using Stanford Software (http://rana.lbl.gov/).
The 2-DE was carried out using Protean II xi 2-D Cell (Bio-Rad Laboratories, Hercules, CA) following the procedures described previously[5]. The protein samples (300 μg) were resuspended in 350 μL of IEF denaturation buffer and were loaded onto the IPG strips (17 cm). The loaded strips were placed on 12% SDS-PAGE gels prepared by a standard protocol[5]. Protein spots were visualized by PowerStain gel staining system and Melanie II software (Bio-Rad Laboratories, Hercules, CA) was used to automate the process of finding protein spots within the image and to quantify the density of the spots in volume basis. To check the reproducibility and to estimate standard deviation, protein

samples were analyzed in duplicate 2-DE gels. Identification of protein spots by Matrix Assisted Laser Desorption/Ionization-Time Of Flight (MALDI-TOF) mass spectrometer (Voyager Biospectrometry, PerSeptive Biosystems Inc., Framingham, MA), was carried out as described previously[1]. ProteinProspector server (http://prospector.ucsf.edu/ucsfhtml3.2/msfit.htm) was used for the identification of protein spots by querying the trypsin digested peptide fragment data. To maintain the highest certainty of protein identification, mass tolerance was set within 50 ppm. Reference database used for the identification of target proteins was SWISS-PROT (http://www.expasy.ch/spot).

Results and discussion

The time profiles of cell growth and concentration of glucose, acetic acid and lactic acid during the HCDC are shown in Fig. 1. During the fed-batch period, the specific growth rate could be successfully controlled at a constant value of 0.14 h^{-1}, and the accumulation of glucose, acetic acid and lactic acid was negligible. Glucose started to accumulate from 24 h of cultivation, which resulted in the deviation of the specific growth rate from the set value. At the end of fed-batch culture (27 h), cell concentration reached 74 g DCW/L. For the analysis of transcriptome and proteome, samples were taken at the cell concentrations of 0.13 (S1), 0.88 (S2), 3.5 (S3), 12.7 (S4), 24 (S5), 40 (S6), 52 (S7), 62 (S8), 72 (S9) and 74 (S10) g DCW/L. The cDNAs made from the samples S2 to S10 were labeled with Cy5. Each of these labeled cDNAs was mixed with a reference cDNA made from sample S1 labeled with Cy3. 695 out of 2,850 genes showing observable gene expression level variation were selected, and these genes were applied to analysis of self-organizing map and hierarchical clustering. Most genes were clustered by the response at the batch stage, exponential stage and stationary stage. Most prominent feature of hierarchical clustering is highly up-regulated expression of transporter genes (*pstA*, *pstABCS*, *phnCDEFGIJKLMN*, *ugpQEC*, *ptsA*, *and phoRB*) which is known to be induced under Pi limitation. Genes involved in central metabolism showed relatively constant gene

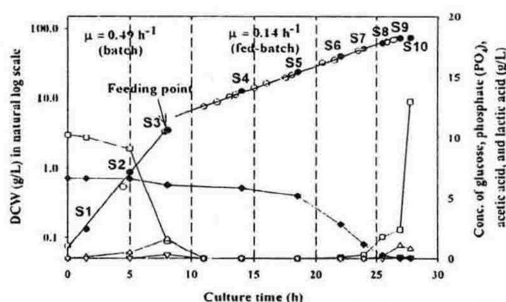

Fig. 1. Time profiles of the concentrations of dry cell weight (○ and ● for sampling), glucose (□), acetic acid (△), phosphate(◆), and lactic acid(▽) during fed-batch fermentation of *E. coli* W3110. In the batch phase, specific growth rate (μ) was 0.49 h^{-1}, and controlled by exponential feeding with μ = 0.14 h^{-1}

expression and down-regulated at entering stationary phase. Most genes involved in TCA cycle were constantly up-regulated after feeding, and down-regulated at entering stationary phase. Population cell density itself was not an inducer of major changes in metabolism, and strongly affect porin regulation, especially *ompF*, and hence the permeability of the outer membrane. At the stationary phase caused by phosphate limitation, rapidly increased gene expression level were found in genes for most of phosphate (P_i) transporter system under Pi limitation, phoE porin, alkylhydroperoxide reductase, *rpoE* regulon (*rpoH, rpoE rseABC*) which are induced by increased level of misfolded OMPs.

In proteome analysis, one of the most distinguishable variations upon high cell density, the Udp, Cdd, and DeoC of pyrimidine salvage pathway were significantly up-regulated during exponential phase. The expression of heat shock proteins (MopA and DnaK) was up-regulated and constitutively synthesized a large amount during exponential phase. Also, *E. coli* trigger factor (Tig), which is a molecular chaperone involved in cell division, was shown to be similar to above heat shock proteins. For oxidative stress defense, the superoxide dismutase (SodA) and alkyl hydroperoxide reductase (AhpC) were gradually increased by cell density. In this paper, to study systematically the variation of proteome and transcriptome in *E. coli* cell under high cell density culture, we combined the techniques of 2D-protein gel electrophoresis and DNA microarray to analyze. Although a little discrepancies were observed in a few genes, the patterns of gene-expression found by proteome and transcriptome analysis were largely similar and this combined techniques served the much more powerful analysis.

Acknowledgments

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