Transcriptome analysis for the production of recombinant protein in *Escherichia coli* using DNA microarray

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Abstract

Transcriptome analysis was performed for the production of recombinant protein in *E. coli* using DNA microarray containing 2,850 genes including all functionally known and putative ones. Changes in transcriptome were analyzed qualitatively and quantitatively to provide their physiological and metabolic meanings.

Introduction

DNA microarray has been used extensively to analyze gene expression in *Escherichia coli* systems. This analysis can provide important information about cell physiology and has the potential to identify connections between regulatory or metabolic pathways that were not previously known. *E. coli* is the most commonly used host for the recombinant protein production and best-studied microorganism. Overexpression of recombinant protein by induction with Isopropyl-1-D-thiogalactopyranoside (IPTG) can complicate the metabolism and physiology of *E. coli*[1]. However, knowledge on cell physiology and metabolism in the induction system is still not sufficient for understanding cell-response from various culture conditions, and this makes it hard to develop efficient way for producing desired product using *E. coli*. In this study, we manufactured DNA microarray containing 2,850 genes including all functionally known and putative ones. Changes in transcriptome level during the production of recombinant protein using induction system, were analyzed qualitatively and quantitatively to provide their physiological and metabolic meanings.

Materials and methods

E. coli BL21(DE3) was used as a host strain for the production of recombinant protein. The recombinant protein was synthesized from the strong T7 promoter by induction with IPTG. Frozen glycerol stock (100 μL) at -70℃ was used to inoculate 50 mL tube containing 10 mL of modified R medium containing 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. Total of 2,850 open reading frames (ORFs) including all

functionally known genes were amplified by polymerase chain reaction (PCR). The resulting 2,850 gene probes were arrayed on poly-L-lysine coated slides using a robotic microarrayer developed in our laboratory. 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 GenePix 4000B (Axon Instruments, Inc. CA). Signal intensities and local background were determined by GenePix Pro 3.0.

Result and discussion

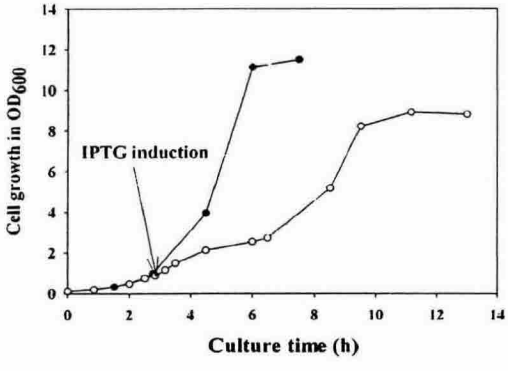

Fig. 1. Time profiles of cell growth for *E. coli* BL21(●) and BL21 producing recombinant protein (○)

Batch cultures of *E. coli* BL21(DE3) and BL21(DE3) producing recombinant protein were carried out (Fig. 1). The cells were induced at cell density of 1 in OD_{600} with 1 mM of IPTG. After induction, the cell growth rate was dramatically decreased, and regained in 4 h afterwards. In transcriptome analysis, many changes in gene expression level of known stress genes related to chaperoning and SOS response were detected. Therefore, overexpression of foreign protein imposed severe metabolic burden and this influence the metabolism and physiology of induced cells. The detailed results and discussion will be presented along with the possible physiological explanation.

Acknowledgments

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References

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