# Study on the variation of cellular physiology of *Escherichia coli* during high cell density cultivation using 2-dimensional gel electrophoresis

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**Abstract**
Physiological changes of *Escherichia coli* during the fed-batch fermentation process were characterized in this study. Overall cellular protein samples prepared at the different stage of fermentation were separated by 2-dimensional gel electrophoresis (2-DE), and differently expressed 15 proteins, Phosphotransferase enzyme I, GroEL, Trigger factor, β subunit of ATP synthase, Transcriptional regulator KDGR, Phosphoglycerate mutase 1, Inorganic pyrophosphatase, Serine Hydroxymethyl-transferase, α subunit of RNA polymerase, Elongation factor Tu, Elongation factor Ts, Tyrosine-tRNA ligase, DnaK suppressor protein, Transcriptional elongation factor, 30S ribosomal protein S6 were identified using matrix-assisted laser desorption / ionization time-of-flight mass spectrometry (MALDI-TOF MS). When bacterial cells grow to high cell density, and IPTG-inducible heterologous protein is produced, expression level of overall cellular proteins was decreased. According to their functions in the cell, identified proteins were classified into three groups, proteins involved in transport process, small-molecule metabolism, and synthesis and modification of macromolecules.

**Introduction**
High cell density cultivation (HCDC) has been developed as a widely used method for the enhancement of productivity of recombinant proteins. To achieve the high protein productivity, various efforts, such as the optimization of growth medium and culture conditions, and the establishment of feeding strategies has been made, along with the construction of optimal protein expression system. However, studies on the changes of cellular physiology, which occur during the HCDC, especially when bacterial strains grow to high cell density, have rarely been done. Since the high reproducibility of 2-dimensional gel electrophoresis (2-DE), combined with the accuracy of mass spectrometry for the identification of particular spots in 2-D gels, was established, proteomics, the study about the protein complement of a genome, has been proposed as a superb tool for the understanding of cellular metabolism at the protein level. The aim of proteomics is to find out what proteins and how much of those proteins are expressed under what conditions. While genome sequencing projects have played a major role in discovering the information about gene sequences, many functional properties of organisms are determined by the variable gene products, not by the static gene sequences. The choice of protein for the characterization of cellular status is persuasive, because cellular phenotype at a particular time is much more dependent on the amount and kind of expressed proteome, rather than transcribed mRNA. In our study, proteome expression patterns of *Escherichia coli* sampled at two different stages of HCDC were demonstrated on 2D-gels. Variations of relative expression level of particular proteins were monitored using software-aided protein quantification tool, and differently expressed proteins were identified using Matrix-

assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry.

## Materials and Methods

### *Bacterial strain, plasmid, and fed-batch fermentation*

The *E. coli* strain used in this work was BL21(DE3) (*E. coli* B F- *dcm ompT hsdS* (rb-mb-) *gal* λ (DE3)). The concentrations of each component in the used media were followed: glucose, 10g/L; $KH_2PO_4$, 6.65g/L; $(NH_4)_2HPO_4$, 2g/L; citric acid, 0.85g/L; MgSO47H2O, 0.8g/L; trace metal solution, 5mL/L. Feeding solution used for the fed-batch culture contains per liter: glucose 500g; $MgSO_47H_2O$, 20g. The dissolved oxygen was maintained above 40% of air saturation by increasing the agitation speed up to 1000rpm and then adding pure oxygen when required. The media pH was controlled at 6.8 by the addition of ammonia water and feeding solution. Nutrient feeding solution was fed by the pH-stat strategy. Cell growth was monitored spectrophotometrically by measuring the absorbance at 600 nm ($OD_{600}$). Cellular dry weights (CDW) were determined as mentioned in the reference.

### *2-Dimensional gel electrophoresis(2-DE)*

The overall method for the 2-DE was based on procedures described previously. SDS-heat method was used for the preparation of 2-DE sample.

### *Image processing*

Coomassie brilliant blue (G250) (Biorad Co.) was used for gel staining. After overnight destaining, gels were scanned by GS710® Calibrated Imaging Densitometer (Biorad Co.). Melanie II® software (Biorad Co.) was used for the quantification of detected protein spots of the scanned 2D-gels. Detected protein spots were digitized, and quantified as volume base (i.e. the integration of spot's optical intensity over the spot's area).

### *Protein identification using MALDI-TOF Mass spectrometry*

Overall procedures for the preparation of MALDI-TOF sample was followed as mentioned in the reference with some modifications by us. The MALDI system used was Voyager™ Biospectrometry (Perseptive Biosystems, Inc., Framingham, MA). Laser intensity for the ionization of sample was 2200, accelerating voltage for the flight of ionized particles was 20,000, and delayed extraction time was 120nsec.

ProteinProspector server (http://prospector.ucsf.edu/ucsfhtml3.2/msfit.htm) was used for the identification of proteins. m/z values of each peptide were entered, and within the 50ppm mass tolerance desired proteins were searched. SWISS-PROT was chosen for the reference database.

## Results and Discussion

### *Fed-batch fermentation*

pH-stat fed-batch fermentation of BL21(DE3) was carried out as indicated in the Materials and Method. Figure 1. shows the time profile of cell growth of BL21(DE3) during the fermentation. To exclude any possibilities of the variation of proteome expression level generated from the shift of external factors, such as pH, temperature, and dissolved oxygen level, each parameters were controlled at 6.8, 37°C, and 40% air saturation respectively. Also in the pH-stat fermentation glucose was present in a constant way to allow the concentration to be minimal after the feeding start. In the fed-batch of BL21(DE3), sample was prepared from cells grown with the high specific growth rate($1.375h^{-1}$). After passing through the exponential growth phase, specific

growth rate was declined to $0.092h^{-1}$, at which time sample✱ was made. Therefore, growth rate dependent variation, along with the cell density effects can be reviewed.

***Expressed protein profiles***

Whole cellular proteins from the sample✂ and sample✱, separated by two parameters, charge and size, are shown in figure 2 and 3 respectively. Protein profiles of these two 2D-gels show that the significant decrease in the amount and number of cellular proteins occurs, when bacterial cells grow to the high cell density. Within our limited gel images, overall proteome profiles are reproducible, and spots are quite distinctive enough to be matched and compared quantitatively. The primary goal of this study was to characterize the physiological changes by identifying differently expressed proteins. Density-derived variation of the protein expression level was verified by the software-aided quantification (Figure 4). We chose 30 proteins which showed different expression level for identification by MALDI-TOF MS, and 15 proteins, which were discovered with the highest certainty, are listed in table 1., and out of 15 proteins only three proteins, phosphoglycerate mutase1 (spot No.11), inorganic pyrophosphatase (spot No.12), and DnaK suppressor protein (spot No.13) showed increase in expression under density-stressed condition.

***Categorized functions of identified proteins***

In order to investigate the physiological changes, identified proteins were categorized according to their functions in the cell (table 1). Interestingly, 15 identified proteins were sorted out into three major functions, and 60% of these proteins were found out to be deeply involved in the process for the synthesis and modification of macromolecules, such as DNA, RNA, and protein.

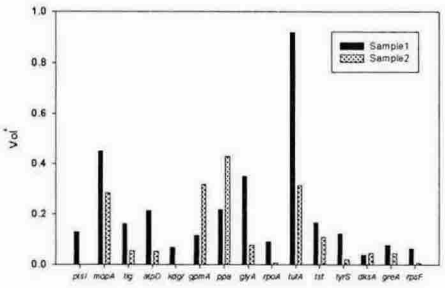


Figure 4. Expression level of identified proteins from sample 1 and 2
*Volumes are the arbitrary value obtained from the integration of spot optical intensity over spot area

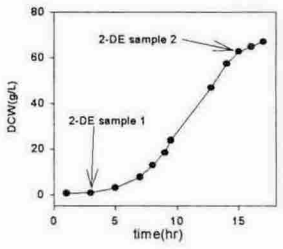


Figure 1. Fed-Batch culture of BL21(DE3)

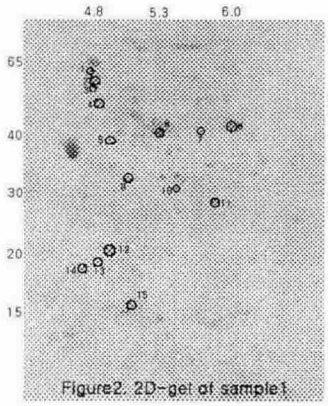


Figure2. 2D-gel of sample1

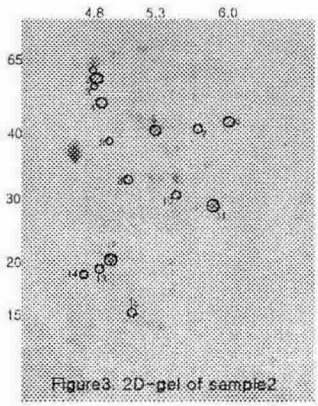


Figure3. 2D-gel of sample2

Table 1. Categorized Functions of Identified Proteins

| Cellular functions and Proteins | Description | Spot No. |
|---|---|---|
| *1. Cell Process* | | |
| Phosphotransferase system enzyme I | Transport | 1 |
| *2. Small Molecule Metabolism* | | |
| ATP synthase, beta subunit | Energy Metabolism/ATP-Protein motive force | 4 |
| Transcriptional Regulator KDGR | Carbon Compound Degradation | 10 |
| Phosphoglycerate mutase I | Energy Metabolism/Glycolysis | 11 |
| Inorganic pyrophosphatase | Central Intermediary Metabolism | 12 |
| Serine Hydroxymethyltransferase | Amino Acid Biosynthesis | 8 |
| *3. Macromolecular Process* | | |
| 60kDa Chaperonine (GroEL) | Chaperone | 2 |
| Trigger Factor | Cell Division | 3 |
| RNA Polymerase, alpha subunit | DNA Transcription | 5 |
| Protein Chain Elongation Factor (EF-Tu) | Protein Translation and Modification | 6 |
| Translation Elongation Factor (EF-Ts) | Protein Translation and Modification | 9 |
| Tyrosine-tRNA Ligase | Aminoacyl tRNA Synthesis | 7 |
| DnaK Suppressor Protein | DNA Replication | 13 |
| Transcription Elongation Factor | Protein Translation and Modification | 14 |
| 30S Ribosomal Protein S6 | Ribosomal Protein Synthesis and Modification | 15 |

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**Reference**

1. **S. Y. Lee,** 1996. High cell density culture of *Escherichia coli.* Trends Biotechnol. **14**:98-105
2. **D. F. Hochstrasser.** 1998. Proteome in Perspective. Clin. Chem. Lab. Med. **36**:825-836.
3. **Alan Dove**. 1999. Proteomics: translating genomics into products? Nat. Biotechnol. **17**:233-236
4. **Peter James**. 1997. Of Genomes and Proteomes. Biochem Biophys Res Commun. **231**:1-6
5. **Michael J. Geisow.** 1998. Proteomics: One small step for a digital computer, one giant leap for humankind. Nat. Biotechnol. **16**:206