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Proteome Analysis of Overproduction of Recombinant Protein in *Escherichia coli* by Fed-Batch Fermentations

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Abstract

Proteomics is a formalized approach for obtaining a rapid snap-shot of the protein complement of a tissue, cell or cell component. Such an approach is powerful in that it allows a parallel assessment of temporal protein fluxes. This is an important concept in view of the dynamic nature of protein expression. Undoubtedly, changes in protein expression are essential in any study aimed at investigating cellular networks. In this study, we analyzed and compared the proteomes of recombinant *E. coli* strain before and after induction. Proteome expression patterns of recombinant *E. coli* were resolved on 2D-gels, and the variations in the relative expression level of particular proteins were examined using software-aided protein quantification tool. We observed above 800 spots on a 2D-gel using Melanie II software. Many proteins which involved in chaperones were significantly up-regulated in recombinant *E. coli*. Therefore, it could be concluded that the expression of recombinant protein in *E. coli* acted as a stress to the cells, which change cells ability to synthesize proteins and induced the expression of various protective proteins.

Introduction

Escherichia coli is one of the most widely used hosts for the production of heterologous proteins and its genetics are far better characterized than those of any other microorganism. The formation of intracellular protein aggregates, referred to as inclusion bodies (IBs), is a phenomenon often accompanying the expression of recombinant proteins in *E. coli*. Inclusion body formation is not limited to damaged, mutant or foreign proteins. Altered conditions of expression of the wild type *E. coli* proteins within the normal host can also result in inclusion body formation. Importantly, recombinant protein overexpression results in a rapid stress response. A key feature of this response is increased

protease activity. Additional features include increased plasmid instability and cell lysis and decreased growth rate. This response is generally referred to as the metabolic burden associated with the production of a nonessential protein. The phenotypic characteristics of this response are similar to the responses *E. coli* have developed to heat shock and amino acid limitation. Interestingly, the stress response of *E. coli* includes an increase in genetic variation (transposition events) which is presumed to provide the host with a tool for adaptation to environmental changes, a feature not desired when maintaining reproducibility among repeated fermentations. Several studies indicate an increase in the levels of heat shock proteins like DnaK, GroEL, and GrpE after overexpression of heterologous proteins. The heat shock-like response during heterologous protein expression may also enhance intracellular proteolytic activities. Furthermore, host stress proteins, like DnaK, GroEL, IbpA, IbpB, and OmpT, have been reported to be associated with the protein aggregates[3].

In this study, the cellular response to overproduction of recombinant protein in high-cell-density cultures is investigated by 2-gels and MALDI-TOF mass spectrometry. The recombinant protein was produced as inclusion bodies in *E. coli*, and the recombinant protein content was as high as 30% of the total protein content.

Material and Method

Cell culture conditions

E. coli BL21(DE3) was used as a host strain. The recombinant protein was synthesized from the strong T7 promoter by induction with isopropyl-β-D-thiogalactopyranoside (IPTG). Cells were cultivated in 250-ml flasks containing 50 ml of Luria-Bertani (LB) medium supplemented with 50 μg of ampicillin per ml in a shaking incubator at 37°C and 200 rpm as the same procedures[2]. Fed-batch cultures were grown in a 6.6-liter jar fermentor (Bioflo 3000; New Brunswick Scientific Co., Edison, N.J.) containing 1.8 liters of R/2 medium. Expression of the *obese* gene was induced by adding IPTG to a final concentration of 1 mM.

Two-dimensional gel electrophoresis (2-DE)

The 2-DE was carried out using Protean II xi 2-D Cell (Bio-Rad Laboratories, Hercules, CA) following the procedures described previously with slight modifications as follows[1]. Culture broth was centrifuged for 5 min at 3,500 × g

and 4℃. The pellet was washed 4 times with low salting washing sample buffer and was then resuspended in a buffer containing 10 mM Tris-HCl pH 8.0, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithiothreitol (DTT), 0.5 mM Pefabloc SC, 0.1% (w/v) sodium dodecyl sulfate (SDS), and stored at -20℃. After the protein quantification by Bradford assay using bovine serum albumin, protein samples (200 μg) were resuspended in IEF denaturation buffer [9 M Urea, 0.5% (w/v) CHAPS 10 mM DTT, 0.2% (w/v) Bio-lyte pH 3-10, 0.001% (w/v) Bromophenol Blue], and the whole final diluted sample was loaded onto the immobilised pH gradient (IPG) strips pH 4-7, 17 cm. Isoelectric-focusing (IEF) was performed using PROTEAN IEF CELL (Bio-Rad Laboratories, Hercules, CA) by a step-wise protocol for 60,000 vh for IPG strips 17 cm. After buffer exchange, the loaded strips were placed onto homogenous 12% SDS-PAGE gels. Silver stained gels were scanned using GS710 Calibrated Imaging Densitometer (Bio-Rad Laboratories, Hercules, CA). 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 (i.e. values were calculated from the integration of spot optical intensity over the spot area). Each 2-DE gel was matched with *E. coli* SWISS 2DPAGE and identified proteins by MALDI-TOF mass spectrometer. To check the reproducibility and to estimate standard deviation, protein samples taken from duplicate cultures were analyzed in duplicate 2-DE gels.

Results and Discussion

Fed-batch cultivation

Fed-batch culture of recombinant *E. coli* was grown as described above. *E. coli* BL21(DE3) without the plasmid was also carried out as controls. The time profile of recombinant *E. coli* was shown Figure 1.

Expressed protein profiles

We prepared for 4 samples at the nearly same concentration from recombinant *E. coli* and controls, respectively. Proteome expression profile of soluble proteins is shown in Figure 2. From two independent experiments, overall proteome profiles are reproducible, and spots are quite distinctive enough to be matched and compared quantitatively. We matched with SWISS 2DPAGE *E. coli* master gel, and database of our laboratory and resulted in identifying with good certainty on 2D-gels. Density-derived variation of the protein expression level

was verified by the software-aided quantification. From a number of identifiable protein spots, we were able to understand global physiological changes for overproduction of the recombinant model protein and this valuable information would be utilized towards the strain improvement and the construction of various databases regarding metabolism.

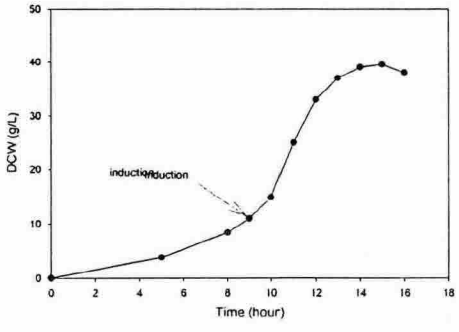

Figure 1. The time profile of recombinant *E. coli*.

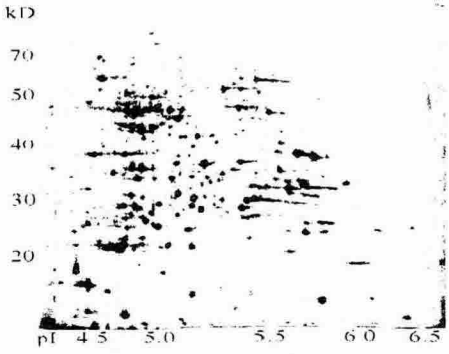

Figure 2. Proteome profile of soluble protein of recombinant *E. coli*.

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