A Proteomic Approach to Engineer Improved Cell Growth and Increased Production of Serine-Rich Proteins in *Escherichia coli*

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1 Introduction

Escherichia coli has been most widely used for the production of various recombinant proteins and as it has been far better characterized than any other microorganism in every aspects. Overexpression of recombinant proteins results in a rapid stress response and changes in the metabolism of *E. coli* [3]. These cellular responses may lead to plasmid instability, ribosome destruction, growth inhibition or even cell lysis, all of which negatively affect recombinant protein production. The yield of a recombinant protein is generally proportional to both the final cell density and the specific protein productivity. Therefore, various strategies have been developed for cultivating *E. coli* to high densities and/or optimizing expression systems for increased specific protein productivity.

Proteome profiling is a systematic and powerful tool for understanding physiological changes and for developing metabolic and cellular engineering strategies. In this study, we studied overexpression of a human leptin in *E. coli* and used proteomics to design a better bioprocess to achieve increased leptin yield.

2 Materials and Methods

2.1 Bacterial Strain, Plasmid and Culture Conditions

E. coli XL1-Blue was used as a host strain for cloning and maintenance of plasmids. *E. coli* BL21(DE3) was used as a host strain for the expression of the obese gene. The mature obese gene in pEDOb5 (2) was expressed from the strong T7 promoter by induction with isopropyl-β-D-thiogalactopyranoside (IPTG). Fed-batch cultures were carried out in a 6.6-liter jar fermentor (Bioflo 3000, New Brunswick Scientific Co., Edison, NJ) containing 1.8 liters of R/2 medium plus 20 g/liter glucose as described by Jeong and Lee [2].

2.2 Analytical Methods

For protein quantification, cells at the same concentrations were harvested by centrifugation at 3,500 × g for 5 min at 4°C. Protein samples were analyzed by electrophoresis on SDS-PAGE gels containing 12% (w/v) polyacrylamide as described by Laemmli [4]. The gels were stained with Coomassie brilliant blue R250 (Bio-Rad, Hercules, CA), and the protein bands were quantified by a GS-710 Calibrated Imaging Densitometer (Bio-Rad). The 2-DE was carried out using Protean II xi 2-D Cell (Bio-Rad Laboratories, Hercules, CA) following the procedures described previously [1]. Protein spots were visualized using a silver staining kit (Amersham Biosciences, Uppsala, Sweden), and the stained gels were scanned by a GS-710 Calibrated Imaging Densitometer (Bio-Rad). Melanie II software (Bio-Rad) was used to identify spots and to quantify spot densities on a volume basis (i.e. integration of spot optical intensity over the spot area). The Matrix Assisted Laser Desorption/Ionization-Time Of Flight mass spectrometer (MALDI-TOF MS) analysis was carried out as reported previously [1].

3 Results and Discussion

Variations in proteome profiles of *E. coli* in response to the overproduction of human leptin, a serine-rich (11.6% of total amino acids) protein, were examined by 2-dimensional gel electrophoresis. The levels of heat shock proteins increased while those of protein elongation factors, 30S ribosomal protein, and some enzymes involved in amino acid biosynthesis decreased after leptin overproduction. Most notably, the levels of enzymes involved in the biosynthesis of serine family amino acids significantly decreased. Based on this information, we designed a strategy to enhance the leptin productivity by manipulating the *cysK* gene, encoding cysteine synthase A. The growth of recombinant BL21(DE3) and leptin production could be enhanced by *cysK* co-expression. Co-expression of the *cysK* gene could increase the biosynthetic flux of serine family amino acids and indirectly repress EF-Tu aggregation by inducing the expression of heat shock proteins, leading to improved cell growth and a three- to four-fold increase in the productivity of serine-rich recombinant proteins. It should be mentioned that the high productivities of serine-rich proteins achieved by *cysK* co-expression are not solely due to the improved cell growth. This is the first report on improving recombinant protein productivity by engineering the metabolic pathways based on the results of proteome analysis. Consequently, we propose a strategy for the rational engineering of metabolic pathways and cellular properties based on the results of proteome profiling. The procedure of this approach is: (i) obtain the proteome profiles of *E. coli* (or recombinant *E. coli*) under different conditions of interest, (ii) identify potentially limiting enzymes in the biosynthetic pathways, (ii) examine theoretically and/or experientially the possible flux changes that can be achieved by amplifying (or knocking out) the activities of the enzymes identified, (iii) select the final candidate enzymes to be amplified (or knocked out), (iv) examine the effects of this metabolic and cellular engineering on achieving the desired objectives, and (v) repeat step (ii) to step (iv) until the objectives are accomplished. This strategy may be extended beyond serine-rich proteins to increase the yield and productivity of other recombinant proteins in industrial bioprocesses.

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