Enhanced Production of Insulin-Like Growth Factor I Fusion Protein in *Escherichia coli* by Coexpression of the Down-Regulated Genes Identified by Transcriptome Profiling

Jong Hyun Choi,¹ Sang Jun Lee,^{1,2} Seok Jae Lee,¹ and Sang Yup Lee^{1,2,3*}

Metabolic and Biomolecular Engineering National Research Laboratory, Department of Chemical and Biomolecular Engineering and BioProcess Engineering Research Center,¹ Department of BioSystems and Bioinformatics Research Center,³ and Center for Ultramicrochemical Process Systems,² Korea Advanced Institute of Science and Technology, Yuseong-gu, Daejeon 305-701, Korea

Received 10 March 2003/Accepted 10 June 2003

The transcriptome profiles of recombinant *Escherichia coli* producing human insulin-like growth factor I fusion protein (IGF-I_r) during the high-cell-density fed-batch culture were analyzed using DNA microarrays. The expression levels of 529 genes were significantly altered after induction. About 200 genes were significantly down-regulated during the production of IGF-I_r after induction. Among these down-regulated genes, we rationally selected and coexpressed in *E. coli* producing IGF-I_r the *prsA* gene (encoding a phosphoribosyl pyrophosphate synthetase) and the *glpF* gene (encoding a glycerol transporter), which are involved in an early key step in the biosynthetic pathway of nucleotides and amino acids (Trp and His) and the first step in glycerol utilization, respectively. As a result, the production of IGF-I_r could be increased from 1.8 ± 0.13 ($\pm$ standard deviation) to 4.3 ± 0.24 g/liter. The volumetric productivity was also increased from 0.36 ± 0.027 to 0.82 ± 0.048 g/liter/h. These results demonstrate that transcriptome profiling can provide invaluable information in designing engineered strains showing enhanced performance.

Escherichia coli has been the workhorse for the production of recombinant proteins (7, 20). Various strategies have been employed for the development of *E. coli* strains which are able to efficiently produce recombinant proteins (14). Once a recombinant host strain is developed, a high level of production of recombinant proteins is generally achieved by high-cell-density fed-batch cultivation (13).

It has been well known that the overproduction of recombinant proteins acts as a stress on the cells, resulting in induction of stress-responsive genes such as *dnaK*, *groEL*, *ibpA*, *ibpB*, and *ompT* (10, 17). Furthermore, the yield and specific productivity of recombinant proteins obtained by high-cell-density culture are generally lower than those obtained by flask cultures (9, 21). Since these problems are caused by unknown complex metabolic events, systematic understanding of the physiology and metabolism altered during the fed-batch culture of recombinant *E. coli*, especially those altered before and after induction, is essential to develop strategies for solving these problems. Transcriptome analysis employing high-density DNA microarrays is well suited for the elucidation of global physiological and metabolic changes. However, most microarray-based studies of transcriptome profiling in *E. coli* have been carried out in flask cultures (18, 19). In flask cultures, cells grow to a relatively low density, and the growth environment is continuously changing. Therefore, the results of transcriptome profiling obtained in flask cultures cannot be extended to high-

cell-density fed-batch cultures, which are industrially relevant processes.

Recently, combined transcriptome and proteome analysis of *E. coli* during high-cell-density culture was reported (21). The expression of many genes including those of tricarboxylic acid cycle enzymes, NADH dehydrogenase, and ATPase was up-regulated during the exponential fed-batch period and was down-regulated afterwards. Interestingly, the expression of most amino acid biosynthesis genes was down-regulated as the cell density increased (21).

In this study, we extended our previous work and applied transcriptome profiling to understand physiological changes of recombinant *E. coli* producing human insulin-like growth factor I fusion protein (IGF-I_r) during the high-cell-density fed-batch culture. We were able to rationally select two down-regulated genes after induction and subsequently used them to develop engineered strains that were capable of enhanced production of IGF-I_r.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1. *E. coli* XL1-Blue was used as a host strain for general cloning works. Recombinant *E. coli* W3110 harboring pYKM-I_r was used for the production of IGF-I_r, which is human insulin-like growth factor I fused to the truncated β -galactosidase (11).

DNA manipulation. PCR was performed with a PCR Thermal Cycler MP TP3000 (Takara Shuzo, Kyoto, Japan) with High Fidelity PCR Master mix (Roche, Indianapolis, Ind.). All DNA manipulations were carried out as described in the work of Sambrook et al. (16). To construct an expression vector containing the *lac* promoter, PCR was performed using pUC19 as a template and oligonucleotide primers 5'-GGAATTCCATATGTGTTTCCTGTGTGAAATGTGT and 5'-TGCTCACATGTTCTTTCCTG, which contain *Nde*I and *Afl*III sites (underlined), respectively. The *Nde*I- and *Afl*III-digested PCR product was cloned into the *Nde*I-*Afl*III-digested pET3a to make pJHlac. The *E. coli prsA* gene, which encodes phosphoribosyl pyrophosphate synthetase (315 amino ac-

* Corresponding author. Mailing address: Department of Chemical and Biomolecular Engineering, Korea Advanced Institute of Science and Technology, 373-1 Guseong-dong, Yuseong-gu, Daejeon 305-701, Korea. Phone: 82-42-869-3930. Fax: 82-42-869-8800. E-mail: leesy@kaist.ac.kr.

TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristics	Reference or source
<i>E. coli</i> strains		
XL1-Blue	<i>SupE44 hsdR17 recA1 endA1 gyrA96 thi relA1 lac F' [proAB⁺ lacI^q lacZΔM15 Tn10(Tet^r)</i>	Stratagene ^a
W3110	Derived from K-12; λ ⁻ F ⁻ ; prototrophic	Lab stock
Plasmids		
pACYC184	4.2 kb; Cm ^r Tc ^r	New England Biolabs ^b
pYKM-I ₁	6.9 kb; Ap ^r ; <i>tac</i> promoter, human insulin-like growth factor I fused to the truncated β-galactosidase gene	11
pJHlac	2.7 kb; Ap ^r ; <i>lac</i> promoter	This study
pJHlac-prsA	5.2 kb; Cm ^r ; <i>lac</i> promoter; <i>prsA</i> gene	This study
pJHlac-prsAg1pF	6.1 kb; Cm ^r ; <i>lac</i> promoter; <i>prsA</i> and <i>glpF</i> genes	This study

^a Stratagene Cloning Systems, La Jolla, Calif.^b New England Biolabs, Inc., Beverly, Mass.

ids), was amplified using the chromosomal DNA of *E. coli* W3110 as a template. The primers used were 5'-GATAGATATCCTGCAGGTCGACCCAAGCGGACATCACCATCATCACCAT and 5'-CCAAGGATCCGATATCCTCGAGACAGAAATGGTGATGATGGTGATG, which contain *NdeI* and *BamHI* restriction sites (underlined), respectively. The *NdeI*- and *BamHI*-digested PCR product was cloned into the *NdeI*-*BamHI*-digested pJHlac. The resulting plasmid pJHlac-prsA was used as a template for the amplification of *lac* promoter plus the *prsA* gene with oligonucleotide primers 5'-GATAGTCTAGAAATATCCTGCAGGTCGACCCAAGCGGACATCACCATCATCACCAT and 5'-CCGGATCCAAGTGCAGGATATCCTCGAGACCAATGGTGATGATGGTGATG, which contain *XbaI* and *BamHI* restriction sites (underlined), respectively. The *XbaI*- and *BamHI*-digested PCR product was cloned into the *XbaI*-*BamHI*-digested pACYC184 to make pJHlac-prsA (Fig. 1A), which is compatible with the IGF-I_r expression plasmid pYKM-I₁. In order to construct a plasmid carrying both the *prsA* and *glpF* genes, the *glpF* gene, which encodes glycerol transporter (291 amino acids), was amplified using the chromosomal DNA of *E. coli* W3110 as a template. The oligonucleotide primers used were 5'-CGGGATCCAGAAAGGATATACATATGAGTCAAACATCAACCTTG and 5'-CTG CAGAA CCGCCGTTACAGCGAAGCTTTTGTTC, which contain *BamHI* and *EagI* sites (underlined), respectively. The *BamHI*- and *EagI*-digested PCR product was cloned into the *BamHI*-*EagI*-digested pJHlac-prsA to make pJHlac-prsAg1pF (Fig. 1B).

Fed-batch cultures. Fed-batch cultures were carried out at 37°C in a 6.6-liter fermentor (Bioflo 3000; New Brunswick Scientific Co., Edison, N.J.) containing 2 liters of R/2 medium (9) supplemented with 20 g of glycerol/liter and 2 g of yeast extract/liter. The seed culture of *E. coli* W3110 harboring pYKM-I₁ was prepared in a 1-liter flask containing the same medium (0.2 liters) at 37°C and 200 rpm in a shaking incubator and transferred into the fermentor. Culture pH was controlled at 6.8, except for the periods of nutrient feeding (see below), by the addition of 28% (vol/vol) ammonia solution. The dissolved oxygen concentration was controlled at 40% of air saturation by automatically increasing the agitation speed up to 1,000 rpm and by changing the pure oxygen percentage. Nutrient feeding solution containing 500 g of glycerol/liter, 50 g of yeast extract/liter, and 15 g of MgSO₄ · 7H₂O/liter was added by the pH-stat feeding strategy (2). When the pH rose to a value greater by 0.05 than that of its set point (6.8) due to the depletion of glycerol, an appropriate volume of feeding solution was automatically added to increase the glycerol concentration in the culture broth to 0.5 g/liter. Expression of the IGF-I_r gene was induced when the cell concentration reached 14 g (dry cell weight [DCW]) per liter by the addition of isopropyl-β-D-thiogalactopyranoside (IPTG; Sigma Chemical Co., St. Louis, Mo.) to give the final 1 mM concentration. Ampicillin and chloramphenicol were added in the

FIG. 1. Schematic drawing of plasmids pJHlac-prsA (A) and pJHlac-prsAg1pF (B). The plasmids are compatible with IGF-I_r expression plasmid pYKM-I₁. Both the *glpF* and *prsA* genes are under the control of the *lac* promoter, which can be induced by IPTG.

initial medium to concentrations of 15 and 50 mg/liter, respectively, depending on the plasmids employed (Table 1). Fed-batch cultures were carried out in duplicates.

Analytical methods. During the fed-batch cultivation, cell growth was monitored by measuring the absorbance at 600 nm (optical density at 600 nm; DU Series 600 spectrophotometer; Beckman, Fullerton, Calif.). DCW (grams per liter) was determined as described previously (1). Protein samples were analyzed by electrophoresis on a sodium dodecyl sulfate-12% (wt/vol) polyacrylamide gel (12). The protein bands were visualized with Coomassie brilliant blue R-250 stain (Bio-Rad Laboratories, Hercules, Calif.). The contents of IGF-I_r in total protein were quantified with a GS710 calibrated imaging densitometer (Bio-Rad). The total protein concentration was determined with a protein assay kit (Bio-Rad) with bovine serum albumin as a standard.

Microarray experiments. We used the Panorama *E. coli* gene array (Sigma-Genosys, Inc., Woodlands, Tex.), which contains all the open reading frames in the *E. coli* chromosome. To make hybridization probes for the *E. coli* DNA microarray, total RNA was isolated from the culture by using the Qiagen RNeasy columns (Valencia, Calif.). The purified RNA (1 μg) was mixed with random hexamer primers for cDNA labeling reaction, which was performed using 50 U of avian myeloblastosis virus reverse transcriptase (Roche, Basel, Switzerland) and 20 μCi of [³²P]dCTP (2,000 to 3,000 Ci/mmol; New England Nuclear, Boston, Mass.) as recommended by Sigma-Genosys, Inc. The cDNA labeling reaction mixture (30 μl) containing 0.5 mM (each) dATP, dGTP, and dTTP and 10 mM dithiothreitol was incubated for 3 h at 42°C. Unincorporated nucleotides were removed by gel filtration through a G-25 Sephadex column (Amersham Biosciences, Piscataway, N.J.).

The Panorama *E. coli* gene array (Sigma-Genosys) was hybridized with labeled cDNA concentrates plus 0.8 mg of salmon sperm DNA per ml (Amersham Biosciences). Prior to hybridization, the solution was boiled for 10 min and then cooled to room temperature. After hybridization for 18 h at 65°C, the Panorama membrane was washed with 0.5× SSPE solution (0.09 M NaCl, 5 mM NaH₂PO₄, and 0.5 mM EDTA [pH 7.7]) and subsequently rinsed with 0.5× SSPE at 65°C for 20 min. The Panorama membrane was scanned with a BAS 1500 (Fuji, Tokyo, Japan) instrument with a pixel size of 100 μm. The signal intensities and local background were determined by Array Vision (Imaging Research Inc., St. Catharines, Ontario, Canada). DNA microarray experiments were carried out in duplicate. Also, the Panorama *E. coli* gene array contains duplicate spots for each gene. Therefore, a total of four measurements were made for each gene. Raw array data from each experimental replicate were exported from Array

Vision into MS Excel for further analysis. The raw data values were normalized and selected as described previously (4). We could select 529 genes that were significantly up- or down-regulated by a value greater than 3 standard deviations of the mean of the log ratios for all genes (99.9% confidence) and those that had a *P* value greater than 0.05% (95% probability that the ratio is significant).

RESULTS AND DISCUSSION

Transcriptome analysis of recombinant *E. coli* producing IGF-I_f fusion proteins. Fed-batch culture of recombinant *E. coli* W3110 (pYKM-I₁) was carried out using glycerol as a carbon source, because it resulted in better production of IGF-I_f than did glucose (3). The time profiles of cell concentration, IGF-I_f concentration, and IGF-I_f content are shown in Fig. 2A. The specific growth rate during the exponential growth was 0.23 h⁻¹. The maximum cell concentration of 48.9 ± 2.93 (± standard deviation) g (DCW)/liter was obtained in 18 h. The concentration of IGF-I_f steadily increased to reach 1.8 ± 0.13 g/liter at 5 h after induction and then slightly decreased towards the end of cultivation. The IGF-I_f content reached the maximum value of 9.5% of total proteins at 2 h after induction. The volumetric productivity of IGF-I_f was 0.36 ± 0.027 g/liter/h. These values are rather low for the recombinant proteins produced by fed-batch culture of *E. coli* (13). Therefore, we decided to identify limiting metabolic enzymes during the IGF-I_f production by transcriptome profiling. For the analysis and comparison of transcriptomes, fermentation samples were taken before IPTG induction and at 6 h after induction and were analyzed by hybridizing the prepared cDNA of these samples to the Panorama *E. coli* gene array containing 4,290 open reading frames of *E. coli*. The calculation of log expression ratios allowed pairwise comparison of relative transcript levels of all genes. Out of 4,290 open reading frames, 529 genes showing significant variations in the gene expression level were identified. Among them, we selected 50 genes that are well characterized and associated with metabolic pathways of interest (Tables 2 and 3).

First, it was notable that the genes associated with protein biosynthetic apparatus, *rplQT*, *rpmB*, and *rpsS* (encoding ribosomal proteins), were considerably down-regulated after IPTG induction. However, the expression of the *rm* operons encoding rRNAs was not changed. The expression of the *rmD* genes (*yhdZ* and *yhdW*) encoding another 5S rRNA was highly up-regulated. These results suggested that protein synthesis could be negatively affected by the ribosome dysfunction which is caused by the lack of ribosomal proteins, rather than rRNAs.

Second, the expression of the genes associated with the biosynthesis of nucleotides and amino acids was down-regulated. For example, the expression of the *metL* gene was most significantly down-regulated after induction (Table 3). It is interesting that the expression of the *metB* gene, which forms an operon with the *metL* gene, was also significantly down-regulated after induction. This suggests the existence of good correlation between the *metBL* operon structure and the transcript levels of the *metB* and *metL* genes. In addition, the expression of the genes in the biosynthetic pathways of serine, cysteine, glutamate, glutamine, arginine, aspartate, threonine, isoleucine, valine, and histidine was slightly down-regulated. It was notable that the expression of the *prsA* gene was severely down-regulated (>2.7-fold). Ribose-phosphate pyrophosphokinase encoded by the *prsA* gene is involved in the first step

for the biosynthesis of purine, pyrimidine, and nicotinamide nucleotides and for the biosynthesis of histidine and tryptophan. Therefore, it was reasoned that nucleic and amino acid depletion may become a bottleneck in protein production during the high-cell-density fed-batch culture of recombinant *E. coli*.

Third, in the energy and carbon metabolism, the genes in the tricarboxylic acid cycle showed a relatively constant expression level, while the expression of the genes in the glycolytic pathway, such as *gapA*, *eno*, and *tpiA*, was up-regulated. In this work, glycerol was used as a carbon source, because it allowed production of more IGF-I_f than glucose did in a previous study (3). The growth rate with glycerol is about 74% of that achievable with glucose (8). It was notable that the expression of the *glpF* gene encoding a glycerol transporter was significantly down-regulated (>2.2-fold) after induction. Therefore, it was thought that the reduced transport of glycerol might have become a limiting factor for the production of IGF-I_f.

Among the other genes that showed altered expression levels, but less-than-twofold changes, were stress-responsive genes including *dnaK*, *ibpA*, and *ibpB*. They were up-regulated by 1.6-, 1.6-, and 1.8-fold, respectively, which has been a well-known phenomenon during recombinant protein production (5, 6, 10). However, the expression level of the *groEL* gene was not changed. Moreover, the expression of the *ompT* gene was significantly down-regulated (Table 3), coinciding with results previously reported (21).

Effect of *prsA* coexpression on IGF-I_f production. Among the down-regulated genes, we selected the *prsA* gene as the first candidate for amplification, as its product ribose-phosphate pyrophosphokinase is a key enzyme for the biosynthesis of nucleic acids and amino acids. The 5-phosphoribosyl-1-pyrophosphate (PRPP) produced by PrsA is of importance for the biosynthesis of purine, pyrimidine, and nicotinamide nucleotides and of histidine and tryptophan. Since the expression level of the *prsA* gene was significantly decreased after induction, PRPP might be limiting. Therefore, it was reasoned that increasing the level of PrsA would result in the sufficient supply of PRPP for synthesis of purine, pyrimidine, and amino acids and consequently increase the protein production during high-cell-density culture. The effect of the *prsA* gene coexpression on IGF-I_f production was investigated by carrying out the pH-stat fed-batch culture of *E. coli* W3110 harboring pYKM-I₁ and pJHlacL-*prsA* (Fig. 2B). The specific growth rate during the exponential phase was 0.16 h⁻¹, which is lower than that of W3110 harboring pYKM-I₁ only. The maximum cell concentration of 34.2 ± 2.12 (± standard deviation) g (DCW)/liter was obtained in 24 h. The IGF-I_f content reached 22% of total protein at 5 h after IPTG induction, which is 2.3 times higher than that obtained without the coexpression of the *prsA* gene, and then was maintained constantly afterwards. The IGF-I_f concentration reached a maximum value of 3.6 ± 0.19 g/liter at 12 h after induction. The volumetric productivity of IGF-I_f was 0.64 ± 0.034 g/liter/h, which is 1.8 times higher than that obtained with W3110 harboring pYKM-I₁ only. Therefore, the coexpression of the *prsA* gene resulted in the increase of recombinant protein production, possibly by increasing the metabolic fluxes towards the biosynthesis of nucleic acids and amino acids. However, the growth rate was lower than that of *E. coli* W3110 harboring pYKM-I₁ only.

(A)

(B)

(C)

FIG. 2. Time profiles of cell density (optical density at 600 nm [●]), DCW (grams per liter [■]), IGF-I_f concentration (grams per liter [○]), and IGF-I_f content (percentage of total proteins [□]) during the fed-batch cultures of *E. coli* W3110 harboring pYKM-I₁ (A), *E. coli* W3110 harboring pYKM-I₁ and pJHlacL-prsA (B), and *E. coli* W3110 harboring pYKM-I₁ and pJHlacL-prsAglpF (C). Vertical dashed lines indicate the time point of induction with 1 mM IPTG. The arrows in panel A are the sampling points for transcriptome analyses.

Enhancement of volumetric productivity of IGF-I_f by the coexpression of *prsA* and *glpF* genes. To further enhance the volumetric productivity of IGF-I_f, we decided to develop a strategy for restoring the growth rate of W3110 harboring

pYKM-I₁ and pJHlacL-prsA to a value close to that of W3110 harboring pYKM-I₁ only. In this study, glycerol was used as a carbon source for the production of recombinant IGF-I_f. Unlike other carbohydrates, glycerol enters the cytoplasm by fa-

TABLE 2. Selected genes that are up-regulated after IPTG induction

Gene	Δ Fold ^a	Gene product	Functional category(ies)
<i>ybbD</i>	3.9	o86; 100% identical to 67 residues	Unknown
<i>lacZ</i>	3.3	β -Galactosidase	Carbon compound catabolism
<i>modF</i>	2.8	Putative molybdenum transport ATP-binding protein ModF	Transport of small molecules
<i>dapE</i>	2.7	Succinyl-diaminopimelate desuccinylase	Amino acid biosynthesis and metabolism
<i>rhsE</i>	2.6	RhsE protein	Transposon-related functions
<i>ptrB</i>	2.4	Protease II	Degradation of proteins, peptides
<i>dsbB</i>	2.4	Disulfide bond formation protein B	Surface structures
<i>ssb</i>	2.4	Single-stranded DNA-binding protein	DNA replication, repair and modification
<i>sspA</i>	2.4	Stringent starvation protein	Global regulatory functions
<i>nagC</i>	2.4	4-Amino-4-deoxychorismate lyase	<i>N</i> -Acetylglucosamine repressor
<i>hycC</i>	2.3	Formate hydrogen lyase subunit 3	Energy metabolism
<i>csgB</i>	2.2	Nucleation component of curlin monomers	Surface structures
<i>bioC</i>	2.3	Biotin synthesis protein BioC	Biosynthesis of cofactors, carriers: biotin
<i>nhaR</i>	2.2	Transcriptional activator protein NhaR	Transport of small molecules: cations
<i>pxdJ</i>	2.2	Pyridoxal phosphate biosynthetic protein PdxJ	Biosynthesis of cofactors, carriers: pyridoxine
<i>pheM</i>	2.2	Phenylalanyl-tRNA synthetase operon leader peptide	Aminoacyl-tRNA synthetases
<i>fruL</i>	2.1	FruR-Sh1 operon leader peptide	Energy metabolism
<i>dinF</i>	2.1	DNA damage-inducible protein F	DNA replication, repair and modification
<i>uxaC</i>	2.1	Glucuronate isomerase	Carbon compound catabolism
<i>ppdD</i>	2.1	Prepilin peptidase-dependent protein D precursor	Unknown
<i>lacY</i>	2.1	Lactose permease	Transport and binding proteins
<i>thrS</i>	2.1	f642; 99% identical to SYT_ECOLI SW: P00955	Aminoacyl-tRNA synthetases
<i>nrdF</i>	2.1	Ribonucleoside-diphosphate reductase 2 beta chain	Central intermediary metabolism
<i>ompX</i>	2.0	Outer membrane protein X precursor	Outer membrane constituents

^a Fold change of gene expression ratio (6 h after IPTG induction/before induction).

cilitated diffusion across the cytoplasmic membrane by use of the facilitator protein GlpF. The expression level of the *glpF* gene was significantly decreased after induction (Table 3). It means that the cell's ability to transport glycerol is significantly decreased after induction. In order to increase glycerol transport, the *E. coli glpF* gene was coexpressed in *E. coli* W3110

harboring pYKM-I₁. Both the *glpF* and *prsA* genes were coexpressed in *E. coli* W3110 harboring pYKM-I₁. The pH-stat fed-batch culture of *E. coli* W3110 harboring pYKM-I₁ and pJHlacL-prsAglpF was then carried out (Fig. 2C). The specific growth rate was 0.22 h⁻¹, which is similar to that of W3110 harboring pYKM-I₁ only. The maximum cell concentration of

TABLE 3. Selected genes that are down-regulated after IPTG induction

Gene	Δ Fold ^a	Function	Functional category(ies)
<i>metL</i>	4.4	Aspartokinase II/homoserine dehydrogenase II	Amino acid biosynthesis and metabolism
<i>atpF</i>	3.7	ATP synthase Fo subunit b	ATP-proton motive force interconversion
<i>artP</i>	3.4	Arginine transport ATP-binding protein ArtP	Transport and binding proteins
<i>cadA</i>	3.3	Lysine decarboxylase	Amino acid biosynthesis and metabolism
<i>rplX</i>	3.2	50S ribosomal subunit protein L20	Ribosomal proteins, synthesis and modification
<i>rpsS</i>	3.0	30S ribosomal subunit protein S19	Ribosomal proteins, synthesis and modification
<i>atpC</i>	2.7	ATP synthase F ₁ epsilon subunit	ATP-proton motive force interconversion
<i>tnaA</i>	2.7	Tryptophanase	Amino acid biosynthesis and metabolism
<i>narK</i>	2.7	Nitrite extrusion protein (nitrite facilitator)	Transport of small molecules: anions
<i>talC</i>	2.7	f451; 93% identical to amino acids 1 to 415 from PTKC_ECOLI SW	Transport and binding proteins
<i>prsA</i>	2.7	Ribose-phosphate pyrophosphokinase	Ribonucleotide biosynthesis
<i>upp</i>	2.6	Uracil phosphoribosyltransferase	Nucleotide biosynthesis and metabolism
<i>hrpB</i>	2.6	ATP-dependent helicase HrpB	DNA replication, repair, and modification
<i>atpL</i>	2.6	ATP synthase subunit	ATP-proton motive force interconversion
<i>ppiB</i>	2.6	Peptidyl-prolyl <i>cis-trans</i> isomerase B	Translation, posttranslational modification
<i>upp</i>	2.6	Uracil phosphoribosyltransferase	Salvage of nucleosides and nucleotides
<i>rplT</i>	2.5	50S ribosomal subunit protein L24	Ribosomal proteins, synthesis and modification
<i>rplT</i>	2.5	50S ribosomal subunit protein L10	Ribosomal proteins, synthesis and modification
<i>emrR</i>	2.5	EmrR protein	Plasmid-related functions
<i>nohB</i>	2.5	DNA-packaging protein	Phage-related functions and prophages
<i>talC</i>	2.4	Transaldolase-like protein	Energy metabolism
<i>atpD</i>	2.4	ATP synthase F ₁ beta subunit	ATP-proton motive force interconversion
<i>rhsD</i>	2.3	RhsD protein precursor	Transposon-related functions
<i>edd</i>	2.3	6-Phosphogluconate dehydratase	Central intermediary metabolism
<i>glpF</i>	2.2	Glycerol transporter	Transport and binding proteins
<i>ompT</i>	2.2	Protease VII precursor	Outer membrane protein

^a Fold change of gene expression ratio (before induction/6 h after IPTG induction).

FIG. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of total proteins of recombinant *E. coli* W3110 harboring pYKM-I₁ (A) and W3110 harboring pYKM-I₁ and pJHlacL-prsAglpF (B). Lanes: M, molecular mass markers; 1, proteins prepared before induction; 2 to 7, 1 to 6 h after IPTG induction, respectively. The arrows indicate recombinant IGF-I_F.

46.2 ± 2.53 (± standard deviation) g (DCW)/liter was obtained in 20 h. The IGF-I_F content reached 19.4% of total protein at 5 h after IPTG induction. The IGF-I_F concentration increased to 4.3 ± 0.24 g/liter at 8 h after induction. The volumetric productivity of IGF-I_F of 0.82 ± 0.048 g/liter/h was obtained at 6 h after induction, which is 2.3 and 1.3 times higher than those obtained with W3110 harboring pYKM-I₁ only and W3110 harboring pYKM-I₁ plus pJHlacL-prsA, respectively. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis clearly shows the improvement of IGF-I_F production by the coexpression of the *prsA* and *glpF* genes (Fig. 3). To examine the effect of GlpF on the productivity of IGF-I_F, the *glpF* gene alone was coexpressed in *E. coli* W3110 harboring pYKM-I₁. In this case, the level of IGF-I_F was not increased, but the specific growth rate was increased (data not shown). Therefore, the increased level of GlpF is responsible for better growth of recombinant *E. coli* producing IGF-I_F.

Transcriptome profiling has recently been widely applied to understand the global physiological changes caused by environmental and/or genetic changes (15, 19). The target genes to be overexpressed or possibly deleted for the achievement of a

desired goal can be rationally selected based on the transcriptome profiling results. This approach, as successfully demonstrated in this study, should prove invaluable in designing engineered microorganisms capable of better performance.

ACKNOWLEDGMENTS

This work was supported by the Basic Industrial Research Project of the Korean Ministry of Commerce, Industry and Energy (MOCIE) and by the Center for Ultramicrochemical Process Systems. Further support from LG Life Sciences Ltd., Samchully Pharmaceutical Co. Ltd., and BioLeaders Co. is also appreciated. Hardware for computational analysis was supported by the IBM Shared University Research Program.

REFERENCES

- Choi, J., S. Y. Lee, K. Shin, W. G. Lee, S. J. Park, H. N. Chang, and Y. K. Chang. 2002. Pilot scale production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) by fed-batch culture of recombinant *Escherichia coli*. *Biotechnol. Bioprocess Eng.* 7:1-4.
- Choi, J. H., K. J. Jeong, S. C. Kim, and S. Y. Lee. 2000. Efficient secretory production of alkaline phosphatase by high cell density culture of recombinant *Escherichia coli* using the *Bacillus* sp. endoxylanase signal sequence. *Appl. Microbiol. Biotechnol.* 53:640-645.
- Chung, B. H., Y. J. Choi, S. H. Yoon, S. Y. Lee, and Y. I. Lee. 2000. Process development for production of recombinant human insulin-like growth factor-I in *Escherichia coli*. *J. Ind. Microbiol. Biotechnol.* 24:94-99.
- Conway, T., B. Kraus, D. L. Tucker, D. J. Smalley, A. F. Dorman, and L. McKibben. 2001. DNA array analysis in a Windows-PC environment. *Bio-Techniques* 32:110-119.
- Gill, R. T., M. P. DeLisa, J. J. Valdes, and W. E. Bentley. 2001. Genomic analysis of high-cell-density recombinant *Escherichia coli* fermentation and cell conditioning for improved recombinant protein yield. *Biotechnol. Bioeng.* 72:85-95.
- Gill, R. T., J. J. Valdes, and W. E. Bentley. 2000. A comparative study of global stress gene regulation in response to overexpression of recombinant proteins in *Escherichia coli*. *Metab. Eng.* 2:178-189.
- Hockney, R. C. 1994. Recent developments in heterologous protein production in *Escherichia coli*. *Trends Biotechnol.* 12:456-463.
- Holms, H. 1996. Flux analysis and control of the central metabolic pathways in *Escherichia coli*. *FEMS Microbiol. Rev.* 19:85-116.
- Jeong, K. J., and S. Y. Lee. 1999. High-level production of human leptin by fed-batch cultivation of recombinant *Escherichia coli* and its purification. *Appl. Environ. Microbiol.* 65:3027-3032.
- Jurgen, B., H. Y. Lin, S. Riemschneider, C. Scharf, P. Neubauer, R. Schmid, M. Hecker, and T. Schweder. 2000. Monitoring of genes that respond to overproduction of an insoluble recombinant protein in *Escherichia coli* glucose-limited fed-batch fermentations. *Biotechnol. Bioeng.* 70:217-224.
- Kim, S. O., and Y. I. Lee. 1996. High-level expression and simple purification of recombinant human insulin-like growth factor I. *J. Biotechnol.* 48:97-105.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680-685.
- Lee, S. Y. 1996. High cell-density culture of *Escherichia coli*. *Trends Biotechnol.* 14:98-105.
- Makrides, S. C. 1996. Strategies for achieving high-level expression of genes in *Escherichia coli*. *Microbiol. Rev.* 60:512-538.
- Richmond, C. S., J. D. Glasner, R. Mau, H. Jin, and F. R. Blattner. 1999. Genome-wide expression profiling in *Escherichia coli* K-12. *Nucleic Acids Res.* 27:3821-3835.
- Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: a laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Schweder, T., H. Y. Lin, B. Jurgen, A. Breitenstein, S. Riemschneider, V. Khalameyzer, A. Gupta, K. Buttner, and P. Neubauer. 2002. Role of the general stress response during strong overexpression of a heterologous gene in *Escherichia coli*. *Appl. Microbiol. Biotechnol.* 58:330-337.
- Tao, H., C. Bausch, C. Richmond, F. R. Blattner, and T. Conway. 1999. Functional genomics: expression analysis of *Escherichia coli* growing on minimal and rich media. *J. Bacteriol.* 181:6425-6440.
- Wei, Y., J. M. Lee, C. Richmond, F. R. Blattner, J. A. Rafalski, and R. A. LaRossa. 2001. High-density microarray-mediated gene expression profiling of *Escherichia coli*. *J. Bacteriol.* 183:545-556.
- Weickert, M. J., D. H. Doherty, E. A. Best, and P. O. Olins. 1996. Optimization of heterologous protein production in *Escherichia coli*. *Curr. Opin. Biotechnol.* 7:494-499.
- Yoon, S. H., M. J. Han, S. Y. Lee, K. J. Jeong, and J. S. Yoo. 2003. Combined transcriptome and proteome analysis of *Escherichia coli* during high cell density culture. *Biotechnol. Bioeng.* 81:753-767.