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ORIGINAL PAPER

Design of a recombinant *Escherichia coli* for producing L-phenylalanine from glycerol

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Abstract A recombinant *Escherichia coli* was engineered to produce the commercially important amino acid L-phenylalanine (L-Phe) using glycerol as the carbon source. Compared to the conventionally used glucose and sucrose, glycerol is a less expensive carbon source. As phenylalanine dehydrogenase (PheDH) activity is involved in the last step of L-Phe synthesis in *E. coli*, a phenylalanine dehydrogenase gene (*phedh*) from the thermotolerant *Bacillus lentus* was cloned into pRSFDuet-1 (pPheDH) and expressed in *E. coli* BL21(DE3). The resulting clone had a limited ability to produce L-Phe from glycerol, possibly because of a poor glycerol uptake by the cell, or an inability to excrete L-Phe, or both. Therefore, *yddG* gene encoding an aromatic amino acid exporter and *glpF* gene encoding a glycerol transport facilitator were coexpressed with the *phedh* in a reengineered *E. coli*. In a glycerol medium, the maximum L-Phe production rates of the clones pPY (*phedh* and *yddG* genes) and pPYF (*phedh*, *yddG* and *glpF* genes) were 1.4- and 1.8-fold higher than the maximum production rate of the pPheDH clone. The better producing pPYF clone was further evaluated in a 5 l stirred-tank fermenter (37 °C, an aeration rate of 1 vvm, an agitation speed of 400 rpm). In the fermenter, the maximum concentration of L-Phe (366 mg/l) was achieved in a much shorter period compared to in the shake flasks. In the latter, the highest titer of L-Phe was only 76 % of the maximum value attained in the fermenter.

Keywords Phenylalanine dehydrogenase · L-Phenylalanine · Glycerol facilitator · Aromatic amino acid exporter · *Bacillus lentus* · *Escherichia coli* BL21(DE3)

Introduction

L-Phenylalanine (L-Phe) is one of the most commercially important amino acids. It is used in the food and pharmaceutical industries and is the most expensive component of the widely used dipeptide artificial sweetener aspartame. In view of its importance, attempts have been made to produce L-Phe in recombinant microorganisms including *Escherichia coli*.

L-Phenylalanine biosynthesis occurs naturally in *E. coli*; however, it can be enhanced by introduction of a foreign phenylalanine dehydrogenase (PheDH, EC 1.4.1.20) into the bacterium. The introduced PheDH activity links to the activities of the three native aminotransferases found in *E. coli*, to enhance the conversion of phenylpyruvate to L-Phe. PheDH is an interesting amino acid dehydrogenase that catalyzes the reversible pyridine nucleotide-dependent reductive amination of phenylpyruvate to form L-Phe (Brunhuber and Blanchard 1994).

Recently, we isolated a thermotolerant PheDH-producing bacterium from a soil sample and identified it to be *Bacillus lentus*. PheDH from this high yielding isolate was

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purified and characterized in detail. This enzyme was shown to be highly thermostable: it retained 50 % of its initial activity after incubation at 50 °C for 3 days (Inkure 2005). In view of this exceptional stability, the nucleotide sequence of phenylalanine dehydrogenase gene (*phedh*) from *B. lentus* (GenBank accession no. EU880599) was obtained and the gene was cloned in pET-17b to produce the plasmid pBLpheDH. The plasmid pBLpheDH was subsequently transformed into *E. coli* BL21(DE3) to create the PheDH producing *E. coli* (Thongchuang 2006). Although the purified PheDH could be used to produce L-Phe in vitro using phenylpyruvate as the substrate, the high cost of the substrate precluded commercial use of this process.

To overcome this problem, a bacterial culture route to production of L-Phe starting from a low-cost carbon substrate was sought. The substrate of interest was glycerol (1,2,3-propanetriol) which is generated in huge quantities as a byproduct of manufacture of biodiesel from vegetable oils and animal fats (da Silva et al. 2009). Production of L-Phe from glycerol using a recombinant *E. coli* BL21(DE3) containing *phedh* from *Acinetobacter lwoffii* had been previously demonstrated (Khamduang et al. 2009), therefore, a similar approach using the *phedh* from *B. lentus* which has a higher substrate specificity on phenylpyruvate appeared feasible.

In *E. coli*, glycerol crosses the cytoplasmic membrane through passive diffusion and facilitated diffusion (Lu et al. 2003). Facilitated diffusion is brought about by a membrane integral protein, the glycerol facilitator GlpF, encoded by *glpF*. Intracellular glycerol is subsequently converted to glycerol 3-phosphate (G3P) by glycerol kinase (GlpK). The intracellular G3P is then oxidized through the action of G3P dehydrogenase to form dihydroxyacetone phosphate (DHAP), which is in turn isomerized to glyceraldehyde 3-phosphate (GA3P) (da Silva et al. 2009). Both DHAP and GA3P are further metabolized in the glycolysis pathway, the pentose phosphate pathway and an aromatic amino acid biosynthesis pathway.

The aromatic amino acid biosynthesis pathway in *E. coli* is initiated by the condensation reaction between phospho*enol*pyruvate (PEP) from the glycolysis pathway and erythrose 4-phosphate (E4P) from the pentose phosphate pathway (Bongaerts et al. 2001). At the last step of L-Phe biosynthesis in *E. coli*, phenylpyruvate is transformed to L-Phe by the activities of three aminotransferases. PheDH activity is able to link to the aromatic amino acid biosynthesis pathway at this step through incorporation of the enzyme via cloning of *phedh* from *B. lentus* into *E. coli*. A recombinant *E. coli* incorporating *phedh* of *B. lentus* was found to produce limited levels of L-Phe in a glycerol medium. This was attributed to a limitation of glycerol uptake by the cells, or a limited ability of the cell to excrete L-Phe, or both.

Two of the enzymes (3-deoxy-D-arabinoheptulosonate-7-phosphate synthase and chorismate mutase/prephenate dehydratase) involved in the biosynthetic pathway of L-Phe are subject to feedback inhibition by L-Phe, the end product of the pathway (Simmonds et al. 1954). An alternative route to relieve the feedback inhibition is to reduce intracellular accumulation of L-Phe in *E. coli* by enhancing the cell's ability to export the aromatic amino acid. The YddG protein in *E. coli* functions as an aromatic amino acid exporter. *E. coli* strains overexpressing *yddG* accumulate less L-Phe within the cell and export L-Phe threefold faster than the isogenic strains containing the wild-type *yddG* (Doroshenko et al. 2007).

In this work, the objective was to improve L-phenylalanine production of a *phedh* expressing-*E. coli* grown on glycerol as the carbon source. The bacterium was engineered to express a glycerol facilitator to improve uptake of glycerol and an aromatic amino acid exporter to reduce feedback inhibition by L-Phe. Thus, *yddG* encoding aromatic amino acid exporter and *glpF* encoding glycerol facilitator were coexpressed with *phedh* of *B. lentus*.

Materials and methods

Strains and plasmids

The strains, plasmids and oligonucleotide primers used for polymerase chain reaction (PCR) amplification are noted in Table 1. *E. coli* BL21(DE3) was the host strain used to overexpress all genes throughout this work. *E. coli* TOP10 was used as the source of genomic DNA for amplification of *yddG* and *glpF* genes. pRSFDuet-1 containing a kanamycin resistance gene as the selectable marker gene, was used as an expression vector. The protein expression by these strains could be controlled by IPTG (isopropyl-β-D-thiogalactopyranoside).

Expression of phedh, yddG and glpF genes in *E. coli* BL21(DE3)

Escherichia coli BL21(DE3) cells containing the recombinant plasmids were cultured in 5 ml of LB medium, pH 7.4, supplemented with 30 μg/ml of kanamycin at 37 °C for 18 h. The resulting cell suspension was used to inoculate (2.5 % v/v) 200 ml of the same medium and incubation was continued. Once the turbidity of the culture at 600 nm reached 0.6, IPTG was added to a final concentration of 1 mM and the cultivation was continued for 3 h as recommended by pET system manual (Novagen).

Table 1 Strains, plasmids and oligonucleotides used

	Description	Source/restriction site
Strains		
BL21(DE3)	F$^-$ *ompT hsdS$_B$*(r_B^- m_B^-) *gal dcm* (DE3)	Novagen, Merck
TOP10	F$^-$ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara-leu*) 7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G	Invitrogen, CA
Plasmids		
pET-17b	Expression vector with T7 promoter	Novagen, Merck
pRSFDuet-1	Expression vector with T7*lac* promoter	Novagen, Merck
pBLPheDH	*B. lentus phedh* gene inserted under T7 promoter of pET-17b	Thongchuang (2006)
pPheDH	*B. lentus phedh* gene inserted under T7*lac* promoter of pRSFDuet-1	This study
pYddG	*E. coli* TOP10 *yddG* gene inserted under T7*lac* promoter of pRSFDuet-1	This study
pGlpF	*E. coli* TOP10 *glpF* gene inserted under T7*lac* promoter of pRSFDuet-1	This study
pPY	Each *phedh* and *yddG* preceded by T7*lac* promoter and ribosome bindging site inserted into pRSFDuet-1	This study
pPYF	Each *phedh*, *yddG* and *glpF* preceded by T7*lac* promoter and ribosome bindging site inserted into pRSFDuet-1	This study
Oligonucleotides		
phedh-*Nde*I	5′-GGA ATT CCA TAT GAG CTT AGT AGA AAA AAC ATC CAT CAT A-3′	*Nde*I
phedh-*Eco*RV	5′-ATC TTA GTT GCG AAT ATC CCA TTT TGG CTT AA-3′	Three bases of *Eco*RV
yddG-*Nde*I	5′-GGA ATT CCA TAT GAC ACG ACA AAA AGC AAC GCT CAT A-3′	*Nde*I
yddG-*Xho*I	5′-CCG CTC GAG TTA ACC ACG ACG TGT CGC CAG CC-3′	*Xho*I
glpF-*Nde*I	5′-GGA ATT CCA TAT GAG TCA AAC ATC AAC CTT GAA AGG-3′	*Nde*I
glpF-*Pac*I	5′-CCT TAA TTA ATT ACA GCG AAG CTT TTT GTT CTG AAG G-3′	*Pac*I
T7-*Asc*I	5′-TTG GCG CGC CAC GGC CGC ATA ATC GAA ATT AAT ACG-3′	*Asc*I
yddG-*Hin*dIII	5′-CCC AAG CTT TTA ACC ACG ACG TGT CGC CAG CC-3′	*Hin*dIII
T7-*Xho*I	5′-CCG CTC GAG ACG GCC GCA TAA TCG AAA TTA ATA CG-3′	*Xho*I

The cells were then harvested by centrifugation (8,000*g*, 5 min). The cell pellet was resuspended in 100 µl of the 5× sample buffer (60 mM Tris–HCl, pH 6.8, 25 % glycerol, 2 % SDS, 0.1 % bromophenol blue and 14.4 mM β-mercaptoethanol) and boiled for 15 min. Eight microliters of each resulting cell sample was analyzed by 12 % SDS-PAGE with Coomassie Brilliant Blue R-250 staining (Bollag et al. 1996). The protein molecular weight markers (Fermentas) were β-galactosidase (116 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45 kDa), lactate dehydrogenase (35 kDa), restriction endonuclease Bsp98I (25 kDa), β-lactoglobulin (18.4 kDa) and lysozyme (14.4 kDa).

Shake flask cultures

Shake flask cultures were used to examine for L-Phe production of pPY and pPYF clones in comparison with production by the basal clone (pPheDH). A single colony of each clone was resuspended in 100 ml of LB medium supplemented with 30 µg/ml of kanamycin at 37 °C, 250 rpm, for 18 h. Overnight seed culture (10 ml) was used to inoculate 200 ml of the production medium (pH 7.4) containing (g/l): glycerol 30; $(NH_4)_2SO_4$ 50; $MgCl_2{\cdot}6H_2O$ 0.81; KH_2PO_4 2.43; K_2HPO_4 2.43; yeast extract 0.085; thiamine-HCl 0.0085; $FeSO_4{\cdot}7H_2O$ 0.002; $MnSO_4{\cdot}H_2O$ 0.002; $CaCl_2{\cdot}2H_2O$ 0.05; $ZnSO_4{\cdot}7H_2O$ 0.01; and 30 µg/ml of kanamycin. When the optical density at 600 nm had reached 0.6, IPTG was added to different final concentrations (0, 0.25, 0.5, 1 mM) and incubation was continued. Samples were taken every 24 h until 240 h. All experiments were carried out in triplicate.

Analytical methods

The biomass concentration was monitored by measuring the optical density at 600 nm using a spectrophotometer. The optical density (OD_{600}) was converted to a dry cell weight (DCW) using the following calibration equation: DCW (g/l) = 0.99 × OD_{600}.

For measuring the concentration of residual glycerol, the broth samples were centrifuged at 8,000*g* for 15 min. The residual glycerol was determined using the periodate oxidation method (Bondioli and Bella 2005) by measurement of absorbance at 410 nm (A_{410}). The concentration of

glycerol was calculated using the following calibration equation: glycerol concentration (g/l) = 0.0487 × A_{410}.

For measuring L-Phe, the culture supernatant obtained was filtered through a 0.2 μm syringe filter. L-Phe concentration was then measured by HPLC using a CROWNPAK CR(+) column (150 mm × 4 mm ID; Daicel Chemical Industries, Ltd., Osaka, Japan) equipped with a UV absorbance detector monitored at 200 nm. The mobile phase was 85: 15 v/v mixture of perchloric acid (pH 1.0) and methanol with a flow rate of 0.8 ml/min.

Fermenter cultures

One colony of the pPYF clone was cultivated in 100 ml of LB medium containing 30 μg/ml of kanamycin at 37 °C, 250 rpm, for 18 h. An overnight culture was diluted with fresh LB medium to bring the optical density (OD_{600}) to 0.6. This served as the inoculum for the fermenter cultures.

Batch fermentations were carried out in a 5 l glass stirred tank bioreactor (BioFlo IIc, New Brunswick Scientific, Edison, NJ, USA). The inoculum (5 % of working volume) was transferred to the bioreactor that had been prefilled with 3.5 l of sterile production medium. The cultivation (37 °C, pH 7.4) was carried out for 84 h at an agitation rate of 400 rpm and an aeration rate of 1 vvm. The dissolved oxygen concentration was monitored using a polarographic electrode (Mettler-Toledo). The pH of the broth was controlled at 7.4 by automated addition of 5 M NaOH in response to signals from the pH electrode (Mettler-Toledo). Foaming was controlled by automated addition of a sterile silicon antifoam agent (Ajax Finechem). Once the OD_{600} reached 0.6, 1 mM of IPTG was added. Samples were taken at 4-h intervals for 84 h after induction.

Results and discussion

Construction of recombinant plasmids

The construction of recombinant plasmids for this work were carried out according to Supplementary methods and their structures are shown in Supplementary Fig. S1. The *phedh* gene from *B. lentus* in a backbone plasmid pET-17b (pBLPheDH) driven by T7 promoter was subcloned into pRSFDuet-1 under the control of the T7*lac* promoter to obtain the recombinant plasmid pPheDH (4,955 bp). The plasmid pPheDH was competent for co-expression with *yddG* and *glpF* genes in contrast to our earlier observations with the plasmid pET-17b.

The transmembrane proteins YddG and GlpF are relatively hydrophobic and are believed to be toxic to cells when expressed at a high level. Therefore, both genes typically need to be placed under stringent regulation. The control by T7*lac* promoter is stricter than that of the T7 promoter. Most toxic proteins are expressed well under control of the T7*lac* promoter in BL21(DE3) (Mierendorf et al. 1994). Thus, pRSFDuet-1 vector consisting of T7*lac* promoter is useful in the BL21(DE3) host to reduce the basal transcription level (leakiness) of the proteins in the absence of induction with IPTG and helps in keeping stable a plasmid containing toxic genes (Mierendorf et al. 1994).

The *yddG* (882 bp) encoding an aromatic amino acid exporter and *glpF* (846 bp) encoding a glycerol transport facilitator were successfully amplified from chromosomal DNA of *E. coli* TOP10. These genes were separately cloned into pRSFDuet-1 resulting in recombinant plasmids pYddG (4,657 bp) and pGlpF (4,546 bp) (Supplementary Fig. S1). The correct insertion of the genes on the plasmid restriction sites was confirmed by restriction enzyme digestion and sequencing. The pYddG and pGlpF were used as templates for amplification of the DNA fragment containing the T7*lac* promoter. Subsequently, the *yddG* gene with its own T7*lac* promoter was directly cloned into pPheDH to produce pPY (5,931 bp) containing *phedh* and *yddG* genes. Similarly, a pPYF (6,817 bp) containing *phedh*, *yddG* and *glpF* genes was created by inserting the *glpF* gene and T7*lac*promoter into pPY. Each recombinant plasmid was expressed in *E. coli* BL21(DE3).

The rate of expression of a gene in a vector is known to depend on the distance between the gene and its promoter. A gene located close to the promoter is expressed with a much higher rate compared to a gene that is located far from the promoter (Weckbecker and Hummel 2004). Therefore, coexpression of two or more genes (i.e. *yddG*, *glpF* and *phedh*) in a single pRSFDuet-1 vector requires each gene to have its own T7*lac* promoter, as in plasmids pPY and pPYF (Supplementary Fig. S1).

Expression of phedh, yddG and glpF genes in *E. coli* BL21(DE3)

The existence of PheDH, YddG and GlpF proteins in recombinant *E. coli* induced by 1 mM IPTG was established by SDS-PAGE. An intense protein band at an apparent molecular weight of ~42 kDa in the cell extract of the pPheDH clone (Fig. 1) was consistent with a molecular weight of 41,329.8 Da estimated for PheDH from the *phedh* nucleotide sequence of *B. lentus* by using the ProtParam tool (http://web.expasy.org/protparam/). In contrast, the amounts of the membrane proteins YddG and GlpF in the membrane fraction of the cell extract were insufficient to be detected on Coomassie Blue stained SDS-PAGE gels. In other studies, the expression of these genes has proved to be detectable by radiolabeling (Seol and

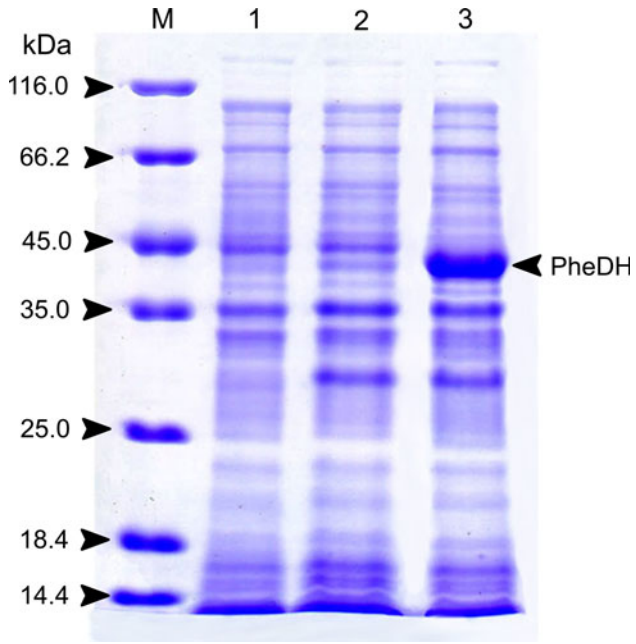

Fig. 1 SDS-PAGE analysis for the expression of *phedh* gene. *Lane M*, standard marker proteins; *lane 1 E. coli* BL21(DE); *lane 2 E. coli* BL21(DE3) harboring pRSFDuet-1; *lane 3 E. coli* BL21(DE3) harboring pPheDH

Shatkin 1992) and also after concentration of His-tagged proteins (Hori et al. 2011).

Shake flask culture

The effects of the added genes on L-Phe production from glycerol were further characterized by comparing the production of the different clones and the parent strain that did not contain the genes. Thus, the clones pPheDH, pPY and pPYF were cultured in a minimal medium containing glycerol and supplemented with IPTG to various final concentrations (0–1 mM). The time elapsed prior to induction was varied in different experiments. The growth of the cells was progressively reduced as the number of inserted genes increased.

The *phedh* expression in each clone was examined by assaying the activity of PheDH (Asano et al. 1987) in the crude extracts of the cells. The results showed that the best level of production of L-Phe did not correspond to the best levels of PheDH activity. This indicates that L-Phe production does not depend only on activity of PheDH but may be affected by the abilities for glycerol uptake, glycerol utilization, L-Phe biosynthesis and excretion of L-Phe. Considering this, only the L-Phe production level was used for a comparative evaluation of the various clones. Similarly, because of the difficulty in directly detecting the membrane-associated protein products of *yddG* and *glpF* genes, only the level of L-Phe production was used to infer about function of these proteins in a given clone.

The growth profiles of *E. coli* clones harboring pPheDH, pPY and pPYF are shown in Fig. 2a–c with and without induction. In the glycerol medium, all the IPTG induced clones generally grew better and attained a higher biomass concentration than the same clones without induction. The concentration of the IPTG inducer (0.25, 0.5 and 1 mM) had no effect on a clone's ability to grow so long as the concentration was at least 0.25 mM. For induced pPheDH clones (Fig. 2a), the induction reduced growth up to 96 h relative to noninduced cells after induction. Afterwards, the induced cells grew faster and attained a higher biomass concentration ($\sim$5 g/l) than the noninduced cells ($\sim$4 g/l). For the noninduced pPY and pPYF clones, the final biomass concentration was in the range of 1.6–2.0 g/l, but with induction it increased by 2- to 2.8-fold depending on the clone (Fig. 2b, c). Although IPTG is known to negatively impact the growth *E. coli* (Law et al. 2002), the IPTG induced pPY and pPYF greatly outperformed the corresponding noninduced (no IPTG) clones. The residual glycerol profiles for the various clones (Fig. 2d–f) were quite consistent with their growth patterns (Fig. 2a–c).

The L-Phenylalanine production profiles for the various clones are shown in Fig. 2. Suitably induced recombinant clones pPheDH, pPY and pPYF produced a final L-Phe concentration of about 250 mg/l by 144 h (Fig. 2g–i), but the clones varied in the inducer concentrations required to achieve this result and the peak of L-Phe production rate. The maximum values of the production rates of L-Phe for the *E. coli* clones pPheDH, pPY and pPYF were 1.6, 2.3 and 2.9 mg/l h, respectively indicating that the maximum L-Phe production rates of the induced pPY and pPYF clones were 1.4-fold ($P < 0.0005$) and 1.8-fold ($P < 0.0005$) higher than the maximum production rate of the pPheDH clone. The additional expression of *yddG* and *yddG*/*glpF* genes resulted in an increased production of L-Phe that was statistically significant at a confidence level of >99.9 %. The pPYF was proved to be the best clone for producing L-Phe and was examined further under more controlled conditions in a bioreactor.

Fermenter cultures

The time profiles of L-Phe production, biomass growth and glycerol consumption by the pPYF clone in a 5 l stirred bioreactor are shown in Fig. 3. The L-Phe production commenced at induction with 1 mM IPTG (0 h, Fig. 3) and the maximum value of 366 mg/l was attained at 56 h after induction. This coincided with exhaustion of glycerol in the medium and consequently there was no further increase either in the concentration of L-Phe or the biomass. In shake flasks, L-Phe was first detected by HPLC at 24 h after induction (Fig. 2i). The production in the bioreactor was detected at the start of induction (0 h, 15.6 mg/l).

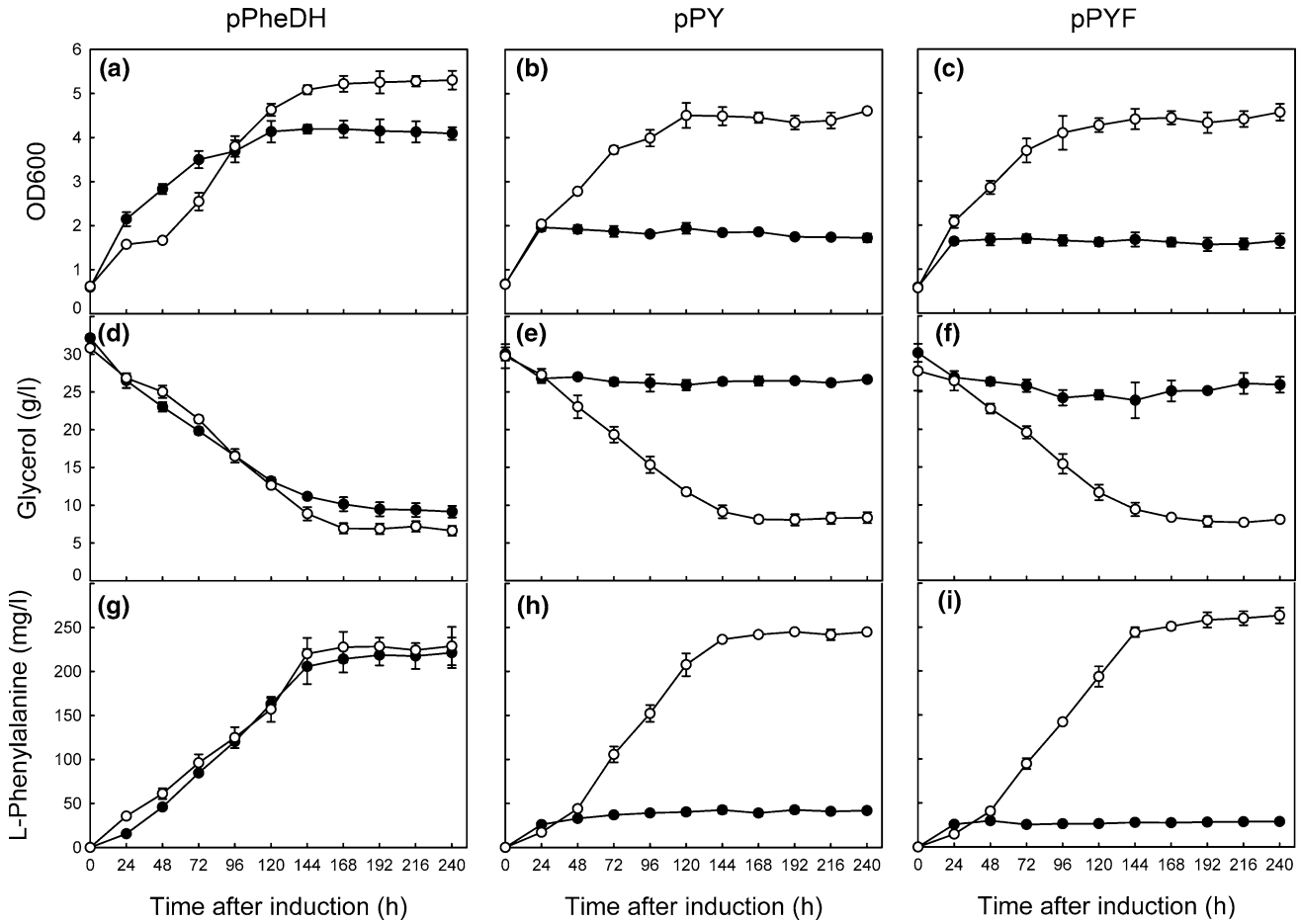

Fig. 2 Time course of cell growth, residual glycerol and concentration of L-Phe for *E. coli* BL21(DE3) harboring pPheDH (**a**, **d** and **g**), pPY (**b**, **e** and **h**) and pPYF (**c**, **f** and **i**) in glycerol medium without induction by IPTG *filled circle* and with a final IPTG concentration of 0.25 mM *open circle* in shake-flask cultures. Data shown are averaged values from three independent experiments. The standard deviation of the measurements was within ±10 % of the average values shown

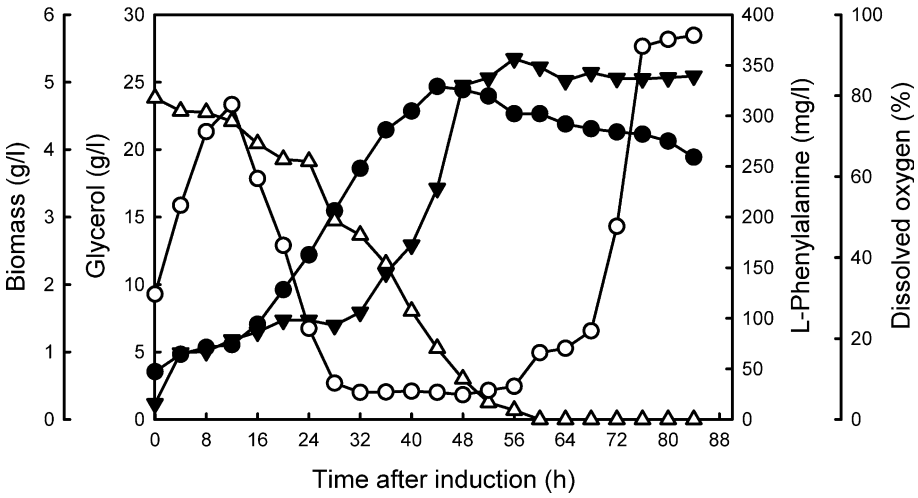

Fig. 3 Fermentation profile of cell growth *filled circle*, residual glycerol *open triangle*, concentration of L-Phe *filled inverted tringle* and dissolved oxygen *open circle* of *E. coli* BL21(DE3) harboring pPYF. Glycerol medium in the fermenter at an aeration rate of 3.5 l/min and an impeller speed of 400 rpm. Data shown are averaged values of two independent experiments

Therefore, in the environment of the bioreactor, the target proteins were expressed and led to L-Phe production/accumulation in the medium prior to induction with IPTG.

In comparison with shake-flasks, the stationary phase of growth occurred earlier (at 64 h from start of fermentation) in the bioreactor because of a highly aerobic environment

(i.e. an aeration rate of 3.5 l/min (1 vvm) and an impeller speed of 400 rpm). Consequently, essentially all glycerol was consumed.

The peak biomass concentration of 4.9 g/l in the bioreactor was not significantly different compared to in the shake flask culture, but the peak L-Phe concentration in the bioreactor (366 mg/l) was nearly 1.3-fold higher than that in the shake flasks (280 mg/l). In the bioreactor, the peak L-Phe concentration was attained at 56 h, but shake flask cultures required 216 h to reach the peak concentration. Thus, L-Phe productivity of the bioreactor was nearly fivefold greater than the shake flask productivity. A better supply of oxygen in the bioreactor explained its higher productivity compared to the shake flasks.

In the present study, the maximum final concentration of L-Phe was less than previously achieved in production from *E. coli* (Leuchtenberger et al. 2005). This was likely because this work focused only on improving transport of the substrate and the excretion of the product, but not on the steps of the tightly regulated biosynthesis pathway of aromatic amino acids. Furthermore, this work used the wild-type *E. coli* BL21(DE3) as producer microorganism whereas other studies have used *E. coli* with extensively engineered pathway of aromatic amino acid biosynthesis.

This work demonstrated the feasibility of enhancing the production of L-phenylalanine from glycerol in an engineered *E. coli* expressing phenylalanine dehydrogenase together with a glycerol transport facilitator and an aromatic amino acid exporter. Coexpression of the aromatic amino acid exporter in *E. coli* containing *phedh* promoted the production of L-Phe and production could be improved a little more by also coexpressing a glycerol facilitator. The production of L-Phe in *E. coli* required highly aerobic conditions and, therefore, the peak concentration of the amino acid was attained more rapidly in a vigorously aerated fermenter compared to in a surface aerated shake-flask.

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