RESEARCH ARTICLE

Enhanced Production of L-Tryptophan with Glucose Feeding and Surfactant Addition and Related Metabolic Flux Redistribution in the Recombinant *Escherichia coli*

Wei Luo, Jin Huang, Xiangcheng Zhu, Lei Huang, Jin Cai, and Zhinan Xu

Abstract The production of L-tryptophan was investigated in a recombinant strain *Escherichia coli* W3110-ZDrr. It was observed that phosphate and feeding strategies are key factors to ensure the good cell growth and high production of L-tryptophan. The simple exponential feeding strategy could only produce 10.6 g/L L-tryptophan due to the improper feeding rate; while the manual glucose-feedback feeding approach could effectively control the substrate and inhibit the formation of acetate, and thus improved the L-tryptophan production to 25.5 g/L. The modified exponential feeding approach avoided overfeeding or underfeeding and achieved high production of L-tryptophan. Moreover, the addition of Tween 60 or PL61 could enhance the cell growth and the production of L-tryptophan in the fed-batch mode. Subsequent metabolic flux analysis showed that more carbon flux was distributed into the biosynthesis of L-tryptophan when Tween 60 or PL61 was supplied. The present work presents one base for further large-scale production of this important amino acid.

Kewords: L-tryptophan, recombinant *Escherichia coli*, feeding strategy, metabolic flux redistribution, surfactant

Wei Luo, Zhinan Xu (✉)
The Key Laboratory of Biomass Chemical Engineering, Ministry of Education, Zhejiang University, Hangzhou 310027, China
Tel/Fax: +86-571-87951220; E-mail: znxu@zju.edu.cn

Jin Huang, Xiangcheng Zhu, Lei Huang, Jin Cai, Zhinan Xu
Institute of Biological Engineering, Department of Chemical and Biological Engineering, Zhejiang University, Hangzhou 310027, China

Wei Luo
The Key Laboratory of Carbohydrate Chemistry & Biotechnology, Ministry of Education, and School of Biotechnology, Jiangnan University, Wuxi 214122, China

Introduction

L-Tryptophan (L-trp) is an aromatic amino acid containing the unique indole side chain. Eukaryotic organisms are not able to synthesize this molecule and have to ingest it from outside for physiological requirement. L-Trp also serves as a fundamental precursor to a number of neurotransmitters in the brain of animal and human, which makes it an important substance for the regulation of appetite, sleep, mood, and pain levels (1). Possessing these distinct traits, L-trp was widely applied in the fields of pharmaceuticals, food additives, and animal feeds.

However, the high cost of L-trp has become the bottleneck for its extensive application and development, especially in the animal feed industry. The increased market requirements of L-trp have stimulated the increasing demands to improve the production of L-trp and reduce its cost. Presently, L-trp is mainly produced via enzymatic synthesis and microbial fermentation. Comparing to the chemical route which produces a mixture of the D- and L-forms of tryptophan, the biological approach is more practical and environmentally friendly by utilizing renewable resources (e.g., glucose, biomass) to synthesis the pure active L-trp. Many efforts have been made to improve the production of L-trp, including the genetic improvement of producing strains, medium formulation, and bioreactor operation optimization, and etc. For example, various complex or chemically defined media have been adopted to support high L-trp productivity (2,3), and the microorganisms used for the fermentative production of L-trp are usually genetically modified, such as *Escherichia coli* (4), *Corynebacterium glutamicum* (5), *Bacillus* sp. (6,7), and *Brevibacterium lactofermentum* (8). In addition, the optimization of fermentation process has suggested that glucose feeding strategy is more efficient than batch operation to improve

the productivity of L-trp (5,9). According to the reported literatures, a relatively high productivity of L-trp in the range of 14.7 to 36.3 g/L could be achieved by applying glucose feeding strategy (4,10-12).

However, comparing to the microbial production of other amino acids like lysine and glutamate, the yield and productivity of L-trp are relatively low even after tremendous efforts on molecular biological and bioprocess engineering trials (13,14). The complex multiple regulations in the biosynthetic pathway of L-trp may contribute to this embarrassment. To achieve the cost-effective production of L-trp, the pathway must be regulated to focus the metabolites flux towards the biosynthesis of L-trp. Another challenge for the overproduction of L-trp is the across-membrane transportation and excretion of L-trp. In the past decades, the fermentative production of aromatic amino acids has been highly improved by the enhanced secretion with the addition of surfactants (15), antibiotics, or local anesthetics (16,17). The contribution of surfactant to the efflux of aromatic amino acid could be ascribed to the alteration of membrane fluidity and permeability (18) or affecting the transport protein (19). Although some L-trp transport systems like permeases have been found in *E. coli* (20), the molecular basis of L-trp efflux remains unclear.

In this paper, a fed-batch production of L-trp using a recombinant strain of *E. coli* has been investigated. A glucose-controlled feeding strategy was adopted to eliminate the formation of acetic acid. The effects of several surfactants and antibiotics on the production of L-trp were evaluated. Finally, the enhanced production of L-trp was elucidated in detail by comparing the metabolic flux analyses with and without the addition of surfactant.

Materials and Methods

Bacterial strain and culture media   The recombinant *E. coli* W3110-ZDrr was derived from *E. coli* K12 strain by the deletion of a functional trp repressor and tryptophanase and the introduction of a composite plasmid which contains the whole trp operon of *E. coli* (21). The preculture medium contained 30 g/L glucose, 8 g/L yeast extract, 5 g/L $(NH_4)_2SO_4$, 5 g/L KH_2PO_4, and 0.5 g/L $MgSO_4 \cdot 7H_2O$ at pH 7.0. The seed medium contained 30 g/L glucose, 5 g/L yeast extract, 2 g/L $(NH_4)_2SO_4$, 5 g/L K_2HPO_4, an 0.5 g/L $MgSO_4 \cdot 7H_2O$. The batch fermentation medium contained 75 g/L glucose, 5 g/L yeast extract, 2 g/L $(NH_4)_2SO_4$, 5 g/L $K_2HPO_4 \cdot 3H_2O$, and 0.5 g/L $MgSO_4 \cdot 7H_2O$. The fed-batch fermentation medium was the same as the batch one, except that initial glucose was 10 g/L and $K_2HPO_4 \cdot 3H_2O$ was 20 g/L. A 650 g/L glucose was supplemented accordingly for the fed-batch process.

Cultivation, batch, and fed-batch fermentation   The colony of *E. coli* W3110-ZDrr was inoculated into a 250-mL Erlenmeyer shake flask containing 30 mL preculture medium (contained 20 mg/L tetracycline and streptomycin), and grew at 37°C and 200 rev/min until the $OD_{600 \, nm}$ reach 5-10.

For normal batch fermentation, 10%(v/v) of such culture was transferred into a 750-mL shake flask containing 100 mL fermentation medium and grew at 35°C for 17 h. For fed-batch fermentation, 10 mL of such culture was further inoculated into a 10-L fermentor (30BS-2-3000; Shanghai Baoxing Bio-engineering Equipment Corp., Shanghai, China) containing 6 L seed medium (contained 20 mg/L tetracycline and streptomycin). The pH of culture was adjusted between 6.8 and 7.0 by automatic addition of 25% NH_4OH. The dissolved oxygen of culture was maintained above 20% via manual adjustment of stirring speed and air flow rate. When seeds were grown to the middle of exponential phase, 10%(v/v) inoculums were transferred into a 10-L bioreactor with 5 L working volume. The temperature was set at 35°C and pH was maintained at 6.5. The dissolved oxygen level was maintained at 10-20% saturation. Commercial silicon antifoam (Sigma-Aldrich, St. Louis, MO, USA) was automatically added into the broth to prevent the formation of foam. After the initial glucose was consumed, the concentrated 650 g/L glucose solution was fed into the broth accordingly by the aid of LongerPump® peristaltic pump (BT100-1F; Baoding Longer Precision Pump Co., Ltd., Baoding, China).

Feeding strategies   Three different feeding strategies were tested during the fed-batch fermentation, to obtain the high cell density and enhanced production of L-trp. When the exponential feeding strategy was applied, the feeding rate was changed according to the predetermined feeding program described by the mass balance equations, to maintain a constant specific growth rate (22). The appropriate glucose feeding rate was depended on an assumed constant yield coefficient and a desired specific growth rate, as shown in Eq. 1 (23):

$$F(t) = \frac{\mu_s V(t_0) X(t_0)}{Y_{X/S}} \exp(\mu_s t) \qquad (1)$$

in which, F is the feed flow rate (g/h), μ_s is the desired specific growth rate (1/h), V is the culture volume (L), X is the cell density (g/L), $Y_{X/S}$ is the cell yield versus the concentration of glucose (g DCW/g glucose), and t_0 is the initial time when feeding is started. Since $X(t_0) = (S_i - S(t_0))Y_{X/S}$, in which S_i is the initial glucose concentration (g/L) and $S(t_0)$ is close to zero, Eq. 1 can be reformed into Eq. 2 (23):

$$F(t) = \mu_s S_i V(t_0)\exp(\mu_s t) \qquad (2)$$

In glucose-feedback feeding approach, the feeding rate was manually adjusted to control the concentration of glucose within a certain low level with the assistance of a biosensor for the timely determination of the titer of residual sugar.

To simplify the feeding process, a modified exponential feeding approach was developed. The feeding rate was determined by the following equation derived from Eq. 2:

$$F(t) = 155\mu_s\exp(\mu_s t) \qquad (3)$$

The multinomial $S_i V(t_0)$ in Eq. 2 was substituted by the empirical constant 155, because feeding rate calculated by the calculation of Eq. 2 would result in the underfeeding and substrate starvation at the initial feeding stage, which were measured by the off-line analysis of glucose.

Experimental analyses   To determine the dry cell weight (DCW), 50 mL fermentation broth was centrifuged at 5,000×*g* for 15 min, and the resulted cell pellets were washed twice with 50 mL distilled water and dried at 105ºC to a constant weight. Simultaneously, the optical density (OD) of culture at 660 nm was measured, and the conversion factor between DCW and $OD_{660\,nm}$ was obtained. Thus, the cell growth could be monitored by measuring the $OD_{660\,nm}$.

The residual glucose concentration in the broth was measured by a biosensor analyzer (SBA-40C; Shangdong Academy of Sciences, Jinan, China). The phosphate concentration of culture was determined according to the procedure described by Lau *et al.* (24).

The analyses of amino acids were performed by HPLC (Agilent 1100; Agilent Technologies, Palo Alto, CA, USA) equipped with a TC-C18 column with 5 μm in particle size (4.6×250 mm, Agilent Technologies). After pre-column derivatization with phenylisothiocyanate (Sigma-Aldrich), amino acids were separated by the reversed phase column and detected by the UV detector at 254 nm. Acetic acid was analyzed by GC (Agilent 6820; Agilent Technologies) equipped with a flame ionization detector and a capillary column (Hp-Innowax, 30 m×0.32 mm i.d., 0.50 μm film thickness; Agilent Technologies).

Plasmid stability assay   To test the stability of recombinant strain, especially for the plasmid, samples were withdrawn at the end of fermentation and appropriately diluted to spread on nonselective LB-agar plates. After incubated at 37ºC for 24 h, 200 colonies from each nonselective plate were spotted onto selective LB-agar plates (supplemented with 20 mg/L tetracycline and streptomycin). The number of survived colonies was recorded to evaluate the stability of plasmid in the strain.

Metabolic flux analysis of L-trp biosynthesis   The metabolic network of L-trp production comprises glycolysis, pentose phosphate pathway (PPP), the tricarboxylic acid (TCA) cycle, biosynthesis of L-trp and other amino acids (Fig. 1). Metabolic flux analyses were based on the pseudo steady-state assumption and calculated by a set of linear equations, which can be expressed in the matrix described by Vallino and Stephanopoulos (25). The flux distribution of generated bio-network was exhibited as the volumetric rates of intracellular metabolite formation at the stationary phase (no consideration of cell growth), which was computed by Matlab (7.1 version; Mathworks Inc., Natick, MA, USA). The flux of glucose uptake during the production of L-trp was set to 100%, and other fluxes in the network were given as the relative molar flux normalized to it.

Statistical analyses   All the experiments were performed independently in triplicate. The obtained values were the means, where the standard deviations (SD) for all the values were less than ±10%.

Results and Discussion

Effect of phosphate   During the fed-batch process, the phosphate and glucose were consumed very quickly at the initial stage (Fig. 2). Since phosphate is the key substance in the glycolysis, it could directly influence the cell growth and fermentation performance (24,26). As shown in Fig. 2, the increase in initial phosphate concentration from 7.5 to 20 g/L could lead to 1.7-fold improvement of L-trp production (from 15.8 to 26.8 g/L). However, further increase in phosphate higher than 25 g/L presented the cell growth inhibition and reduction of L-trp production (data not shown). In this study, the initial phosphate concentration of 20 g/L was sufficient for the fed-batch fermentation, since there was a small quantity of phosphate remained in the medium until fermentation end-point (Fig. 2B).

Evaluation of different feeding strategies   After consumption of the initial glucose, indicated by the increasing dissolved oxygen, the feeding process was started at that time. Four different specific growth rates (0.1, 0.15, 0.2, and 0.25) were applied to evaluate the fermentation performance, since the threshold growth rate of *E. coli* for the formation of acetic acid is in the range of 0.14-0.17/h in the defined media (27). The listed results suggested that the good fermentation performance could be obtained when the desired specific growth rate was set at 0.15 or 0.20 (Table 1). However, the titer and average productivity of L-trp were still relatively low when compared with other reports (9,12).
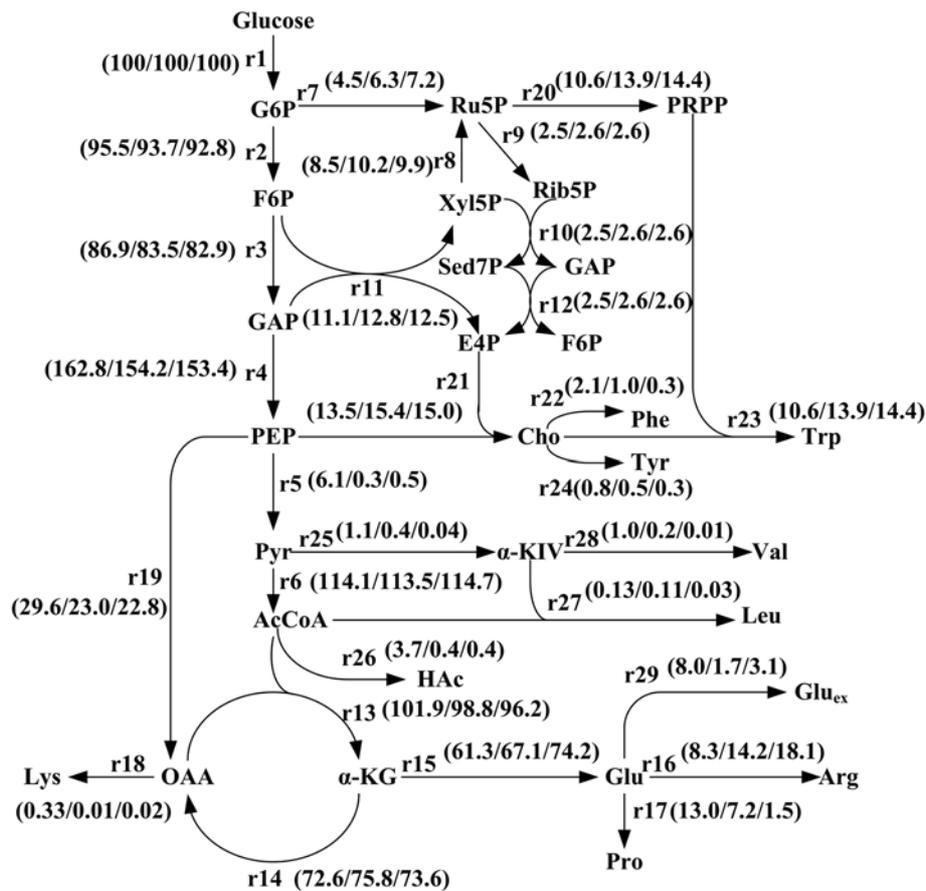
Fig. 1. Metabolic pathways and flux distribution related to the biosynthesis of L-trp. Values from left to right were calculated metabolic flux distribution in fermentation culture without surfactant, with the presence of Tween 60 and with the presence of PL61, respectively.

In contrast, the glucose feedback feeding was operated manually according to the concentration of glucose detected each hour. As shown in Fig. 3A, the cell density (OD$_{660\,nm}$) could reach nearly 70 at 24 h, and the titer and productivity of L-trp were 25.5 g/L and 0.487 g/L·h, respectively. The concentration of glucose was maintained at a low level (1 g/L) to eliminate the formation of acetate, which could inhibit the cell growth and L-trp production. Although the fermentation performance of this strategy was much better than that of exponential feeding method, the operation was kind of laborious.

When conducted by the modified exponential feeding approach, the titer of L-trp was 24.9 g/L and the average productivity was 0.586 g/L·h (Fig. 3B), which were comparable to those from the glucose feedback feeding method but more applicable.

To achieve high production of the target metabolite L-trp, several practical feeding strategies were evaluated in our work. Although the exponential feeding method was easily adopted for L-trp production, the lag response of this method towards the fermentative changes may lead to the improper feeding rate, and thus caused the low cell density

and titer of L-trp. The improved biomass and production of L-trp via the manual glucose-feedback feeding approach have supported our above assumption. Zhang *et al.* (28) had argued that the growth of microorganism was complex, a slight variation of cell growing environment would lead to a significant deviation of residual glucose concentration when the feeding is conducted via the exponential feeding mathematical model. So they have developed a pH feedback-controlled feeding method to control the residual glucose in the fermentation broth with an expected level and obtained a good performance for lactic acid production. Moreover, to avoid the overfeeding and/or underfeeding during the fed-batch fermentation due to the varying actual specific growth rate, Lee *et al.* (23) had also modified the exponential feeding strategy to allow the correction of feeding rate based on the actual specific growth rate. So a modified exponential feeding strategy was developed. The equation for the calculation of specific growth rate was reformed, and the empirical constant was calculated base on the data from glucose-feedback feeding experiments. This feeding strategy presented better control of glucose supply to achieve the optimal cell density and titer of L-trp,
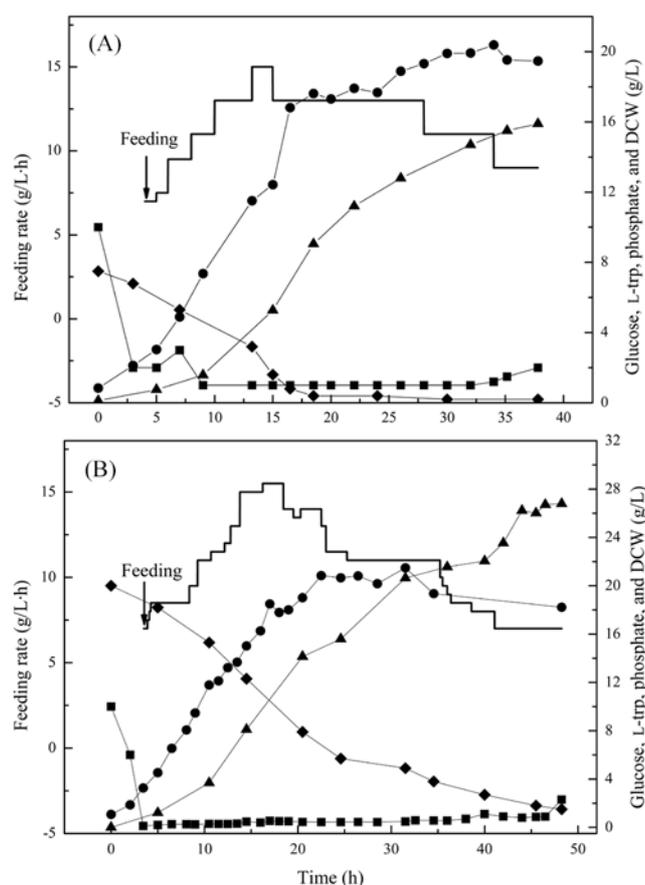
Fig. 2. Profiles of fed batch fermentation concerning about biomass, consumption of glucose and phosphate, and production of L-trp. Initial phosphate concentration was 7.5 g/L (A) and 20 g/L (B). ● DCW, ■ glucose, ◆ phosphate, and ▲ L-trp; line represents the feeding rate throughout the fed-batch process.

Table 1. Effect of the nominal specific growth rate on cell growth and L-trp production in the exponential feeding culture

Nominal specific growth rate (1/h)	Maximum DCW (g/L)	Titer of L-trp (g/L)	Average productivity (g/L·h)
0.10	11.3	7.40	0.31
0.15	15.5	10.6	0.44
0.20	17.9	8.50	0.47
0.25	6.80	4.60	0.22

and should provide some useful information for large scale production of L-trp.

Influence of surfactants and antibiotics on the production of L-trp Surfactants and cell wall inhibitors such as antibiotics have been widely applied for the production improvement of various amino acids (16,29-31). Thus several surfactants and antibiotics were selected to investigate their effects on the accumulation of L-trp in the batch culture, respectively. The antibiotics, including neomycin, streptomycin, carbenicillin, kanamycin, penicillin,
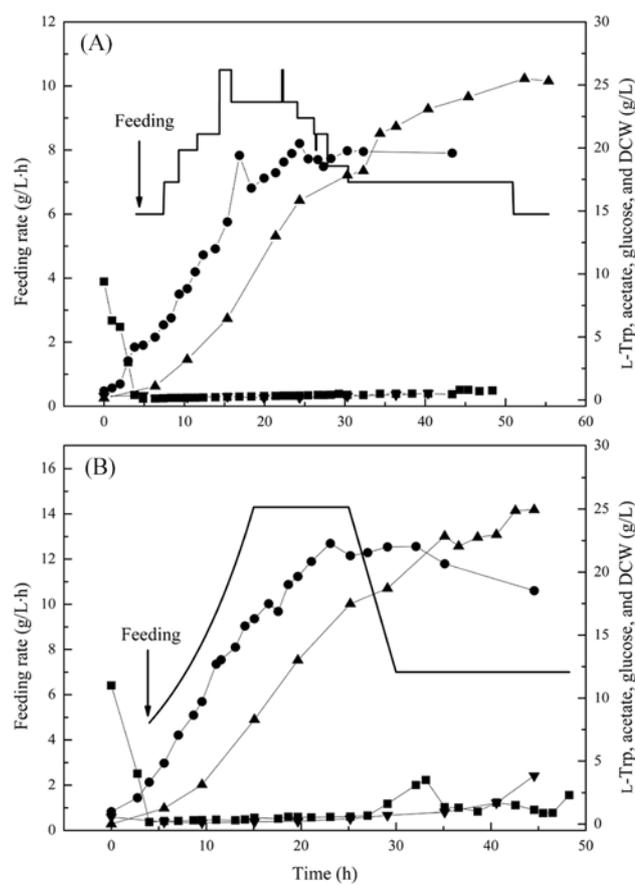
Fig. 3. Profiles of fed-batch culture with glucose-feedback feeding strategy (A) and modified exponential feeding method (B). ● DCW, ■ glucose, ◆ phosphate, and ▲ L-trp; line represents the feeding rate throughout the fed-batch process.

Table 2. Effect of various surfactants on cell growth and L-trp production in batch culture

Surfactants	Maximum DCW (g/L)	L-Trp (g/L)	Average productivity (g/L·h)
Tween 80	7.55	5.36	0.32
PL61	4.19	2.43	0.15
PEG-10000	5.87	3.70	0.22
Triton X-114	8.31	5.15	0.31
Polyvinyl alcohol	4.87	3.49	0.21
Span 80	6.33	3.39	0.21
Control	4.10	2.67	0.16

and 2 cell wall inhibitors (SDS and CTAB), were respectively tested to determine their critical inhibition concentrations. Then each antibiotic was supplemented into the medium to evaluate its contribution to the production of L-trp. However, no obvious improvement was observed among all tested antibiotics (data not shown). In contrast, surfactants such as Tween 80 and Triton X-114 could enhance the production of L-trp up to 2-fold in batch culture; while PL61 had no effect on the titer of L-trp
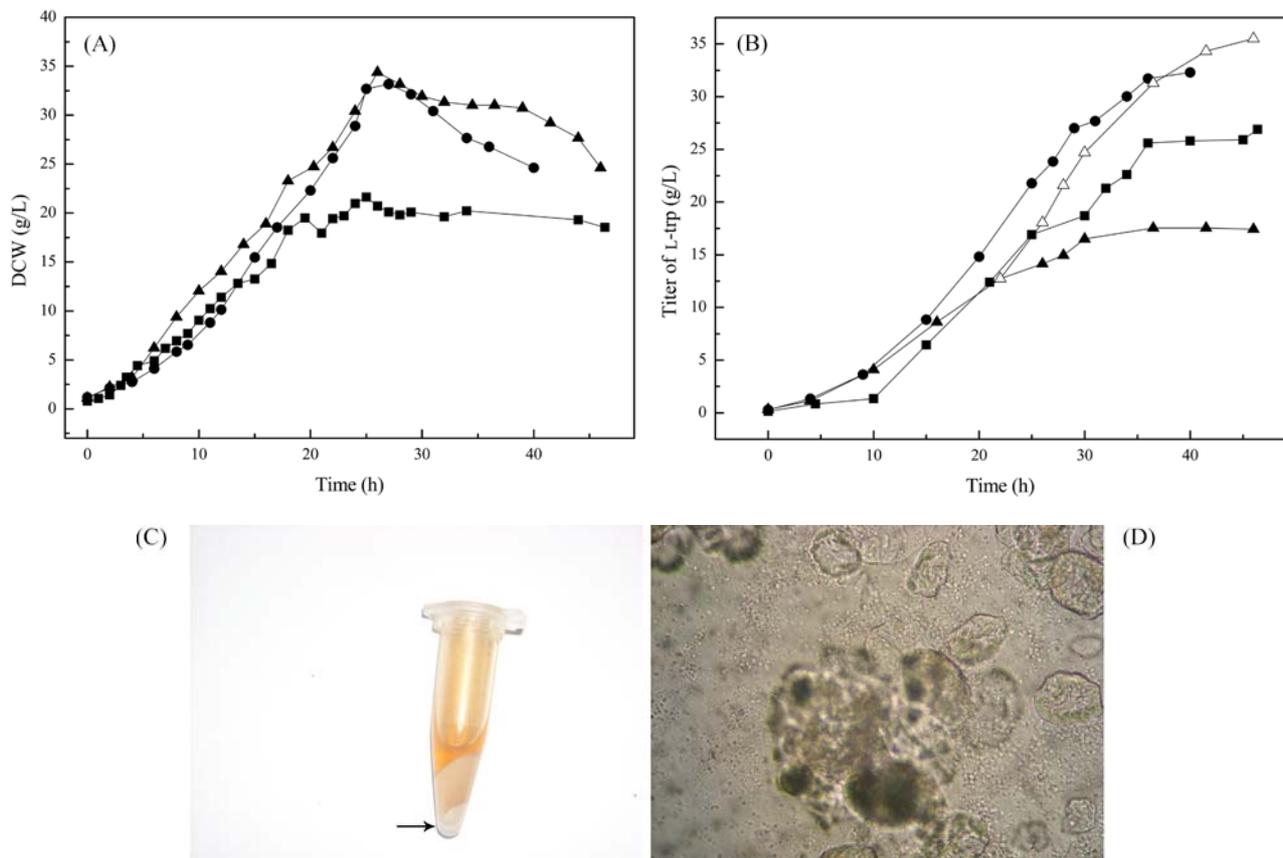
Fig. 4. Time courses of cell growth (A) and titer of L-trp under different conditions (B). (■) without surfactant, (●) addition of Tween 60, and (▲) addition of PL61, (△) the total titer of L-trp including the amount of crystalline L-trp in the presence of PL61. (C) L-Trp crystal sediments at the bottom (arrow shows) of an Eppendorf tube after centrifugation of culture broth; (D) photomicrograph of L-trp crystals in the fermentation broth

(Table 2). Considering about large quantities of foam formation caused by Triton surfactant, the Tween family series (Tween 20, 40, 60, and 80) were evaluated, and Tween 60 was the best candidate to support L-trp production (data not shown).

Subsequently, Tween 60 and PL61 were further chosen to investigate their effects on the fed-batch process. The results indicated that supplementation of either Tween 60 or PL61 could improve the cell growth and the production of L-trp (Fig. 4A,4B). The concentration of L-trp could steadily increase to 32 g/L with the addition of Tween 60, while the addition of PL61 up to 0.5%(v/v) could even result in the crystallization of L-trp with the highest titer of 35.5 g/L (Fig. 4C,4D).

From the above results, Tween 60 gave a good performance in both batch and fed batch modes; while PL61 presented distinctively difference in each mode. Hence, the underlying mechanism for enhanced production of L-trp via the addition of varied surfactants was different. Kim *et al.* (29) had reported that the addition of Tween 40 could trigger the enhanced production of glutamate from 4 to 18 g/L in *C. glutamicum*. Koníček (15) also observed that the production

of L-lysine was increased 23-25% by using Tween 80 (0.2%, w/v) in the regulatory mutant strains of *C. glutamicum*. A recent viewpoint had suggested that the conformation of specific transmembrane exporter was altered when the cells was exposed to the surfactant such as Tween 60, which in return triggered the improved efflux of target product (32). So the positive effect of Tween 60 on the fermentation may ascribe to its assistance of L-trp export, which reduces the negative feedback of product and induced the better cell growth, resulting in the improvement of L-trp production. Since PL61 could not affect the fermentation in the batch mode, it should apply a different mechanism to enable the improvement of L-trp production in the fed-batch mode. Considering about the higher productivity and observed crystallization of L-trp in fed batch mode, we concluded that PL61 could significantly reduce the solubility of L-trp at high concentration and lead to the resulted crystallization of L-trp in broth. Thus, the overall mechanism of surfactants supplementation was to decrease the concentration of L-trp in cells, so the product-feedback inhibition on cell growth and L-trp production was reduced. But instead of facilitating the secretion of L-
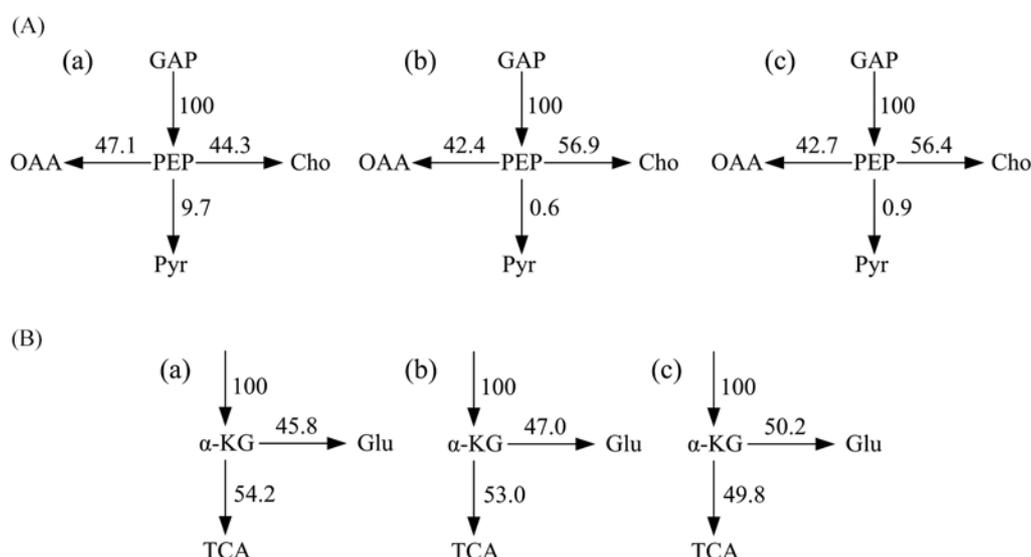
Fig. 5. Metabolic flux distribution at PEP node (A) and α-KG node (B) under 3 different conditions. (a) without surfactant, (b) addition of Tween 60, and (c) addition of PL61

trp via addition of Tween 60, PL61 executed its function by reducing the external concentration of L-trp.

Metabolic flux redistribution with the addition of surfactants Based on the previously developed metabolic flux analysis (25), a mass balance was applied to examine the intracellular metabolic flux distribution during the production of L-trp (Fig. 1). With the addition of Tween 60 or PL61, the metabolic fluxes were clearly redistributed within the overall network. The distinct increase of flux distribution towards the branch of L-trp biosynthesis should directly boost the yield of L-trp. As shown in Fig. 1, the metabolic flux was redistributed at the G6P principal node with the presence of surfactants. Comparing to the control value of 4.5% without surfactant, 6.3% (with the addition of Tween 60) or 7.2% (with the addition of PL 61) of the carbon flux was directed to the pentose phosphate pathway (PPP). Finally, the resulted carbon flux distribution to the L-trp biosynthesis was 13.9% (with the addition of Tween 60) or 14.4% (with the addition of PL 61), evidently higher than the control value of 10.6% without surfactant. Moreover, the addition of surfactants not only just affected the pathway of L-trp, the biosynthetic pathways of other amino acids were also influenced. Overall, the synthesis of glutamate and arginine were boosted with the addition of surfactants; while the production of lysine, proline, leucine, valine, tyrosine, and phenylalanine were impaired.

To gain the clear insight about the metabolic network related to the amino acids, 2 important PEP node and α-KG node were further analyzed. Setting the incoming carbon flux of each principal node as 100%, the flux from PEP node to the Cho-branch, which is the direct precursor of L-trp, was improved from 44.3 to over 56% (56.9% for

Tween 60 and 56.4% for PL61) in the presence of surfactants (Fig. 5A). Similarly, the flux from the α-KG node to the Glu-branch was also enhanced with the addition of surfactants (Fig. 5B).

Thus, the carbon fluxes to the possible precursors of L-trp, either in the PPP pathway from G6P node to PRPP node, or in the Cho-branch from PEP node to Cho node, were both increased with the supplementation of surfactants. In return the enhanced biosynthesis of precursors (PRPP and Cho) could directly improve the production of L-trp. It is reasonable to find that the biosynthesis of most amino acids, such as lysine, proline, leucine, valine, tyrosine and phenylalanine, were limited to centralize the carbon flux to L-trp. But the accumulation of glutamate and arginine derived from α-KG node was also increased. So an alternative way to improve the production of L-trp could be proposed here by limiting or even eliminating these competitive flux distributions in the metabolic network.

Overall, our efforts for the improvement of L-trp production have gained some preliminary results. With the development of feeding strategy and supplementation of surfactants, the titer of L-trp was significantly increased in the recombinant *E. coli* strain. However, the feeding strategy could still be upgraded to harmonize the fermentation process and may further improve the productivity of L-trp; the product feedback inhibition could be further minimized by concentration change and various combinations of surfactants; the central carbon metabolic flux could be more focused on the biosynthesis of L-trp. The other issue is the plasmid instability. Remarkable loss (40%) of plasmid from host strain at the end of the fermentation was observed when cells were cultivated without antibiotic selective pressure. Subsequent research addressing these issues shall

supplement the industrialization of L-trp production and other amino acids.

References

1. Kocabaş P, Çalik P, Özdamar TH. Fermentation characteristics of L-tryptophan production by thermoacidophilic *Bacillus acidocaldarius* in a defined medium. Enzyme. Microb. Tech. 39: 1077-1088 (2006)

2. Ikeda M, Okamoto K, Katsumata R. Cloning of the transketolase gene and the effect of its dosage on aromatic amino acid production in *Corynebacterium glutamicum*. Appl. Microbiol. Biot. 51: 201-206 (1999)

3. Nam SW, Kim BK, Son KH, Kim JH. Continuous culture of a recombinant *Escherichia coli* and plasmid maintenance for tryptophan production. Biotechnol. Lett. 9: 489-494 (1987)

4. Zhao ZJ, Chen S, Wu D, Wu J, Chen J. Effect of gene knockouts of L-tryptophan uptake system on the production of L-tryptophan in *Escherichia coli*. Process. Biochem. 47: 340-344 (2012)

5. Ikeda M, Katsumata R. Transport of aromatic amino acids and its influence on overproduction of the amino acids in *Corynebacterium glutamicum*. J. Ferment. Bioeng. 78: 420-425 (1994)

6. Yajima Y, Sakimoto K, Takahashi K, Miyao K, Kudome Y, Aichi K. L-Tryptophan-producing microorganism and production of L-tryptophan. Japan Patent 02,190,182 (1990)

7. Ikeda M. Towards bacterial strains overproducing L-tryptophan and other aromatics by metabolic engineering. Appl. Microbiol. Biot. 69: 615-626 (2006)

8. Matsui K, Ishida M, Tsuchiya M, Sano K. Construction of tryptophan-producing recombinant strains of *Brevibacterium lactofermentum* using the engineered trp operons. Agr. Biol. Chem. Tokyo 52: 1863-1865 (1988)

9. Cheng LK, Wang J, Xu QY, Xie XX, Zhang YJ, Zhao CG, Chen N. Effect of feeding strategy on L-tryptophan production by recombinant *Escherichia coli*. Ann. Microbiol. 62: 1625-1634 (2012)

10. Kawasaki K, Yokota A, Tomita F. L-Tryptophan production by a pyruvic acid-producing *Escherichia coli* strain carrying the *Enterobacter aerogenes* tryptophanase gene. J. Ferment. Bioeng. 82: 604-606 (1996)

11. Zhao ZJ, Zou C, Zhu YX, Dai J, Chen S, Wu D, Wu J, Chen J. Development of L-tryptophan production strains by defined genetic modification in *Escherichia coli*. J. Ind. Microbiol. Biot. 38: 1921-1929 (2011)

12. Liu Q, Cheng Y, Xie X, Xu Q, Chen N. Modification of tryptophan transport system and its impact on production of L-tryptophan in *Escherichia coli*. Bioresource Technol. 114: 549-554 (2012)

13. Jetten M, Sinskey A. Recent advances in the physiology and genetics of amino acid-producing bacteria. Crit. Rev. Biotechnol. 15: 73-103 (1995)

14. Krämer R. Genetic and physiological approaches for the production of amino acids. J. Biotechnol. 45: 1-21 (1996)

15. Koníček J, Smékal F, Koníčeková-Radochová M. Effect of Tween

16. Radmacher E, Stansen K, Besra G, Alderwick L, Maughan W, Hollweg G, Sahm H, Wendisch V, Eggeling L. Ethambutol, a cell wall inhibitor of *Mycobacterium tuberculosis*, elicits L-glutamate efflux of *Corynebacterium glutamicum*. Microbiology 151: 1359-1368 (2005)

17. Ekwealor IA, Obeta JAN. Antibiotic and surfactant effects on lysine accumulation by *Bacillus megaterium*. Afr. J. Biotechnol. 7: 1550-1553 (2008)

18. Nampoothiri KM, Hoischen C, Bathe B, Möckel B, Pfefferle W, Krumbach K, Sahm H, Eggeling L. Expression of genes of lipid synthesis and altered lipid composition modulates L-glutamate efflux of *Corynebacterium glutamicum*. Appl. Microbiol. Biot. 58: 89-96 (2002)

19. Duperray F, Jezequel D, Ghazi A, Letellier L, Shechter E. Excretion of glutamate from *Corynebacterium glutamicum* triggered by amine surfactants. BBA-Biomembranes 1103: 250-258 (1992)

20. Yanofsky C, Horn V, Gollnick P. Physiological studies of tryptophan transport and tryptophanase operon induction in *Escherichia coli*. J. Bacteriol. 173: 6009-6017 (1991)

21. Luo W. High efficient production of purine nucleoside phosphorylase and L-tryptophan with recombinant *Escherichia coli*. PhD thesis, Zhejiang University, Hangzhou, China (2011)

22. Korz D, Rinas U, Hellmuth K, Sanders E, Deckwer W. Simple fed-batch technique for high cell density cultivation of *Escherichia coli*. J. Biotechnol. 39: 59-65 (1995)

23. Lee J, Lee SY, Park S. Fed-batch culture of *Escherichia coli* W by exponential feeding of sucrose as a carbon source. Biotechnol. Tech. 11: 59-62 (1997)

24. Lau J, Tran C, Licari P, Galazzo J. Development of a high cell-density fed-batch bioprocess for the heterologous production of 6-deoxyerythronolide B in *Escherichia coli*. J. Biotechnol. 110: 95-103 (2004)

25. Vallino JJ, Stephanopoulos G. Metabolic flux distributions in *Corynebacterium glutamicum* during growth and lysine overproduction. Biotechnol. Bioeng. 41: 633-646 (1993)

26. Kelle R, Hermann T, Weuster-Botz D, Eggeling L, Krämer R, Wandrey C. Glucose-controlled L-isoleucine fed-batch production with recombinant strains of *Corynebacterium glutamicum*. J. Biotechnol. 50: 123-136 (1996)

27. Eiteman MA, Altman E. Overcoming acetate in *Escherichia coli* recombinant protein fermentations. Trends Biotechnol. 24: 530-536 (2006)

28. Zhang Y, Cong W, Shi SY. Application of a pH feedback-controlled substrate feeding method in lactic acid production. Appl. Biochem. Biotech. 162: 2149-2156 (2010)

29. Kim J, Hirasawa T, Sato Y, Nagahisa K, Furusawa C, Shimizu H. Effect of *odhA* overexpression and *odhA* antisense RNA expression on Tween-40-triggered glutamate production by *Corynebacterium glutamicum*. Appl. Microbiol. Biot. 81: 1097-1106 (2009)

30. Asakura Y, Kimura E, Usuda Y, Kawahara Y, Matsui K, Osumi T, Nakamatsu T. Altered metabolic flux due to deletion of *odhA* causes L-glutamate overproduction in *Corynebacterium glutamicum*. Appl. Environ. Microb. 73: 1308-1319 (2007)

31. Eggeling L, Sahm H. The cell wall barrier of *Corynebacterium glutamicum* and amino acid efflux. J. Biosci. Bioeng. 92: 201-213 (2001)

32. Nakamura J, Hirano S, Ito H, Wachi M. Mutations of the *Corynebacterium glutamicum* NCgl1221 gene, encoding a mechanosensitive channel homolog, induce L-glutamic acid production. Appl. Environ. Microb. 73: 4491-4498 (2007)