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ORIGINAL PAPER

# Enhancing the production of γ-aminobutyric acid in *Escherichia coli* BL21 by engineering the enzymes of the regeneration pathway of the coenzyme factor pyridoxal 5′-phosphate

**Ping Yu[1] · Jian Ma[1] · Pengzhi Zhu[1] · Qingwei Chen[1] · Qili Zhang[1]**

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**Abstract**
The compound γ-aminobutyric acid (GABA) was widely used in various fields. To enhance the production of GABA in *Escherichia coli* BL21(DE3), the enzymes of the regeneration pathway of the coenzyme factor pyridoxal 5'-phosphate (PLP) were engineered. The recombinant *E. coli* strain was screened and identified. The initial concentrations of L-monosodium glutamate (L-MSG) had an obvious influence on the production of GABA. The highest concentration of GABA in recombinant *E. coli* BL21/pET28a-gadA was 5.54 g/L when the initial L-MSG concentration was 10 g/L, whereas it was 8.45 g/L in recombinant *E. coli* BL21/pET28a-gadA-SNO1-SNZ1 at an initial L-MSG concentration of 15 g/L. The corresponding conversion yields of GABA in these two strains were 91.0% and 92.7%, respectively. When the initial concentrations of L-MSG were more than 15 g/L, the concentrations of GABA in *E. coli* BL21/pET28a-gadA-SNO1-SNZ1 were significantly higher as compared to those in recombinant *E. coli* BL21/pET28a-gadA, and it reached a maximum of 13.20 g/L at an initial L-MSG concentration of 25 g/L, demonstrating that the introduction of the enzymes of the regeneration pathway of PLP favored to enhance the production of GABA. This study provides new insight into producing GABA effectively in *E. coli* BL21(DE3).

**Keywords** Metabolic engineering · *Escherichia coli* BL21 · γ-aminobutyric acid · Pyridoxal 5'-phosphate · Coenzyme factor · Regeneration pathway

## Introduction

The compound γ-aminobutyric acid (GABA) is a four-carbon non-protein amino acid and a natural active ingredient, which has the biological activities of improving brain blood circulation, lowering blood pressure, treating epilepsy and enhancing liver and kidney function (Li and Cao 2010; Luo et al. 2021; Shelp et al. 1999). Nowadays, GABA is used considerably in pharmaceutical, medical, cosmetics and feed industries (Barrett et al. 2012; Li et al. 2010; Luo et al. 2021; Park et al. 2005). It also was used as a major active constitute in foods, such as gammalone, cheese, gabaron tea, and shochu (Chamba and Irlinger 2004; Chemler and Koffas 2008; Chen et al. 2005).

✉ Ping Yu
yup9202@hotmail.com

[1] College of Food Science and Biotechnology, Zhejiang Gongshang University, 149 Jiaogong Road, Hangzhou, Zhejiang Province 310035, People's Republic of China

Because GABA has numerous commercial uses, researchers have attempted to develop an efficient method for producing it (Park and Oh 2006; Shi et al. 2013; Yu et al. 2018). GABA can be not only produced by chemical synthesis and bioproduction, but also produced by plants enrichment and enzymatic method (Choi et al. 2006; Inoue et al. 2003; Luo et al. 2021). The chemical synthesis of GABA often suffers from a long process, waste of raw materials and many by-products. Thus, its biosynthesis may be more promising than the chemical synthesis since it has a simple reaction procedure, high catalytic efficiency, mild reaction condition and environmental compatibility (Huang et al. 2007).

GABA can be synthesized by a one-step reaction of decarboxylating L-glutamate catalyzed via glutamate decarboxylase (Gad) (Fig. 1a) (Li and Cao 2010). However, this method has a critical problem that must be solved before becoming a competitive process for GABA production. It needs coenzyme factor pyridoxal 5′-phosphate (PLP) (Storici et al. 2004), and its supply is critical to enhance the production of GABA. PLP is the active form of vitamin $B_6$, and is a coenzyme factor in a

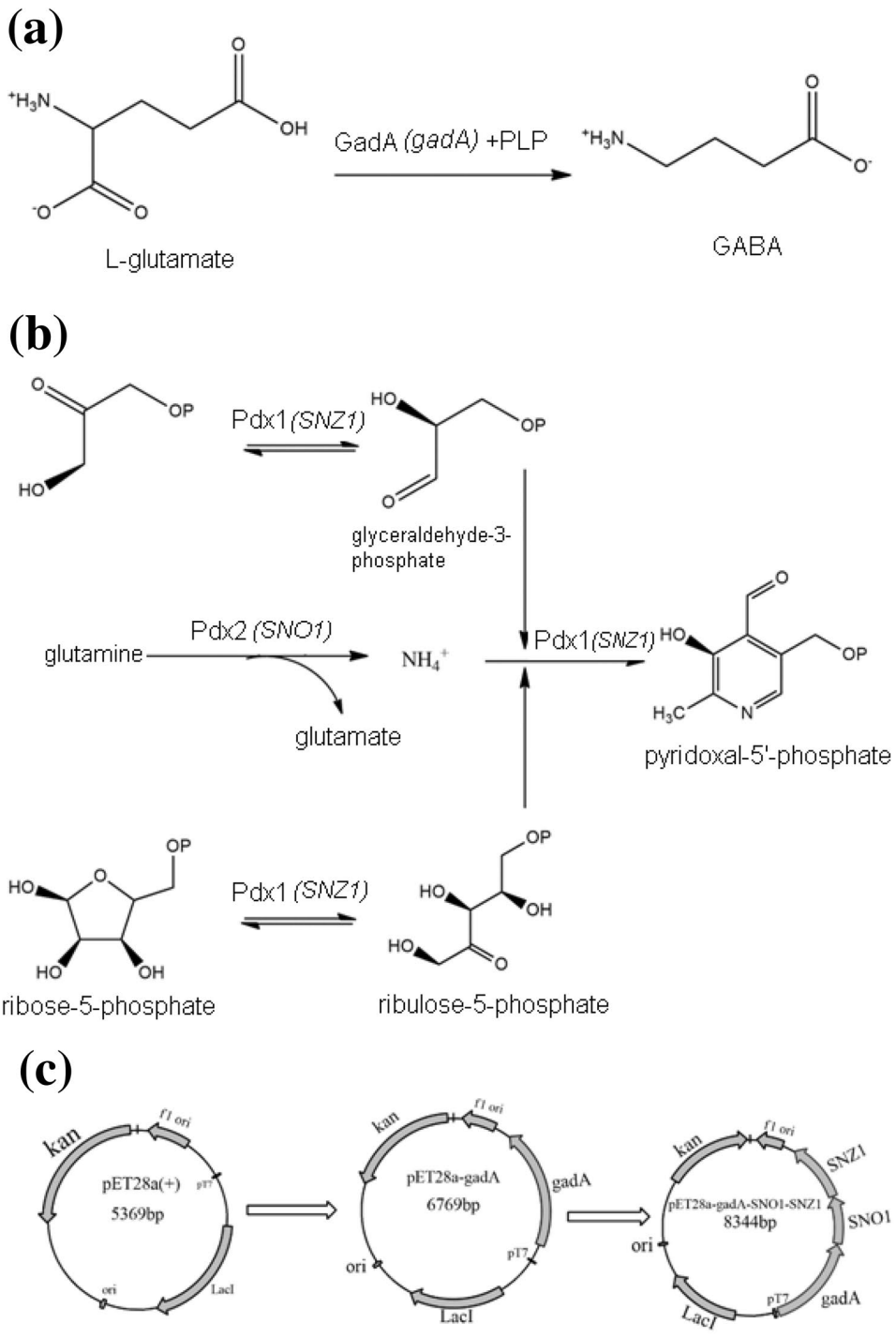


**Fig. 1** Biosynthetic pathways of γ-aminobutyric acid and pyridoxal 5'-phosphate, and conctruction of recombinant plasmids. **a** Bioconversion of L-glutamate to γ-aminobutyric acid; **b** biosynthetic pathway of pyridoxal 5'-phosphate; **c** construction of recombinant plasmids pET28a-gadA and pET28a-gadA-SNO1-SNZ1 The gene *gadA* encodes glutamate decarboxylase A (GadA). Two genes, *SNZ1* and *SNO1*, respectively encode two subunits of pyridoxal 5'-phosphate synthase, Pdx1 and Pdx2. Three genes are in italic. PLP: pyridoxal 5'-phosphate; GABA: γ-aminobutyric acid

variety of enzymatic reactions including decarboxylation, transamination and racemization (Vacca et al. 2008). In the previous study, pyridoxal kinase was overexpressed in *Lactobacillus plantarum* CCTCC M209102 to enhance PLP production by the ATP- dependent phosphorylation of pyridoxal. Thus, this strain could efficiently convert L-glutamate to GABA (Zhang et al. 2014). *E. coli* cells overexpressing glutamate decarboxylase A (GadA) were also used as a whole-cell biocatalyst for catalyzing L-glutamate into GABA (Ke et al. 2016). However, the maintenance of the proper function of the PLP-dependent glutamate decarboxylase A (GadA) was needed during biocatalysis because of the low level of in vivo PLP, which required that the in vitro expensive PLP was supplemented. Thus, it is interesting to investigate if the introduction of the enzymes of the regeneration pathway of PLP into *E. coli*

cells can enhance the production of GABA from L-glutamate by increasing endogenous PLP.

PLP biosynthetic pathway is widely distributed in bacteria, fungi and plants (Ehrenshaft et al. 1999; Mittenhuber 2001). However, it varies widely in different organisms. The native biosynthetic pathway of PLP in *E. coli* is involved in many key enzymes, and so it is difficult to engineer it for enhancing the production of PLP. In *Saccharomyces cerevisiae* SC288, PLP can be synthesized by only 5'-phosphate synthase that contains two subunits Pdx1 and Pdx2. PLP biosynthesis by Pdx1 requires the substrates ribose 5-phosphate (R5P) and glyceraldehyde 3-phosphate (Fitzpatrick et al. 2007). The heterocyclic nitrogen is derived from the hydrolysis of L-glutamine by Pdx2 (Tanaka et al. 2000). These two subunits form the PLP synthase complex, functionally classified as a glutamine aminotransferase (Guédez et al. 2012) (Fig. 1b). Genes *SNO1* and *SNZ1* respectively encode Pdx2 and Pdx1 in *S. cerevisiae* SC288 that participate in PLP biosynthesis (Dong et al. 2004).

In this study, the enzymes of the regeneration pathway of PLP from *S. cerevisiae* SC288 were attempted to introduce into *E. coli* BL21as a approach to synthesize endogenous PLP from its precursors in order to achieve a high production of GABA from L-glutamate. This approach was based on the PLP biosynthesis, in which Pdx1 and Pdx2, respectively encoded by *SNZ1* and *SNO1*, were used to synthesize PLP to produce GABA, together with glutamate decarboxylase A (GadA). As a result, a system was developed using a salvage biosynthetic pathway to increase the cellular level of PLP, resulting in higher GABA production. To the best of our knowledge, this is the first report on enhancing the production of GABA in *E. coli* BL21 by engineering the enzymes of the regeneration pathway of PLP by introducing the pyridoxal 5'-phosphate synthase from *S. cerevisiae* SC288.

## Materials and methods

### Strains, plasmids and growth conditions

The strains and plasmids used in this study are listed in Table 1. *E. coli* BL21 (DE3) was used as the host strain for the expression of target genes. *E. coli* DH5α was used as the host strain for constructing recombinant plasmids. *E. coli* BL21/pET32a-gadABC was constructed before in our laboratory (Yu et al. 2018). Unless otherwise specified, all strains were cultured at 37 °C in a Luria–Bertani medium (LB: 10 g/L tryptone, 5 g/L yeast extract and 10 g/L NaCl, pH 7.2). Ampicillin (100 mg/L), kanamycin (50 mg/L) and isopropyl $\beta$-D-thiogalactopyranoside (IPTG, 0.1 mM) were added for the plasmid stability maintenance and protein expression if required. IPTG, tryptone, yeast extract, NaCl, L-monosodium glutamate (MSG), ampicillin (Amp) and kanamycin (Kan) were purchased from the BBI Co. Ltd, USA. The plasmid purification kit, bacterial genomic DNA extraction kit, DNA fragment purification kit, agarose gel DNA extraction kit, restriction endonucleases and $T_4$ DNA ligase were purchased from the TaKaRa, Co. Ltd, Japan. All other reagents were of analytical grade.

**Table 1** Strains and plasmids

| Strains and plasmids | Description | Sources |
|---|---|---|
| Strains | | |
| *E. coli* BL21(DE3) | *F- ompT gal dcm lon hsdSB(rB- mB-)* | Invitrogen, USA |
| *E. coli* DH5α | *supE44ΔlacU169(φ80lacZΔM15)hsdR17recA endA 1gyrA96 thi- relA1* | Invitrogen, USA |
| *Saccharomyces cerevisiae SC288* | | Sangon, Shanghai, China |
| *E. coli* BL21(DE3)/ pET32a-gadABC | *E. coli* BL21(DE3) with pET32a-gadABC | (Yu et al. 2018) |
| *E. coli* BL21(DE3)/ pET28a-gadA | *E. coli* BL21(DE3) with pET28a-gadA | This work |
| *E. coli* BL21(DE3)/ pET28a-gadA-SNO1 | *E. coli* BL21(DE3) with pET28a-gadA-SNO1 | This work |
| *E. coli* BL21(DE3)/ pET28a-gadA-SNO1-SNZ1 | *E. coli* BL21(DE3) with pET28a-gadA-SNO1-SNZ1 | This work |
| Plasmids | | |
| pET28a(+) | $T_7$ promoter, pBR322 ori, $Kan^r$ | Sangon, Shanghai, China |
| pET32a-gadABC | pET32a by inserting *gadA*, *gadB* and *gadC* | (Yu et al. 2018) |
| pET28a-gadA | pET28a by inserting *gadA* | This work |
| pET28a-gadA-SNO1 | pET28a by inserting *gadA* and *SNO1* | This work |
| pET28a-gadA-SNO1-SNZ1 | pET28a by inserting *gadA, SNO1* and *SNZ1* | This work |

**Table 2** Primer sequences

| Primers | Sequences (5'-3') | Digested sites |
|---|---|---|
| $F_1$-gadA | TCGCGGATCCATGGACCAGAAGCTGTT | *Bam*HI |
| $R_1$-gadA | CTTCGAGCTCTTAGGTGTGTTTAAAGCT | *Sac*I |
| $F_1$-SNO1 | CTAAGAGCTCGAAGGAGATATACCATGCACAAAACCCACAGTACAATGT | *Sac*I |
| $R_1$-SNO1 | CCTTCGCGGCCGCTTAATTAGAAACAAACTGTCTGATA | *Not*I |
| $F_1$-SNZ1 | CCTAAGCGGCCGCGAAGGAGATATACCATGACTGGAGAAGACTTTAAGATCA | *Not*I |
| $R_1$-SNZ1 | AAGCTTCTCGAGGTTACCACCCAATTTCGGAAAGTCTT | *Xho*I |

## Construction and transformation of recombinant plasmids

All primer sequences are listed in Table 2, and were synthesized by Shanghai Sangon Biotech Co. Ltd, China. PCR reaction mixture includes 10 µL PCR buffer, 4 µL dNTP mixture, 2 µL template (100 ng/µL), 1 µL forward primer (20 µM), 1 µL reverse primer (20 µM), and 0.5 µL PrimeSTAR™ HS DNA polymerase. PCR amplification was performed for 30 cycles. Each cycle consisted of denaturation at 95 °C for 30 s, annealing at 55 °C for 15 s, and extension at 72 °C. The extension time varied with the length of PCR product (1 kb/min). The plasmid pET28a(+) was used to construct pET28a-gadA and pET28a-gadA-SNO1-SNZ1 (Fig. 1c).

To construct pET28a-gadA, the gene *gadA* was amplified using a primer set $F_1$-gadA and $R_1$-gadA. The plasmid pET32a-gadABC was extracted from recombinant *E. coli* BL21(DE3)/pET32a-gadABC (Yu et al. 2018), and was used as the template. The PCR product was digested with *Bam*HI and *Sac*I, and was ligated into the *Bam*HI-*Sac*I digested plasmid pET28a(+). The resultant plasmid was designated as pET28a-gadA (Fig. 1c).

The genomic DNA of *S. cerevisiae* SC288 was extracted as described by Looke et al. (2011), and was used as the template for amplifying the genes *SNO1* and *SNZ1*. The PCR procedure was the same as that of *gadA* amplication except for primer sets. Primer sets, $F_1$-SNO1/$R_1$-SNO1 and $F_1$-SNZ1/$R_1$-SNZ1, were respectively used for the amplification of the genes *SNO1* and *SNZ1*. The PCR products were sequentially ligated into pET28a-gadA to form pET28a-gadA-SNO1-SNZ1 (Fig. 1c). After being verified by DNA sequencing, the plasmid pET28a-gadA and pET28a-gadA-SNO1- SNZ1 were respectively transformed into *E. coli* BL21 (DE3) using $CaCl_2$-heat shock method (Maniatis et al. 1982). The resultant strains were designated as *E. coli* BL21/pET28a-gadA and *E. coli* BL21/pET28a-gadA-SNO1-SNZ1, respectively.

## Strain culture

Two hundred microliter of the glycerol-stocked cell cultures were inoculated into 10 mL LB medium, and were cultured overnight at 37 °C and 180 rpm for preparing seeds. Five percent of the cell cultures were transferred into 250 mL conical flasks pre-equipped with 50 mL fermentation medium (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl, and different concentrations of L-MSG, pH 7.4), and were cultured for 36 h at 37 °C and 180 rpm. The pH of fermentation broth was adjusted to 7.0 by 5.0 M NaOH during the first 6 h of culture. Afterwards, IPTG (1 mM) was added when the $OD_{600}$ of the cells was 0.8, and then pH was adjusted to 4.2 by 4 M HCl for producing GABA. One milliliter of the samples were taken for determining the cell optical density ($OD_{600}$) and the concentrations of L-MSG and GABA. For investigating the effect of different concentrations of L-MSG on the production of GABA, L-MSG (5, 10, 15, 20 and 25 g/L) were used.

## SDS-PAGE analysis of recombinant proteins

The recombinant protein SDS-PAGE analysis was performed using 15% separating gel and 5% stacking gel. Protein samples were prepared using a 10 × SDS buffer. After the sample was vortexed adequately, it was heated at 100 °C for 10 min, and was cooled in a ice water bath. The sample was centrifuged for 10 min at 15,000 × g. Twenty microliter of the samples were used for electrophoresis in a 5–15% polyacrylamide gel. After electrophoresis, the protein sample was stained with coomassie brilliant blue R250 (Laemmli 1970).

## Determination of the cell optical density and concentrations of L-MSG and GABA

The cell optical density of *E. coli* was determined by measuring $OD_{600}$ with UV-1800 spectrophotometer. The

concentrations of the L-MSG and GABA were determined by HPLC after derivatization (Meeploy and Deewatthanawong 2015; Yu et al. 2019). One milliliter of the fermentation broth was taken and centrifuged for 5 min at 8000 × g. One hundred microliter of the supernatant was transferred into 1.5 mL centrifuge tube. Two hundred microliter of 1 M sodium carbonate-sodium bicarbonate buffer (pH 10.0) was added to the centrifuge tube. Afterwards, One hundred microliter of 80 g/L solution of dansyl chloride in acetone was added to the sample, followed by 600 µL of distilled water to form 1 ml of reaction system. Next, the sample derivatization was conducted by incubating it in a water bath for 40 min at 80 °C. The reaction was halted by adding 100 µL of 10% acetic acid. The sample was centrifuged for 5 min at 12,000 × g. The resultant supernatant was filtered by a 0.22 µm filter membrane.

The operation conditions for HPLC determination of L-MSG and GABA were as follows (Cho et al. 2007; Kim et al. 2009): the mobile phase A was methanol, and the mobile phase B contained 50 mM sodium acetate: methanol: tetrahydrofuran [1:15: 84, v/v] (pH 6.2). The gradient program was 80% A, 0–5 min; 80–45% A, 5–21 min; 45–0% A, 21–22 min; 0% A, 22–25 min. The flow rate of the mobile phase was 1 mL/min. The chromatographic column and UV detection wavelength were an Agilent Zorbax Eclipse Plus C18 (4.6 × 250 mm, 5 µm) and 254 nm, respectively. The injection volume was 15 µL and the column temperature was 30 °C.

The conversion yield of GABA was calculated according to the following equation:

$$Y = \frac{B}{A} \times 100\% \quad (1)$$

where $Y$ is the conversion yield of GABA from L-MSG, $A$ is the theoretically calculated maximal mass concentration of GABA when L-MSG is depleted, and $B$ is the mass concentration of the produced GABA.

### Statistical analysis

Experiments were performed in triplicate, and the results are represented as the mean ± standard deviation. The statistical analysis of data was performed by the SPSS 17.0 software. The graphing was performed by the Origin 8.5 software.

## Results

### SDS-PAGE analysis of the protein expression of GadA, Pdx1 and Pdx2

The SDS-PAGE analysis of the recombinant protein expression is shown in Fig. 2. The genes *gadA*, *SNZ1* and *SNO1* encode the enzyme proteins GadA, Pdx1 and Pdx2, respectively. Their theroretically calculated molecular weights were 52.6, 38.3 and 27.8 kDa, respectively. It could be seen from Fig. 2 that the molecular weights of the expressed proteins GadA, Pdx1 and Pdx2 were in coincide with their respectively calculated ones, indicating that these three proteins were correctly expressed in recombinant strains. The enzyme GadA is responsible for the conversion of L-MSG to GABA. The other two enzymes, Pdx1 and Pdx2, are responsible for the biosynthesis of pyridoxal 5'-phosphate. The overexpression of these two enzymes could enhance the production of pyridoxal 5'-phosphate, and then improve the activity of GadA. When the activity of GadA was enhanced, it could catalyze the conversion of more L-MSG to GABA.

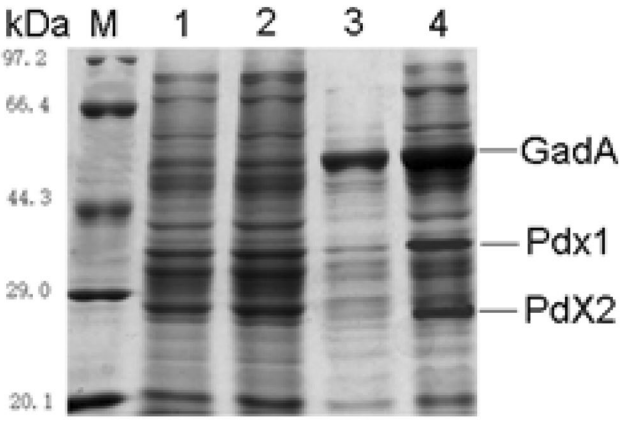


**Fig. 2** SDS-PAGE analysis of recombinant proteins lane M: protein standard sample; lane 1: the sample from the strain *E. coli* BL21; lane 2: the sample from the strain *E. coli* BL21/pET28a; lane 3: the sample from the strain *E. coli* BL21/pET28a-gadA; lane 4: the sample from the strain *E. coli* BL21/pET28a-gadA-SNO1-SNZ1

### Production profiles of GABA in recombinant *E. coli BL21/pET28a-gadA*

The cell optical density ($OD_{600}$) and the concentrations of L-MSG and GABA were respectively determined during fermentation in recombinant *E. coli* BL21/pET28a-gadA and *E. coli* BL21/pET28a-gadA-SNO1-SNZ1. A major factor affecting GABA production by *E. coli* fermentation is the concentration of L-MSG. Thus, the effect of the L-MSG concentration on the production of GABA was investigated in detail.

Figure 3 shows the effect of different concentrations of L-MSG on the production of GABA in recombinant *E. coli* BL21/pET28a-gadA. Overall, the concentration of L-MSG decreased as the fermentation time prolonged. When the initial concentration of L-MSG was 5.0 g/L, it was basically depleted at 9 h of fermentation. During this stage, the cell optical density and the concentration of GABA were

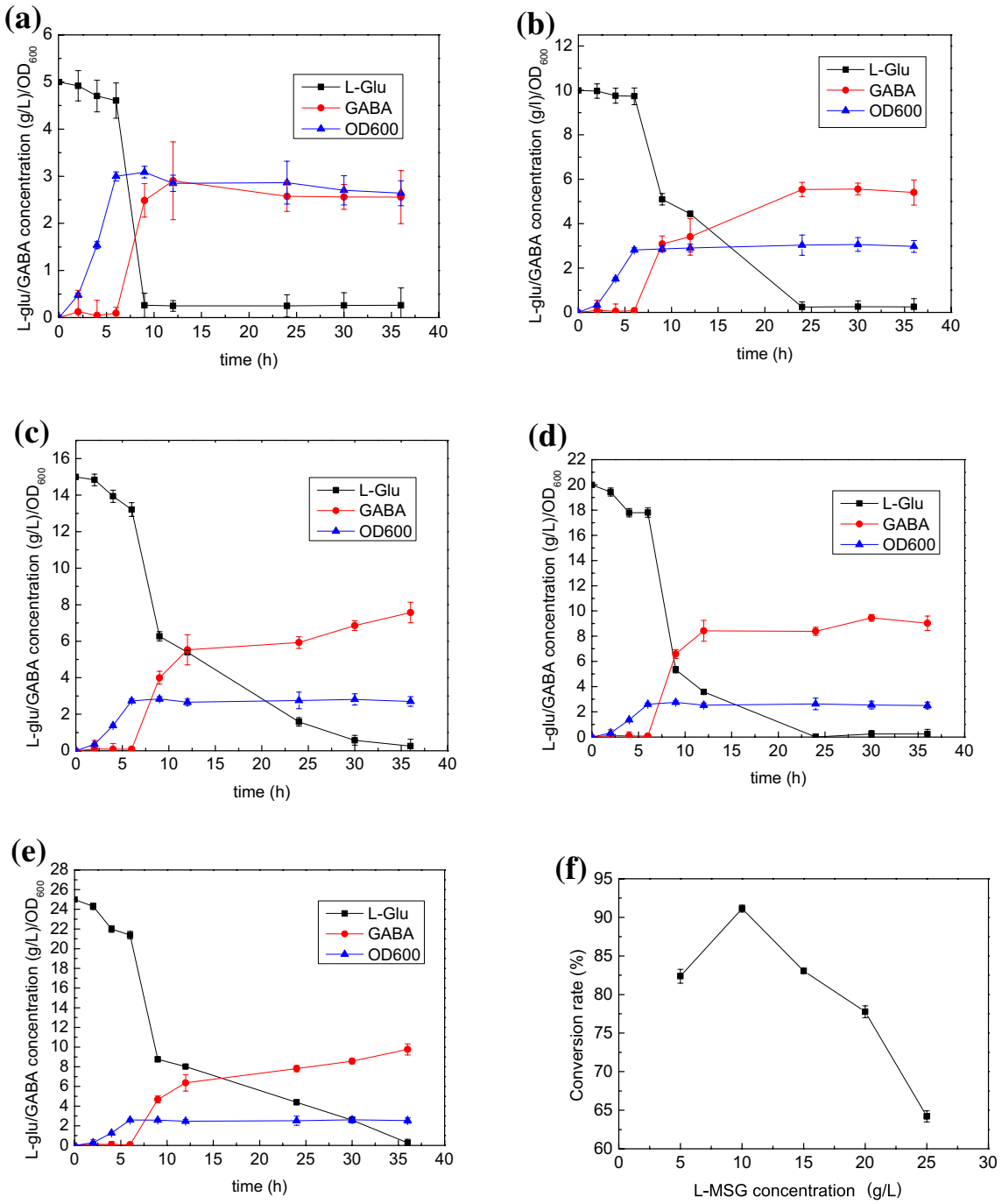


**Fig. 3** Production profiles of GABA in *E. coli* BL21/pET28a-gadA at different concentrations of L-MSG. **a** 5 g/L of L-MSG; **b** 10 g/L of L-MSG; **c** 15 g/L of L-MSG; **d** 20 g/L of L-MSG; **e** 25 g/L of L-MSG; **f** the conversion yield of GABA at different concentrations of L-MSG

increased, and reached 3.09 and 2.49 g/L, respectively. Afterwards, they kept relatively constant as the fermentation time prolonged (Fig. 3a). As the increase in the concentrations of L-MSG, the time when it was depleted was much prolonged. When the initial concentrations of L-MSG were 10 g/L, L-MSG was depleted at 25 h of fermentation. Meanwhile, the cell optical density and the concentration of GABA respectively reached maximum of 3.3 and 5.54 g/L (Fig. 3b), whereas they were 3.6 and 7.57 g/L at 35 h of fermentation when the initial concentration of L-MSG was 15 g/L (Fig. 3c). The highest concentrations of GABA of 9.03 and 9.76 g/L ($p < 0.05$) were respectively achieved at the initial L-MSG concentrations of 20 and 25 g/L (Fig. 3d-e). The conversion yield of GABA in recombinant *E. coli*

BL21/pET28a-gadA is shown in Fig. 3f. The conversion yield of GABA was increased when the concentrations of L-MSG varied from 0 to 10 g/L. It reached the highest value of 91% ($p < 0.05$) at an initial L-MSG concentration of 10 g/L. Afterwards, it was decreased as the concentrations of L-MSG were further increased.

## Production profiles of GABA in recombinant *E. coli BL21/pET28a-gadA-SNO1- SNZ1*

The effect of different concentrations of L-MSG on the production of GABA was investigated in recombinant *E. coli* BL21/pET28a-gadA-SNO1-SNZ1 containing the enzymes

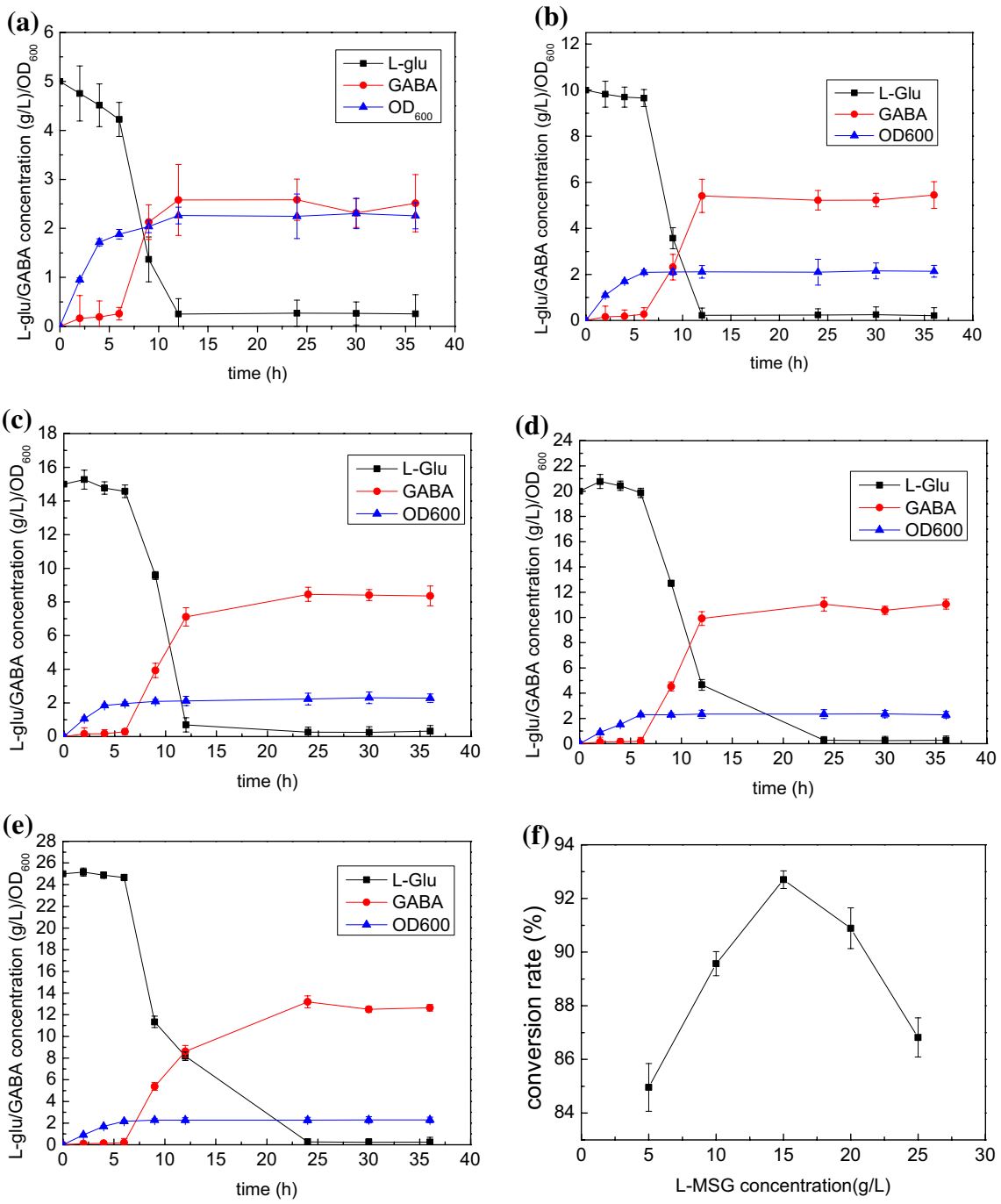


**Fig. 4** Production profiles of GABA in *E. coli* BL21/pET28a-gadA-SNO1-SNZ1 at different concentrations of L-MSG. **a** 5 g/L of L-MSG; **b** 10 g/L of L-MSG; **c** 15 g/L of L-MSG; **d** 20 g/L of L-MSG; **e** 25 g/L of L-MSG; **f** the conversion yield of GABA at different concentrations of L-MSG

of the regeneration pathway of PLP, and the results are shown in Fig. 4. Overall, the concentrations of L-MSG had an obvious influence on the production of GABA. As the increase in the initial concentrations of L-MSG from 5 g/L to 25 g/L, the highest concentrations of GABA increased from 2.58 g/L to 13.20 g/L. When the initial concentration of L-MSG was 5 g/L, it was depleted at 12 h of fermentation. The concentration of GABA and cell optical density also reached maximum of 2.58 g/L and 2.26, respectively. Afterwards, they basically kept relatively constant (Fig. 4a). When the concentrations of L-MSG varied from 10 g/L to 20 g/L, the highest concentrations of GABA were increased from 5.41 g/L and 11.05 g/L (Fig. 4b–d). When the concentration of L-MSG was 25 g/L, it was depleted at 25 h of fermentation, and the concentration of GABA reached the highest value of 13.20 g/L ($p < 0.05$). The cell optical density was increased from 5 to 25 h of fermentation, and reached a maximum of 3.54 (Fig. 4e). Figure 4f shows the variation in the conversion yield of GABA at different concentrations of L-MSG. When the concentrations of L-MSG increased from 5 g/L to 15 g/L, the conversion yield of GABA increased from 84.96% to 92.7% ($p < 0.05$). The maximal GABA conversion yield of 92.7% was achieved at an initial L-MSG concentration of 15 g/L. Afterwards, it declined as the increase in the concentrations of L-MSG.

### Comparison of the GABA concentrations

The highest concentrations of GABA obtained at different initial concentrations of L-MSG were compared, and the result is shown in Fig. 5. When the initial concentrations of L-MSG were 5 and 10 g/L, the highest concentrations of GABA showed no obvious difference in recombinant *E. coli* BL21/pET28a-gadA and *E. coli* BL21/pET28a-gadA-SNO1-SNZ1. However, when the initial concentrations of L-MSG were more than 10 g/L, it was significantly observed that the concentrations of GABA were higher in recombinant *E. coli* BL21/pET28a-gadA-SNO1-SNZ1 than those in recombinant *E. coli* BL21/pET28a-gadA. Especially, when the initial concentration of L-MSG was 25 g/L, the concentration of GABA reached 13.20 g/L in recombinant *E. coli* BL21/pET28a-gadA-SNO1-SNZ1, which was increased by 35.2% ($p < 0.05$) as compared to that (9.76 g/L) in recombinant *E. coli* BL21/pET28a-gadA. Taken together, these results demonstrated that the introduction of the enzymes of the regeneration pathway of PLP in recombinant *E. coli* BL21/pET28a-gadA-SNO1- SNZ1 was beneficial to enhance the production of GABA.

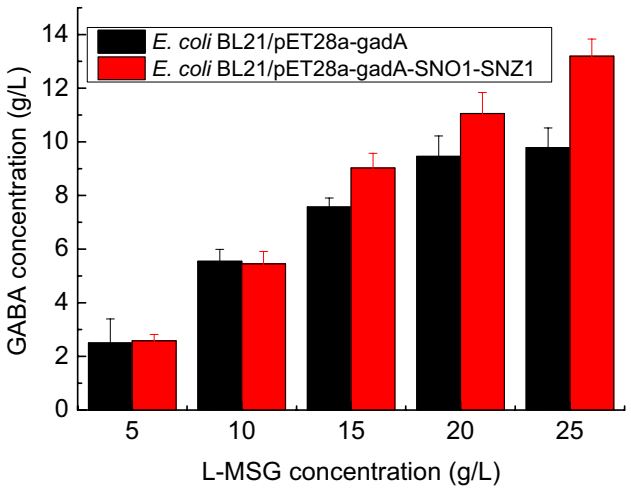


**Fig. 5** Comparison of the GABA production in *E. coli* BL21/pET28a-gadA and *E. coli* BL21/pET28a-gadA-SNO1-SNZ1

## Discussion

Pyridoxal 5'-phosphate is the coenzyme factor of glutamate decarboxylase that can catalyze the conversion of the substrate L-MSG to GABA (Spink et al. 1985; Strausbauch and Fischer 1970). In *S. cerevisiae* SC288, pyridoxal 5'-phosphate synthase, which contained two subunits Pdx1 and Pdx2, plays a key role in the regeneration of the coenzyme factor PLP (Strohmeier et al. 2006; Zhang et al. 2010). In order to enhance the bioconversion of L-MSG to GABA, pyridoxal 5'-phosphate synthase was overexpressed in *E. coli* BL21 in this study by introducing its two subunits-encoding genes *SNZ1* and *SNO1*. *E. coli* BL21 contains the substrates that are required for the biosynthesis of PLP, glyceraldehyde-3-phosphate and glutamine (Käck et al. 1999; Mukherjee et al. 2011). Thus, the entire regeneration pathway of PLP could be established in *E. coli* BL21. The regeneration of PLP could provide the adequate coenzyme factor for enhancing the activity of glutamate decarboxylase A. As a result, the recombinant strain *E. coli* BL21/pET28a-gadA-SNO1-SNZ1 produced 13.20 g/L of GABA, which was 35.2% ($p < 0.05$) higher as compared to that (9.76 g/L) in the strain *E. coli* BL21/pET28a-gadA. Thus, we succeed in enhancing the bioconversion of L-MSG to GABA by introducing the enzymes of the regeneration pathway of PLP in *E. coli* BL21. This process does not require the addition of in vitro expensive coenzyme factor PLP, and so displays an enormous potential towards reducing the production cost of GABA. Besides pyridoxal 5'-phosphate synthase used in this study, pyridoxal kinase from *L. plantarum* is an ideal alternative that can catalyze the conversion of pyridoxal to PLP. *Corynebacterium glutamicum* G01 can efficiently convert glucose to the L-glutamate, but it can not acculumate GABA directly from L-glutamate because it lacks of glutamate decarboxylase and pyridoxal kinase. In

order to realize the efficient one-step production of GABA from glucose without the addition of exogenous PLP, the metabolic pathway from L-glutamate to GABA from *L. plantarum* was grafed into *C. glutamicum* by overexpressing glutamate decarboxylase and pyridoxal kinase. As a result, the recombinant *C. glutamicum* produced 70.6 g/L of GABA (Zhang et al. 2014). Although the production strain was different from *E. coli*, the idea for the GABA production was somewhat similar. Taken together, these results demonstrate that pyridoxal 5'-phosphate synthase and pyridoxal kinase are two good candidates for enhancing the biosynthesis of the endogenous PLP in microorganisms, a cofactor of glutamate decarboxylase required for the efficient conversion of L-glutamate to GABA.

Owing to considerable uses of GABA in many fields, various studies have been conducted to enhance its production. Through the expression of rice glutamate decarboxylase in *Bifidobacterium longum*, GABA (0.1 g/L) was obtained from 30 g/L of L-MSG (Park et al. 2005). GABA (0.4 g/L) was produced from 30 g/L of L-MSG in *Bacillus subtilis* by expressing glutamate decarboxylase from *Lactobacillus brevis* (Park and Oh 2006). By expressing glutamate decarboxylase from *L. plantarum* in *L. sakei*, the conversion yield of GABA was enhanced by 1.35-fold (Kook et al. 2010). When *E. coli* XL1 glutamate decarboxylase B was introduced into *E. coli* XB, the recombinant strain produced 5.09 g/L of GABA (Le Vo et al. 2012). In the above-mentioned studies, only glutamate decarboxylase was expressed in heterologous host cells. Moreover, only endogenous PLP from native metabolic pathways of host cells was used during the bioconversion of L-MSG to GABA. These led to a low conversion yield of GABA from L-MSG. Besides expressing glutamate decarboxylase A, the enzymes of the regeneration pathway of its coenzyme factor PLP from *S. cerevisiae* SC288 were introduced into *E. coli* BL21 in this study. This not only enhances the activity of glutamate decarboxylase A, but also eliminates the use of in vitro expensive PLP. Through these efforts, we succeeded in producing 13.20 g/L of GABA in this study.

In conclusion, the production of GABA in *E. coli* BL21(DE3) was enhanced by introducing the enzymes of the regeneration pathway of the coenzyme factor PLP from *S. cerevisiae* SC288. Thus, our study provides a new method that may be very useful for the bioconversion of L-MSG to GABA by a single recombinant *E. coli* strain containing the enzymes of the regeneration pathway of PLP. This study also lays good foundation for the industrial production of GABA from L-MSG by recombinant *E. coli* in future.

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## Declarations

**Conflict of interest** The authors declare that they have no competing interests.

**Research involving human participants and/or animals** This article does not contain any studies with human participants or animals performed by the author.

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