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Original Research Article

High-level production of γ-aminobutyric acid via efficient co-expression of the key genes of glutamate decarboxylase system in *Escherichia coli*

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ABSTRACT

Biosynthesis of the functional factor γ-aminobutyric acid (GABA) in bacteria involves two key proteins an intracellular glutamate decarboxylase (GadB) and a membrane-bound antiporter (GadC). Efficient co-expression of suitable GadB and GadC candidates is crucial for improving GABA productivity. In this study, $gadB_{\Delta C11}$ of *Lactiplantibacillus plantarum* and $gadC_{\Delta C41}$ of *Escherichia coli* were inserted into the designed double promoter (P_{T7lac} and P_{BAD}) expression system. Then, *E. coli* Lemo21(DE3) was chosen as the host to minimize the toxic effects of $GadC_{\Delta C41}$ overexpression. Furthermore, a green and high-efficiency GABA synthesis system using dormant engineered Lemo21(DE3) cells as biocatalysts was developed. The total GABA yield reached 829.08 g/L with a 98.7% conversion ratio within 13 h, when engineered *E. coli* Lemo21(DE3) cells were concentrated to an OD_{600} of 20 and reused for three cycles in a 3 M *L*-glutamate solution at 37 °C, which represented the highest GABA productivity ever reported. Overall, expanding the active pH ranges of GadB and GadC toward physiological pH and employing a tunable expression host for membrane-bound GadC production is a promising strategy for high-level GABA biosynthesis in *E. coli*.

1. Introduction

γ-Aminobutyric acid (GABA) is a nonprotein amino acid [1] that has been utilized as a health-promoting ingredient in various foods and pharmaceutical products due to its potential to lower blood pressure, improve sleep and stimulate immune cells in humans [2–5]. In addition, GABA has been used as a feed stock for the synthesis of 2-pyrrolidone, a precursor in the production of the heat-resistant and biodegradable polymer nylon-4, which creates an avenue for its industrial applications [6]. GABA can be produced by several approaches, including biosynthesis and chemical synthesis [2]. Featuring high catalytic efficiency, mild reaction conditions and attractive environmental compatibility, the biosynthesis of GABA has gradually attracted increasing attention [7,8].

A widely accepted model for GABA synthesis in microorganisms involves two key proteins: pyridoxal 5′-phosphate (PLP)-dependent glutamate decarboxylase (GAD, encoded by *gadA* or *gadB*), which catalyzes the α-decarboxylation of *L*-Glu-to form GABA and CO_2, and a cognate membrane-bound antiporter GadC (encoded by *gadC*), which exchanges intracellular GABA with extracellular *L*-Glu [9,10]. Currently, the biosynthesis of GABA is mainly performed by employing microbial whole-cell systems or isolated GADs, either in free or immobilized form [7,11-16]. In particular, whole-cell biocatalysts are preferred in this process, which eliminates the need for tedious cell disruption and subsequent GAD purification [14,17]. However, owing to the permeability barriers imposed by their unique cell envelopes, whole-cell biocatalysts generally exhibit much slower reaction rates than do the use of free GADs. Overcoming the mass transfer limitations has thus become an important issue for the application of whole-cell biocatalysts to produce GABA.

E. coli is the most common and widely used prokaryotic host for GAD system key gene expression owing to its genetic and biochemical stability, rapid growth rate and high protein expression level [18,19]. In our previous work, several GAD genes derived from *Levilactobacillus brevis, Lactiplantibacillus plantarum* and *Lactococcus lactis* were expressed in *E. coli* BL21(DE3) and characterized in terms of specific activity, optimum pH, optimum temperature and thermal stability [11,12,20]. Meanwhile,

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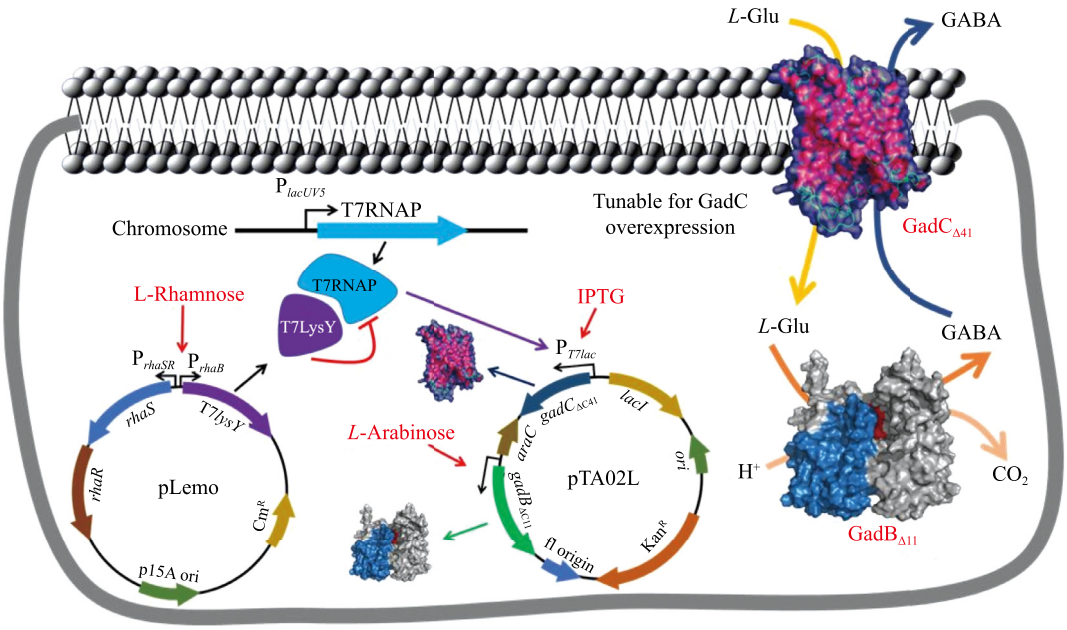

Fig. 1. Tunable control of GadBΔC11 and GadCΔC41 overexpression in *E. coli* Lemo(DE3).
Recombinant *E. coli* Lemo(DE3) strain harboring two plasmids, pLmeo and pTA02L. The expression of *gadC*ΔC41 and *gadB*ΔC11 was under the control of P*T7lac* and P*BAD*, respectively.

the GABA productivity of recombinant *E. coli* strains has been determined. Moreover, organic solvents and surfactants have been utilized to increase the membrane permeability of engineered *E. coli* cells [21]. Unfortunately, permeabilization with organic solvents and surfactants dramatically improved the cell-associated GAD activity, whereas an obvious decrease in cell viability was also observed.

Recently, another elegant remedy to improve mass transport efficiency is the overexpression of the antiporter GadC to facilitate transport of the reaction substrate *L*-Glu-and its product GABA through the cells [21–23]. Nevertheless, problems remain, and recombinant GadC production is associated with severe toxicity for the host strain *E. coli* BL21(DE3), thereby leading to low final biomass and volumetric yield [21,24]. In particular, similar to other membrane proteins, the cytotoxicity caused by GadC overexpression is an issue which has not been systematically addressed. Therefore, improvements of the GadC itself via protein engineering or of the expression host via metabolic engineering might be helpful in solving the problem of low cellular productivity. Moreover, almost all reported microbial GadC and GadB enzymes exhibit catalytic activities only under acidic environments and rapidly lose their robustness when shifted to near-neutral pH [21–23]. Thus, expanding the active pH range of GadB and GadC toward the physiological range of intracellular pH is also needed.

To address this issue, in the present work, a double-promoter expression system (P_{T7lac} and P_{BAD} promoters) was constructed with pET28a as the backbone. Then, the GadC mutant ($GadC_{\Delta C41}$) gene of *E. coli* [24,25] and the GadB mutant ($GadB_{\Delta C11}$) gene of L. *plantarum* [20] were inserted into the designed vector and overexpressed under the control of P_{T7lac} and P_{BAD}, respectively. To further minimize the toxic effects of $GadC_{\Delta C41}$ production, *E. coli* Lemo21(DE3) [26], an *E. coli* BL21(DE3) derivative that precisely modulates the activity of T7 RNA polymerase (T7 RNAP) by titratable control of its natural allosteric inhibitor T7 lysozyme (T7Lys) expression, was selected as the host strain (Fig. 1). Subsequently, a green and high-efficiency GABA synthesis system using dormant engineered *E. coli* Lemo21(DE3) cells as the biocatalyst and pure water as the sole solvent was designed, and its GABA productivity was determined. The current work sheds new light on the process of appropriate expression vector and host strain selection to optimize key GAD system protein production, which is essential to achieve a high yield of GABA.

2. Materials and methods

2.1. Bacterial strains, plasmids and growth conditions

The strains and plasmids used in this work are listed in **Table S1**. *E. coli* strains were grown aerobically in Luria-Bertani (LB) broth at 37 °C with constant shaking at 220 rpm. The L. *plantarum* strain was grown in MRS medium at 37 °C without shaking. Solid LB agar plates for plating were prepared by adding 1.5% (w/v) agar to the corresponding liquid media. When needed, 50 μg/mL of kanamycine was used for the isolation of transformants and subsequent stable plasmid maintenance.

2.2. Reconstruction of the GAD system in E. coli

For tunable control of GadC and GadB expression in *E. coli*, a double promoter expression system (P_{T7lac} promoter and P_{BAD} promoter) was designed and constructed. Firstly, the *araC*-P_{BAD} gene fragment was cloned from pG-KJE8 plasmid with primers P_{BAD}-F and P_{BAD}-R, and inserted into the linearized pET28a. Subsequently, the *gadC* and *gadB* was cloned from the genomic DNA of *E. coli* BL21(DE3) and L. *plantarum* Yll.03, respectively, and then ligated into the above designed vector. Information on the primers is listed in **Table S2**. The sequences of the promoters and genes used in this work are listed in **Table S3**. The restriction enzyme digestion, ligation reaction and seamless cloning process were performed according to the instructions. *E. coli* DH5α was routinely used as the intermediate host. After confirmed by DNA sequencing, recombinant plasmids were further transformed into *E. coli* BL21(DE3) and *E. coli* Lemo21(DE3) cells.

2.3. Tunable control of GadC and GadB overexpression in E. coli in shake flasks

Recombinant *E. coli* BL21(DE3) cells were grown in LB broth containing 50 μg/mL of kanamycin at 37 °C and 220 rpm. After the culture reached an OD_{600} of approximately 0.6, 4.0 g/L *L*-arabinose and

of various concentrations of IPTG were added to induce the GadC and GadB overexpression at 30 °C and 180 rpm. As for recombinant *E. coli* Lemo21(DE3), the cells were grown in LB medium supplemented with 50 μg/mL kanamycin and 30 μg/mL chloramphenicol. Similarly, 50 μM IPTG, 4.0 g/L *L*-arabinose and various concentrations of *L*-rhamnose were utilized as the inducers to signal the programmed cells to synthesize the target proteins.

2.4. Cell-associated GAD activity assay

Cell-associated GAD activity was analysed by determing the GABA formed in the reaction mixture containing 0.2 M sodiumacetate buffer (pH 4.7), 100 mM *L*-Glu-and 0.1 mg (dry cell weight)/mL cell biomass. After incubation for 15 min at 37 °C, the reaction was terminated by boiling for 5 min. The GABA and *L*-Glu-contents were determined by HPLC method [27].

2.5. Fed-batch cultivation of engineered E. coli strain

Engineered *E. coli* Lemo21(DE3)/pTA02L strain was inoculated into 200 mL of LB medium supplemented with appropriate antibiotics and grown at 37 °C in a shaker incubator overnight. Then, the seed culture was transferred to 3 L of fermentation medium (20 g/L glucose, 5 g/L yeast extract, 9 g/L KH_2PO_4, 4 g/L $(NH_4)_2HPO_4$, 0.6 g/L $MgSO_4$) in a 5 L bioreactor (Biotech-5JGZ, Shanghai, China). During the cultivation, temperature was set at 30 °C, and the pH was maintained around 6.7 by adding 25% (v/v) $NH_3{\cdot}H_2O$. The dissolved oxygen (DO) level was maintained at approximately 20% (v/v) by adjusting the agitation speed from 200 rpm to 600 rpm with an air flow rate of 1.0 vvm. The cells were grown to OD_{600} of about 4.0, then induced with 50 μM IPTG, 4.0 g/L *L*-arabinose and 0.5 mM *L*-rhamnose. When the initial carbon source was depleted, 80% (w/v) glucose solution was fed to maintain its concentration at 0.5–5 g/L. After culture for 15 h, the mixture was centrifuged at 5000×*g* for 20 min at 4 °C, and the supernatant was discarded. The resulting cell pellets were resuspended in 50 mM sodium phosphate buffer (pH 7.2) and washed 3 times by repeated centrifugations.

2.6. High level synthesis of GABA by using dormant engineered E. coli cells

After re-suspending the cell pellets in 200 mL deionized water (OD_{600} = 20), 88.3 g of *L*-Glu-powder was added to start the reaction. The flasks were incubated on a rotatory shaker at 37 °C and 150 rpm under the pH un-controlled conditions. To scale-up GABA production, the bioconversion process was further performed in a 3.7 L reactor (Bioengineering KLF) by 20 OD cells suspended in 1.5 L deionized water with initial addition of 0.663 kg of *L*-Glu (3 M) at 37 °C and 500 rpm under the pH un-controlled conditions. The time variations of GABA synthesis in the above reaction systems were analyzed by HPLC [27].

3. Results and discussion

3.1. Efficient co-expression of key genes of the GAD system in E. coli

Besides the decarboxylase reaction, another rate-limiting step in GABA biosynthesis is the transport of the substrate *L*-Glu-and its product GABA through the cells [21,24]. However, GadC production is associated with severe toxicity for the *E. coli* host, most previous works have only focused on the overexpression of various wild-type GadB proteins and catalytic performance-improved mutants for enhancing GABA production [14,19,21,28]. To minimize the toxic effects of *gadB* and *gadC* coexpression mediated by a single promoter, the *araC*-P_{BAD} gene fragment was cloned from pG-KJE8 and inserted into pET28a. Then, *gadC* of *E. coli* and $gadB_{\Delta C11}$ of L. *plantarum* were successively inserted into the designed vector (Fig. S1). The recombinant vector was termed pTA01L, and the expression of *gadC* and $gadB_{\Delta C11}$ was under the control of P_{T7lac} and P_{BAD}, respectively.

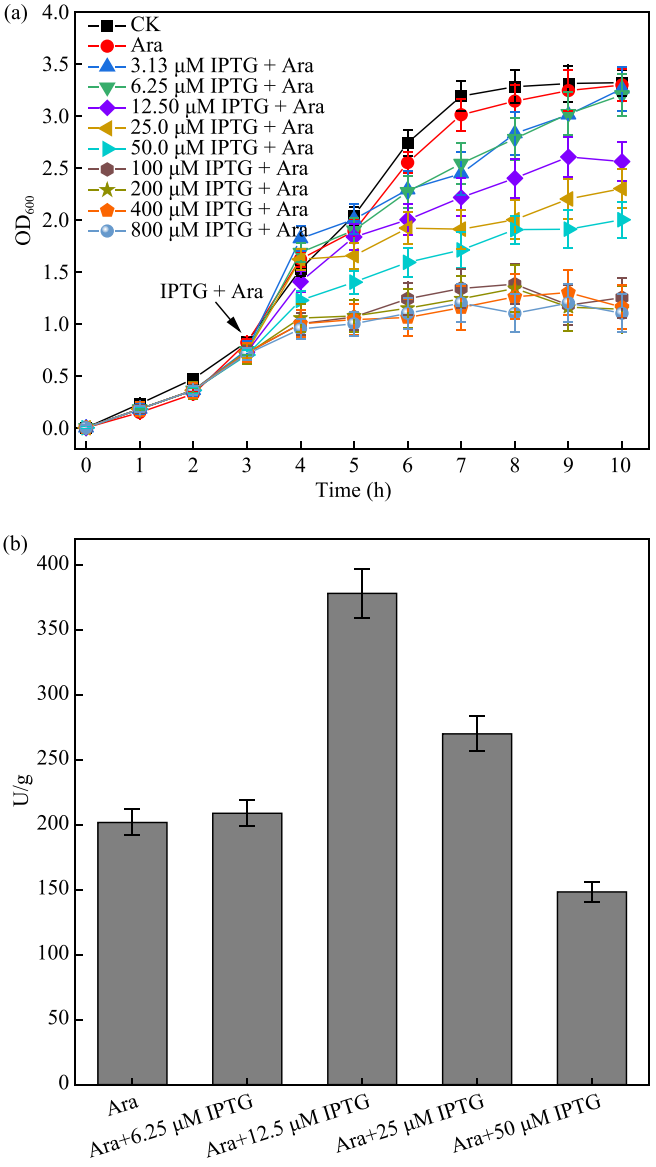

Fig. 2. Analysis of the cell growth and cell-associated GAD activity of strain *E. coli* BL21(DE3)/pTA01L.
(a) When the cells reached an OD_{600} of 0.6, 4.0 g/L *L*-arabinose and various concentrations of IPTG were added to the cultures and then incubated at 30 °C and 180 rpm for 7 h. (b) The cell-associated GAD activity of *E. coli* BL21(DE3)/pTA01L induced with 4.0 g/L *L*-arabinose and various concentrations of IPTG.

As shown in Fig. 2, overexpression of $GadB_{\Delta C11}$ via the addition of 4.0 g/L *L*-arabinose did not significantly inhibit the growth of *E. coli* BL21(DE3), as was the case in previous work [19]. However, overexpression of GadC by the addition of various concentrations of IPTG (3.13–800 μM) affected cell growth to different degrees. After 8 h of culture, the control strain (CK) without *L*-arabinose and IPTG addition reached an OD_{600} value of 3.22 ± 0.16, while the OD_{600} value of the strain supplied with 4 g/L of *L*-arabinose and 50 μM of IPTG was only 1.91 ± 0.14. Meanwhile, the cell-associated GAD activity of the engineered strain was also determined. As expected, the GAD activity of 378.27 ± 24.46 U/g DCW was achieved upon induction with 4.0 g/L *L*-arabinose and 12.5 μM IPTG, whereas a GAD activity of only 201.83 ± 10.54 U/g DCW was observed in the absence of IPTG. Collectively, these results strongly suggest that the tunable control of overexpression of $GadB_{\Delta C11}$ and GadC in *E. coli* BL21(DE3) made much

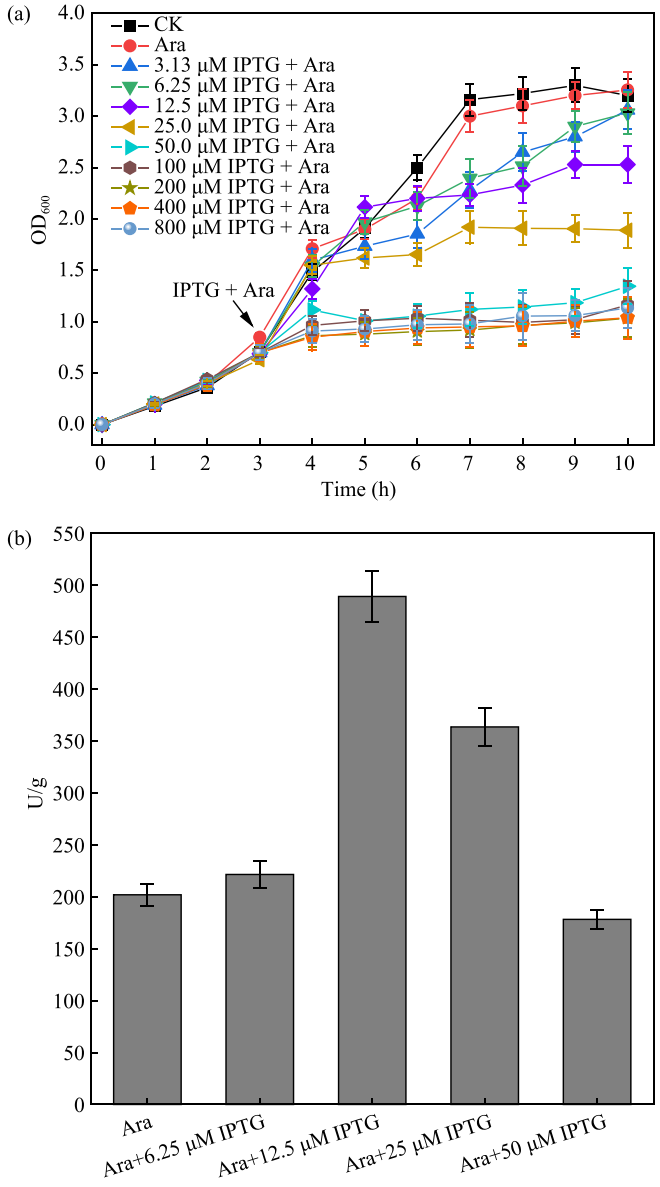

Fig. 3. Analysis of the cell growth and cell-associated GAD activity of strain *E. coli* BL21(DE3)/pTA02L.
(a) When the cells reached an OD_{600} of 0.6, 4.0 g/L *L*-arabinose and various concentrations of IPTG were added to the cultures, which were then incubated at 30 °C and 180 rpm for 7 h. (b) The cell-associated GAD activity of *E. coli* BL21(DE3)/pTA02L induced with 4.0 g/L *L*-arabinose and various concentrations of IPTG.

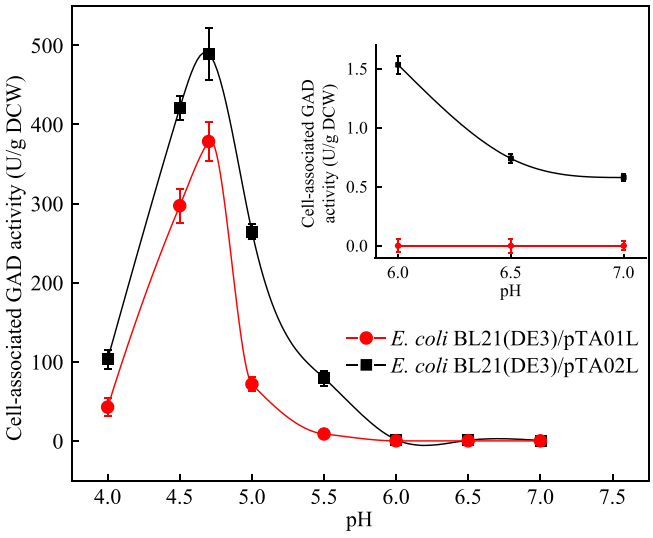

Fig. 4. Effects of pH on the cell-associated GAD activity of the engineered *E. coli* strain.
The cell-associated GAD activity was analyzed by measuring the GABA formed at 37 °C in the 0.2 M sodium acetate buffer (pH range 4.0 to 5.5) or 0.1 M sodium phosphate buffer (pH range 6.0 to 7.0). One unit (U) of GAD activity was defined as 1 μM GABA produced per minute at 37 °C in the above reaction solution.

greater contributions to the whole-cell catalytic activity than the individual overexpression of $gadB_{\Delta C11}$, although GadC production resulted in negative effects on cell growth.

3.2. Engineering a C-terminal truncated GadC variant to further improve cell-associated GAD activity

Similar to GadB, GadC also has a C-plug tail, which blocks the *L*-Glu/GABA transport pathway in near-neutral pH conditions [25,29]. To further improve GAD activity, an engineered mutant of GadC ($GadC_{\Delta C41}$), which is relatively active in a near-neutral pH environment, was employed. The corresponding recombinant vector was named pTA02L (Fig. S2). As shown in Fig. 3, the recombinant strain harboring pTA02L maintained growth behaviors similar to those of *E. coli* BL21(DE3)/pTA01L. After 10 h of culture, the strain supplied with 4.0 g/L *L*-arabinose and 50 μM IPTG reached an OD_{600} value of only 1.35 ± 0.23. Remarkably, the cell-associated GAD activity for strain *E. coli* BL21(DE3)/pTA02L increased to 489.24 ± 33.01 U/g DCW upon induction with 4.0 g/L *L*-arabinose and 12.5 μM IPTG, which was 1.29-fold higher than that of *E. coli* BL21(DE3)/pTA01L. Obviously, efficient co-expression of C-terminal truncated GadC and GadB variants has a distinct advantage for further favoring the *E. coli* cell-associated decarboxylation reaction.

3.3. Co-expression of C-terminal truncated GadC and GadB variants extends the cell-associated GAD activity toward near-neutral pH

Moreover, the effect of pH on the cell-associated GAD activity of engineered *E. coli* was investigated. Similar to free GadB, [1,30] the pH profiles demonstrated that the whole-cell catalytic activity was sensitive to pH, being observed at pH values below 5.5 and 6.0 for strain *E. coli* BL21(DE3)/pTA01L and strain *E. coli* BL21(DE3)/pTA02L, respectively (Fig. 4). Significantly, the latter exhibited higher cell-associated GAD activity between pH 4.0 and pH 6.0, although the optimum pH was pH 4.7 for both strains. As expected, the cell-associated GAD activity of *E. coli* was further enhanced by employing a more efficient *L*-Glu/GABA transport system ($GadC_{\Delta C41}$) within a neutral pH range.

3.4. Minimizing the toxic effects of antiporter production by employing the host E. coli Lemo21(DE3)

As described above, GadC is a key protein for *L*-Glu/GABA transportation in *E. coli* under acidic conditions [25,29]. However, antiporter production resulted in marked cell growth inhibition [21,24]. Recently, an *E. coli* BL21(DE3) derivative Lemo21(DE3), which could balance the synthesis speed of membrane proteins within the cell's capacities via modulating T7 RNAP activity has been constructed. In this strain, tunable expression is achieved by varying the level of T7 lysozyme, the natural inhibitor of T7 RNAP. The *t7lys* gene is expressed from the pLemo plasmid using the titratable *L*-rhamnose promoter (Fig. 1).

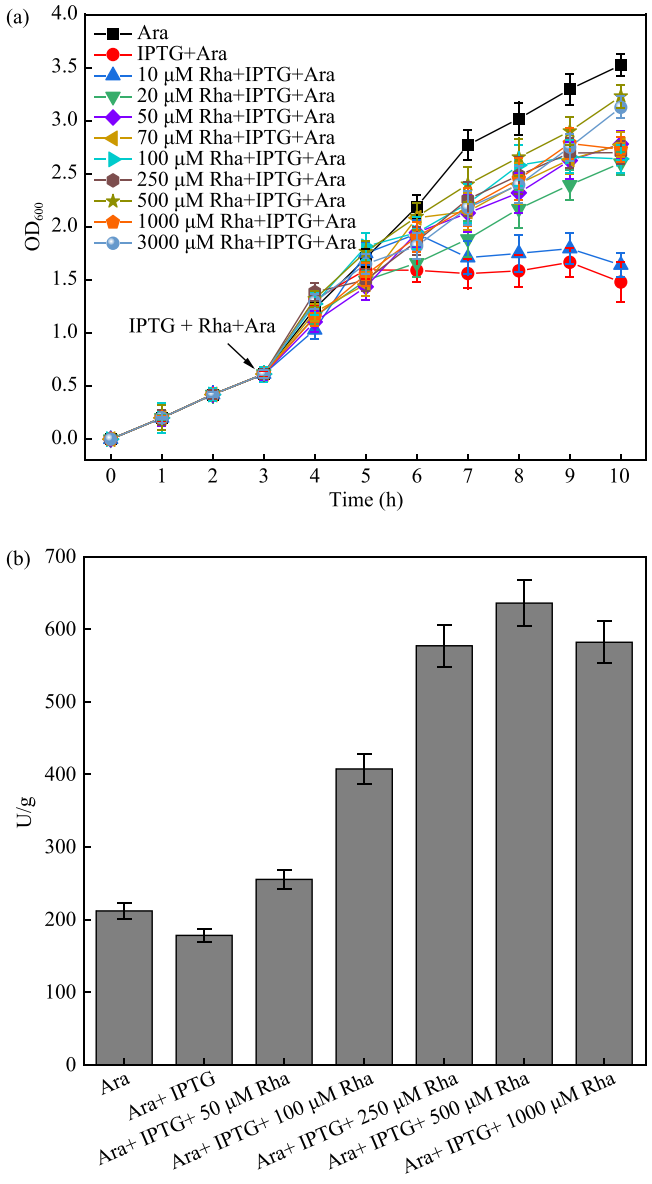

Fig. 5. Analysis of the cell growth and cell-associated GAD activity of strain *E. coli* Lemo21(DE3)/pTA02L.
(a) When the cells reached an OD_{600} of 0.6, 50 μM IPTG, 4.0 g/L *L*-arabinose and various concentrations of *L*-rhamnose were added to the cultures, which were then incubated at 30 °C and 180 rpm for 7 h. (b) The cell-associated GAD activity of *E. coli* Lemo21(DE3)/pTA02L induced with 4.0 g/L *L*-arabinose, 50 μM IPTG and various concentrations of *L*-rhamnose.

Several studies have shown that Lemo21(DE3) was an excellent host for the expression of difficult constructs, including toxic proteins, membrane proteins and proteins prone to insoluble expression [26]. To minimize the toxic effects of $GadC_{\Delta C41}$ overexpression, *E. coli* Lemo21(DE3) was also selected as the host strain. Similarly, the engineered strain *E. coli* Lemo21(DE3)/pTA02L was grown in LB broth at 37 °C and 220 rpm. SDS-PAGE analysis demonstrated that both $GadC_{\Delta C41}$ and $GadB_{\Delta C11}$ were strongly overexpressed in *E. coli* Lemo21(DE3) (Fig. S3). Significantly, after 10 h of culture, the strain *E. coli* Lemo21(DE3)/pTA02L with 4.0 g/L *L*-arabinose, 50 μM IPTG and 500 μM *L*-rhamnose added reached an OD_{600} value of 3.23 ± 0.11, while the OD_{600} value of the strain without *L*-rhamnose added was only 1.49 ± 0.19 (Fig. 5a). Moreover, the GAD activity of 635.83 ± 31.79 U/g DCW was achieved upon induction with 4.0 g/L *L*-arabinose, 50 μM IPTG and 500 μM *L*-rhamnose, whereas a GAD activity of only 178.31 ± 8.92 U/g DCW was observed in the absence of *L*-rhamnose (Fig. 5b). As expected, *E. coli* Lemo21(DE3) is an elegant alternative host for minimizing the toxic effects of membrane-bound $GadC_{\Delta C41}$ production.

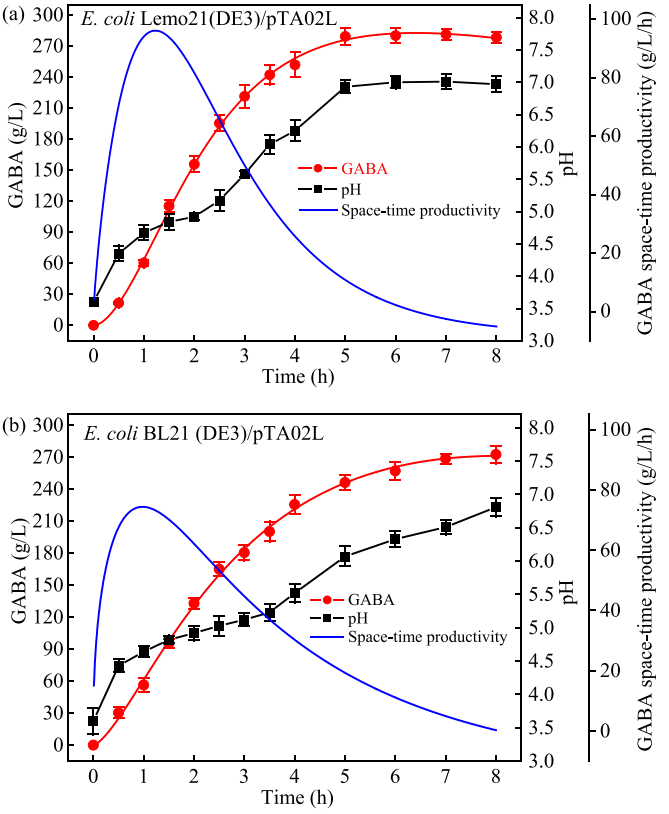

Fig. 6. Time courses of GABA synthesis using engineered *E. coli* Lemo21(DE3)/pTA02L (a) and *E. coli* BL21(DE3)/pTA02L (b).

3.5. *High-level synthesis of GABA by using dormant engineered* E. coli *cells*

To document the progress of the reduced toxic effects of $GadC_{\Delta C41}$ production on GAD activity, the GABA synthesis capacities of *E. coli* BL21(DE3)/pTA02L and *E. coli* Lemo21(DE3)/pTA02L were further compared. In the initial period, most of the substrate was insoluble owing to its limited water solubility [17]. As the reaction proceeded, the precursor gradually dissolved into the solution, accompanied by the dramatic bubbling of CO_2. As indicated in Fig. 6a, the volumetric rate of GABA formation of *E. coli* Lemo21(DE3)/pTA02L increased rapidly as the pH of the solution increased to 4.7 from its initial value of 3.6 within the first hour and began to decrease thereafter. Indeed, this is consistent with the decarboxylation reaction mediated by the GAD system, which consumes intracellular protons via GadB and exchanges the extracellular substrate (*L*-Glu) for the more alkaline intracellular product (GABA) by GadC [7,31]. After reaction for 5 h, the concentration of GABA reached 279.31 ± 8.12 g/L with a final pH value of up to 6.9 and a conversion ratio of 99.4%. However, engineered *E. coli* Lemo21(DE3)/pTA02L without induction was an inefficient whole-cell biocatalyst for GABA production, which resulted in the synthesis of 1.03 ± 0.05 g/L GABA within 5 h (Fig. S4). For strain *E. coli* BL21(DE3)/pTA02L, a similar variation tendency was observed for GABA production and consequent pH change (Fig. 6b). However, its GABA space-time productivity was much lower than that of *E. coli* Lemo21(DE3)/pTA02L within the first 3.5 h. After 5 h of transformation, the GABA level reached a level of 246.13 ± 6.90 g/L with an ultimate pH value of 6.1.

Table 1
The GABA production performance of engineered strains via whole-cell conversion strategy.

Microorganism	Engineering strategy	Substrate	Operation mode and strategy	OD_{600}	Concentration (g/L)	Conversion ratio	Conversion time (h)	Productivity (g/L/h)	References
B. *subtilis* BS-T710	*gadB* (*Streptococcus salivarius* ssp. *thermophilus*)	*L*-MSG	Whole-cell conversion	N/S	5.26	N/S	12	0.44	32
S. cerevisiae BJ5464	*gadB* (*Streptomyces* sp.)	*L*-Glu	Repeated whole-cell conversion process	30	62.6	60.8%	12	0.522	34
E. coli BL21 (DE3)	$gadB_{\Delta C11}{}^{K17T/D294G/E312S/Q346H}$ (L. *brevis*)	*L*-Glu	Whole-cell conversion	20	270.42	99%	7.5	36.06	33
E. coli BW25113	Δ*gadC*, Δ*gadA*, Δ*gadB* (*E. coli*), *gadB* (L. *lactis*), *groES-groEL*	*L*-Glu	Whole-cell conversion	15	308.26	99.6%	12	44.04	24
E. coli BW25113	Δ*gadAB* (*E. coli*), *gadB* (L. *lactis*)	*L*-Glu	Repeated whole-cell conversion process	15	614.15	99%	12	40.94	14
E. coli Lemo21(DE3)	$gadC_{\Delta C41}$ (*E. coli*), $gadB_{\Delta C11}$ (L. *plantarum*), *T7lysY*	*L*-Glu	Repeated whole-cell conversion process	20	829.08	98.7%	13	63.78	This work

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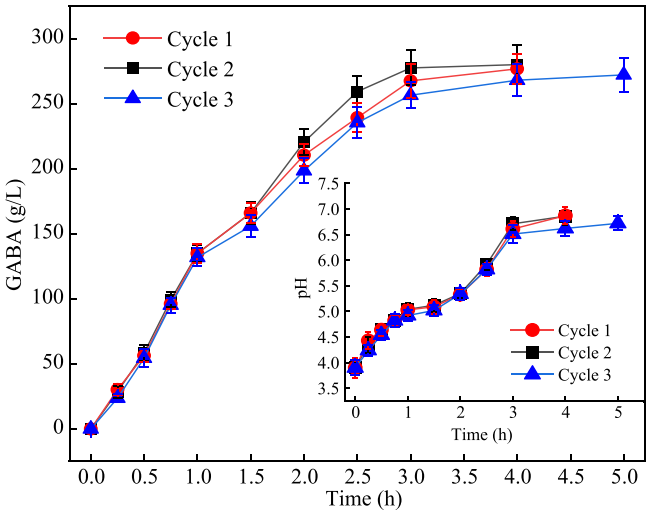

Fig. 7. Time profiles of GABA synthesis via repeated use of engineered *E. coli* Lemo21(DE3)/pTA02L cells from a 3 M *L*-Glu-solution.

3.6. Scaled-up production of GABA by engineered E. coli Lemo21(DE3)/pTA02L

During the fed-batch cultivation, the time-course of biomass (OD_{600}), dissolved oxygen (DO), pH and glucose concentration for engineered *E. coli* Lemo21(DE3)/pTA02L in a 5 L fermenter has been determined, as shown in Fig. S5. After 15 h cultivation, an optical density (OD_{600}) of 20.47 corresponding to 25.39 g/L of wet biomass was obtained. To scale up GABA production, the volume of the reaction system was increased to 1.5 L. The same batch of *E. coli* Lemo21(DE3)/pTA02L cells was reused three times in 3 M *L*-Glu-solution each time to synthesize GABA. Particularly, it is difficult to estimate the volume of the reaction solution accurately because *L*-Glu, which is insoluble at the beginning of the reaction, gradually dissolves and changes its volume as the reaction progresses. However, after reaction for 2.5 h, *L*-Glu-was completely dissolved. The final volume of the reaction solution was increased to approximately 1.65 L. As shown in Fig. 7, the GABA titer reached 276.86 ± 11.47 g/L at 4 h in cycle 1, corresponding to a 98.9% conversion ratio. A similar variation tendency was observed in cycle 2; almost all of the substrate was consumed, with a GABA yield of 280.09 ± 15.04 g/L within 4 h (Fig. S6). However, in cycle 3, after reaction for 5 h, the corresponding GABA level and conversion ratio were reduced to 272.13 ± 12.89 g/L and 97.2%, respectively. Notably, the total GABA yield reached 829.08 g/L with an average specific productivity of 63.78 g/L/h, which was much higher than that previously reported [14,24,32-34] (Table 1). Strain *E. coli* Lemo21(DE3)/pTA02L is, thus, capable of large-scale GABA production under uncontrolled pH conditions. Remarkably, the trace amounts of precursor residue and utilization of deionized water as the sole medium would greatly contribute to the development of cost-effective and environmentally friendly processes for GABA production. In addition, although IPTG ($ 1.59/g), *L*-rhamnose ($ 0.20/g) and *L*-arabinose ($ 0.085/g) were used to induce the tunable control system, the corresponding cost of GABA (market price $ 125/kg) increased only $ 0.45/kg. Comparatively, the method had a significant improvement in the productivity and yield with fewer operating steps. Therefore, it had great potential for the industrial-scale GABA production from *L*-Glu. In the future, the GABA biosynthesis system using dormant *E. coli* Lemo21(DE3)/pTA02L cells as the biocatalyst needs to be further optimized and amplified.

4. Conclusion

In this work, a double-promoter P_{T7lac} and P_{BAD} expression system was constructed with pET28a as the backbone. Then, $gadC_{\Delta C41}$ of *E. coli* and $gadB_{\Delta C11}$ of L. *plantarum* were inserted into the designed vector under the control of P_{T7lac} and P_{BAD}, respectively. To minimize the toxic effects of $GadC_{\Delta C41}$ production, *E. coli* Lemo21(DE3) was selected as the host. Furthermore, a GABA biosynthesis system using pure water as the sole reaction medium and dormant *E. coli* Lemo21(DE3)/pTA02L cells as the catalyst was designed. The total GABA yield reached 829.08 g/L with a 98.7% conversion ratio within 13 h when engineered *E. coli* Lemo21(DE3)/pTA02L was concentrated to an OD_{600} of 20 and reused for three cycles in the 3 M *L*-Glu-solution at 37 ˚C, which represented the highest GABA productivity ever reported.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.engmic.2023.100077.

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