

## Production of $\gamma$ -aminobutyric acid from monosodium glutamate using *Escherichia coli* whole-cell biocatalysis with glutamate decarboxylase from *Lactobacillus brevis* KCTC 3498

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**Abstract**— $\gamma$ -Aminobutyric acid (GABA), an important fine chemical in pharmacotherapy and food industries, is used as a novel material in the nylon industry and has attracted attention for its potential application in large scale production. Search for new genes and strains, development of efficient reaction systems, such as fermentation and bioconversion, and use of cheap starting material like monosodium glutamate (MSG) can make GABA production using less expensive bulk chemicals possible. Therefore, in this study, we constructed a recombinant *Escherichia coli* whole-cell system for GABA production that expressed glutamate decarboxylase (GAD) from *Lactobacillus brevis* and used MSG as the starting material. We also optimized the reaction conditions for MSG to GABA conversion, such as citrate buffer concentration, pyridoxal 5'-phosphate concentration, temperature, MSG concentration, and cell density (OD<sub>600</sub>). The optimized whole-cell system converted MSG to GABA via seven repetitive cycles resulting in an average conversion rate of 86% (71.7 mM/h) within 42 h.

**Keywords:**  $\gamma$ -Aminobutyric Acid, Monosodium Glutamate, Glutamate Decarboxylase, Whole-cell Bioconversion, *Lactobacillus brevis*

### INTRODUCTION

$\gamma$ -Aminobutyric Acid (GABA) is a non-protein amino acid that acts as a major inhibitory neurotransmitter in higher animals [1]. It is used to treat sleep, panic, and anxiety disorders and is also used as a food supplement. Recent studies have demonstrated the application of GABA as a platform chemical in the production of 2-pyrrolidone and nylon 4; its use is expanding [2–5].

GABA is produced in vivo by the GABA shunt pathway [6]. The first step is the production of glutamate from  $\alpha$ -ketoglutarate via a transamination reaction catalyzed by glutamate dehydrogenase. The next step is the decarboxylation of glutamate to GABA, which is catalyzed by glutamate decarboxylase (GAD, EC:4.1.1.15). In GABA synthesis, GAD is the rate-limiting enzyme that requires pyridoxal phosphate (PLP) as a cofactor [7]. GABA is mostly synthesized from L-glutamic acid (L-Glu) or monosodium glutamate (MSG) by GAD, which is the sole enzyme that can catalyze the reaction [8,9]. *Escherichia coli* GAD is a well-known hexameric enzyme with significant structural differences from the mammalian GAD, and it exists in two isoforms, GADA and GADB [1,7,10].

Recently, efficient production of GABA from L-Glu using lactic acid bacteria has been reported in several studies (Table 1) [11–13]. For instance, 1,005.8 mM of GABA was produced by *L. brevis*

NCL912 in 48 h [14], 526.3 mM of GABA was produced by *L. brevis* CGMCC 1306 in 72 h [15], and 251 mM of GABA was produced by *L. buchneri* [16]. Previous studies also used recombinant *E. coli* strains as whole-cell biocatalysts to produce 1,338.2 mM GABA from 1,359.3 mM L-Glu (99% conversion) within 35 h (*E. coli* GADK10) [17], 2,996 mM of GABA from 3,000 mM L-Glu (99.9% conversion) within 12 h (*E. coli* BW25113) [18], and 738.9 mM (62.4% conversion) within 2 h from MSG (recombinant *E. coli* XL1-Blue) [5]. Although wild type *Lactobacillus* shows high yield, it requires a long reaction time. Additionally, recombinant microorganisms that use GAD for GABA production from MSG have also shown a low yield. However, not many studies have used high concentrations of MSG as the starting material despite its low conversion yield to GABA in comparison to that of L-Glu [19–23]. This could be due to the increase in pH with high concentration of MSG, causing the pH to become higher than what is optimal for GAD. However, MSG is an attractive starting material for a robust GABA production system because it is cheaper than L-Glu and is available in large quantities (2.75 million tons per year) [24–26]. In addition, MSG is easy to melt and is more soluble than L-Glu (8.57 g/L) in water (739 g/L) at 25 °C [27,28].

Whole-cell biocatalysts can produce value-added chemicals from cheap feedstock by avoiding complex cellular metabolism, and they show better stability than enzymes or purified enzymes. Therefore, manufacturers focus on optimizing precursor supply, cofactor balance, and reaction conditions to enhance the efficiency of biocatalysts [29–33].

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**Table 1. GABA production reported in previous studies**

Substrate	Strains	Origin of GAD enzyme	Strategy	Time (h)	Titer (mM)	Productivity (mM/h)	Ref.
L-Glu	<i>E. coli</i> BW25113	<i>L. lactis</i> GadB	Bioconversion	12	2,996	249.7	[18]
L-Glu	<i>E. coli</i> GADK10	<i>E. coli</i> gadA	Bioconversion	35	1,338.2	38.2	[17]
L-Glu	<i>L. brevis</i> NCL912	<i>L. brevis</i> NCL912	Fed-batch	48	1,005.8	21.0	[14]
MSG	<i>E. coli</i> XL1-Blue	<i>L. lactis</i> subsp. <i>lactis</i> Il1403 (GadB)	Bioconversion	2	738.9	369.5	[5]
MSG	<i>E. coli</i> UT481	<i>L. brevis</i> OPK-3	Bioconversion	24	19.62	0.8	[20]
MSG	<i>L. brevis</i> CGMCC 1306	<i>L. brevis</i> CGMCC 1306	Fed-batch	72	526.3	7.3	[15]
MSG	<i>E. coli</i> BL21	<i>L. brevis</i> (KCTC 3498)	Repetitive Bioconversion	6*	430*	71.7*	This study

\*These values were obtained from the average of seven repetitive reactions.

Hence, we developed a whole-cell system with a gene from an active source like *Lactobacillus* and used a cheap starting material, such as MSG, although most previous recombinant systems have preferred L-Glu. We constructed a whole-cell GABA production system using GAD from *L. brevis* in a recombinant strain and optimized the reaction conditions. We achieved more than 80% conversion of 500 mM MSG to GABA in seven repetitive reactions of the whole-cell system, resulting in an average conversion rate of 86% with 71.7 mM/h within 42 h from seven repetitive reactions. Our approaches appear to work, considering our goals to decrease reaction time and use of MSG for GABA production.

## MATERIALS AND METHODS

### 1. Chemicals

Restriction enzymes and DNA polymerase were purchased from Enzymomics (Daejeon, Korea). MSG and GABA were purchased

from Tokyo Chemical Industry Co. (Tokyo, Japan). PLP was purchased from Sigma-Aldrich, Inc. Sodium citrate dihydrate was purchased from Daejung Chemical & Metals Co., Ltd. Citric acid was purchased from Duksan pure chemical Co., Ltd. Dimethyl ethoxymethylenemalonate was purchased from Fluka (Japan) for the derivatization reaction. Sodium acetate trihydrate (98.5%) was purchased from SAMCHUN. Acetic acid (glacial) was purchased from Merck. Acetonitrile was purchased from Fisher Brand.

### 2. Bacterial Strains, Plasmids, and Media

*L. brevis* KCTC 3498 was purchased from KCTC (Daejeon, South Korea). *E. coli* DH5 $\alpha$  was used as the cloning host, and *E. coli* BL21(DE3) was used as the expression host. pCDFDuet plasmid was used for cloning purposes (Table 2). *E. coli* strains were grown in the Luria Bertani (LB; MILLER) medium with or without antibiotics. *L. brevis* strain was grown in the *Lactobacilli* MRS medium.

### 3. Construction of Plasmid and Culture Conditions

The *gad* gene from *L. brevis* was inserted into the pCDFduet

**Table 2. Details of the strains, plasmid, and primers used in the study**

Strains, plasmid, and primers	Details	Source
Strains		
<i>Lactobacillus brevis</i> KCTC 3498	Wild type	KCTC
<i>E. coli</i> K12 (MG1655)	Wild type	Lab stock
<i>E. coli</i> DH5 $\alpha$	F <sup>−</sup> $\phi$ 80lacZ M15 <i>endA</i> <i>recA</i> <i>hsdR</i> ( <i>rk-mk</i> -) <i>supE</i> <i>thi</i> <i>gyrA</i> <i>relA</i> $\Delta$ ( <i>lacZYA-argF</i> )U169	Lab stock
<i>E. coli</i> BL21 (DE3)	F <sup>−</sup> <i>ompThsdSB</i> ( <i>rB-mB</i> -) <i>gal</i> <i>dcm</i>	Novagen
GADA	<i>E. coli</i> K12 glutamate decarboxylase Alpha ( <i>gadA</i> )	In this study
GADB	<i>E. coli</i> K12 glutamate decarboxylase Beta ( <i>gadB</i> )	In this study
LBGAD	<i>L. brevis</i> glutamate decarboxylase ( <i>gad</i> )	In this study
Plasmids		
pCDFDuet- <i>gadA</i>	<i>gadA</i> of <i>E. coli</i> K12 inserted into pCDF duet 1	In this study
pCDFDuet- <i>gadB</i>	<i>gadB</i> of <i>E. coli</i> K12 inserted into pCDF duet 1	In this study
pCDFDuet- <i>LBgad</i>	<i>gad</i> of <i>L. brevis</i> inserted into pCDF duet 1	In this study
Primers		
F: <i>gadA</i> -BamHI	5'-CTGCTGGGATCCGATGGACCAGAAGCTGTAA-3'	In this study
R: <i>gadA</i> -SacI	5'-CTGCTGGAGCTCGTCAGGTGTGTTTAAAGCTG-3'	In this study
F: <i>gadB</i> -BamHI	5'-CTGCTGGGATCCGATGGATAAGAAGCAAGTAACG-3'	In this study
R: <i>gadB</i> -SacI	5'-CTGCTGGAGCTCGTCAGGTATGTTTAAAGCTGTTCTG-3'	In this study
F: <i>LBgad</i> -BamHI	5'-CTGCTGGGATCCGATGAATAAAAACGATCAGGAAACA-3'	In this study
R: <i>LBgad</i> -SacI	5'-CTGCTGGAGCTCTTAACCTCGAACGGTGGTCTTG-3'	In this study

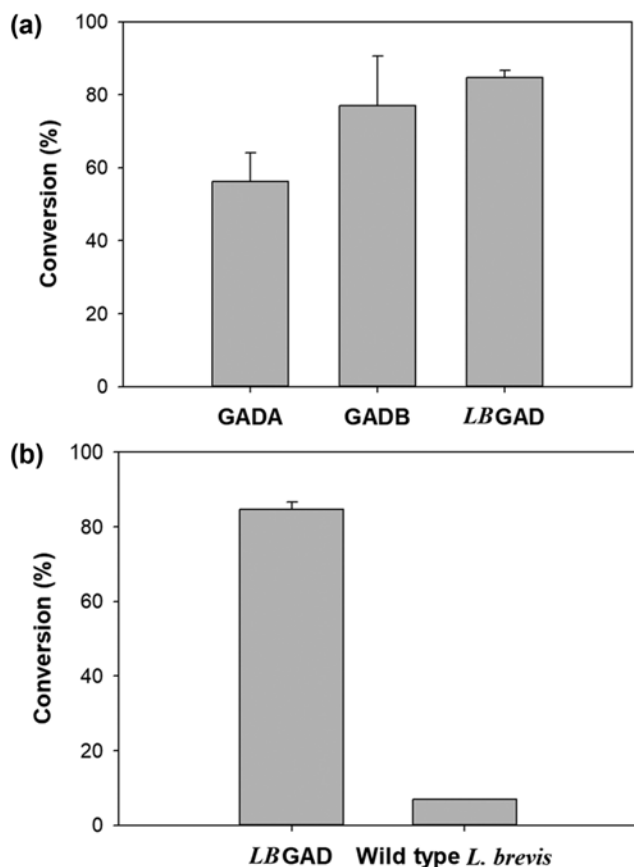


Fig. 1. Comparison of the MSG to GABA conversion rates using different recombinant systems (*E. coli* BL21 (pCDFDuet1::*gadA*), *E. coli* BL21 (pCDFDuet1::*gadB*), and *E. coli* BL21 (pCDFDuet1::*LBgad*)) (a), using *E. coli* BL21 (pCDFDuet1::*LBgad*) and wild type *L. brevis* (b) at 30 °C with 50 mM MSG, 50 mM citrate buffer, and 0.1 mM PLP within 15 h.

vector at MCS1 (Supplementary Table 1 and Supplementary Fig. 1). Primer details are provided in Table 2. The PCR product and vector were cut with restriction enzymes BamH1 and SacI and ligated using T4-ligase. The sequences of the insert and vector were confirmed by sequencing from Bionics (Seoul, South Korea). The constructed vectors were transformed in *E. coli* BL21(DE3). A single colony of each recombinant *E. coli* strain was picked from the agar plate and precultured in 5 mL LB broth containing 10 g/L tryptone, 5 g/L yeast extract, 10 g/L sodium chloride, and antibiotics on a shaking incubator (Han-Beak Science Co., Bucheon, Gyeonggi-do, Korea) at 200 rpm, overnight. The pre-culture was then inoculated in 50 mL LB in a 250 mL baffled Erlenmeyer flask with antibiotics and incubated at 37 °C with shaking. Induction was carried out with 0.5 mM isopropyl  $\beta$ -D-thiogalactopyranoside after the optical density of the culture at OD<sub>600</sub> reached 0.6-0.7. After overnight incubation at 25 °C, the culture was harvested by centrifugation at 4,000 rpm for 10 min at 4 °C, and the pellet was washed twice using deionized water.

#### 4. Whole-cell Reaction

Cell concentration was monitored by measuring the OD<sub>600</sub> by ultraviolet-visible spectrophotometry (SpectraMax M2). The prepared GAD cells were used for the whole-cell reaction. The reac-

tion was performed with 500 mM MSG, 50 mM citrate buffer, and 0.1 mM PLP. Each condition was varied to identify the optimal reaction condition. The static reaction using the prepared reaction mixture was carried out at 30 °C for 6 h. The reaction was stopped by heating at 95 °C for 5 min. The reaction solution was then diluted to an appropriate concentration for high-performance liquid chromatography (HPLC) analysis (Prominence-i LC-2030, Shimadzu, Kyoto, Japan).

#### 5. Derivatization and HPLC Analysis

For measuring the concentration of MSG and GABA, amines present in MSG and GABA were measured. The amine derivatives were prepared in a mixture of 300  $\mu$ L borate buffer (50 mM, pH 9), 100  $\mu$ L methanol, 47  $\mu$ L distilled water, 50  $\mu$ L target sample, and 3  $\mu$ L DEEMM [34]. The derivatization reaction was performed to derivatize MSG and GABA at 70 °C for 2 h, followed by HPLC (Prominence-i LC-2030, Shimadzu, Kyoto, Japan) analysis of the reaction products at a UV-absorbance of 284 nm. Chromatographic separation was conducted using a reverse-phase C18 column (ZORBAX SB-C18 column, 4.6 $\times$ 250 mm, 5  $\mu$ m particle size; Agilent Technologies, Santa Clara, CA, USA), and the column temperature was maintained at 35 °C. The mobile phase consisted of 100% acetonitrile (solvent A) and 25 mM sodium acetate buffer pH 4.8 (solvent B). The flow rate was maintained at 1 mL/min, and the composition of solvent A to B (A : B, v/v) changed as per the following gradient program: 0 min (20 : 80), 2 min (25 : 75), 32 min (60 : 40), 37 min (20 : 80), 40 min (20 : 80).

#### 6. Repeated Batch Process

The cells were prepared in advance, and the reaction was set as per the optimized conditions. After 6 h, the samples were centrifuged at 13,000 rpm (Hanil Co, Smart 13) for 1 min. The reaction mixture was collected from the supernatant and the cells were used for an additional round of reaction. Fresh reaction mixture was added to the cells and this procedure was repeated seven times with the samples in duplicate. MSG to GABA conversion was calculated from each round [35].

## RESULTS

### 1. GABA Production by Recombinant *E. coli* Strains and Wild Type *L. brevis*

*E. coli* GAD, a hexameric enzyme with significant structural differences from mammalian GAD, is of two types, GADA and GADB [1,7,10]. Likewise, the GAD system from a strain of *L. brevis* was also reported to have two isoforms of GAD, namely GADA and GADB, which catalyzed the conversion of L-Glu to GABA [36]. However, as per the Kyoto Encyclopedia of Genes and Genomes, GADA and GADB have not been classified in *L. brevis* KCTC 3498 and it has only one *gad* annotated gene. Therefore, we constructed GABA systems using the *L. brevis gad* gene (*LBgad*) as well as *gadA* and *gadB* from *E. coli* as controls.

GABA production was compared between *E. coli* strains overexpressing *gadA* (GADA), *gadB* (GADB), and *LBgad* (LBGAD) cloned in pCDFDuet. Whole-cell conversion of MSG to GABA showed that the highest GABA conversion rate was 84.7%, which was achieved within 15 h with LBGAD. The GABA conversion rate with GADB and GADA was 77.0% and 56.3%, respectively, under

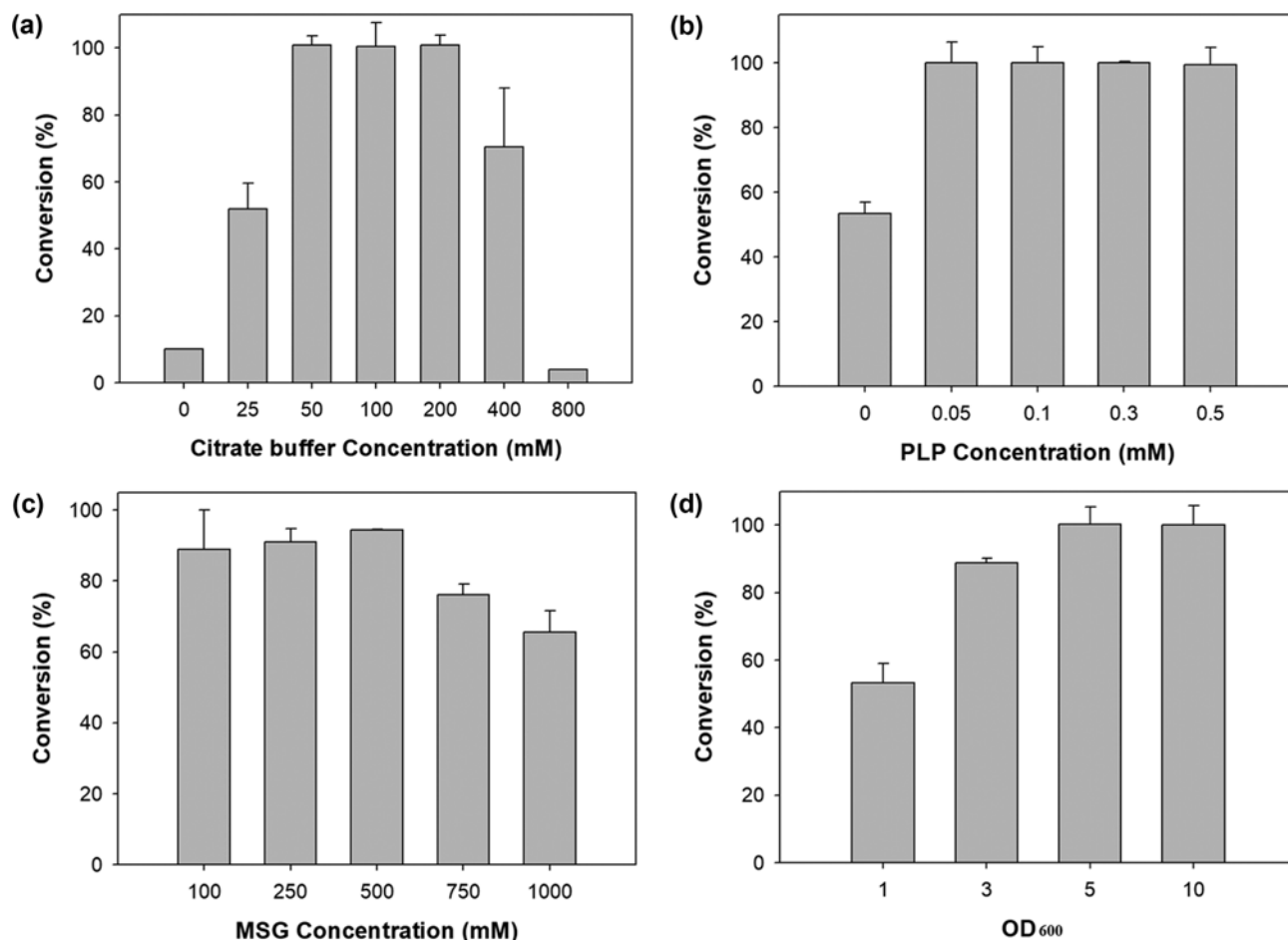


Fig. 2. Optimization of conditions for GABA whole-cell reaction, such as concentration of citrate buffer (a), PLP (b), MSG (c), and cell density (OD<sub>600</sub>) (d). The reactions were performed with the change in citrate, PLP, and MSG concentrations starting from 50 mM of citrate, 500 mM MSG, 0.1 mM PLP, and OD<sub>600</sub> of 5 within 6 h at 30 °C.

the same conditions (Fig. 1(a)). We observed that the GABA conversion rate of wild type *L. brevis* was only 7.0% (Fig. 1(b)). Based on these results, we constructed an pCDFDuet::LBgad system in BL21(DE3) and used it for further experiments.

## 2. Optimization of Reaction Conditions

To identify optimum reaction conditions, we evaluated GABA conversion from L-Glu at different concentrations (0 mM to 800 mM) of citrate buffer. We observed that the GABA conversion with 0 mM citrate buffer was 10% from 500 mM MSG. Greater than 90% conversion of MSG to GABA was achieved on increasing the concentration of citrate buffer from 50 mM to 200 mM (Fig. 2(a)). Further, with more than 400 mM citrate buffer, GABA conversion dropped sharply and almost no conversion was observed at a higher concentration of citrate buffer. Therefore, we selected 50 mM citrate buffer for further experiments. As PLP is a coenzyme for decarboxylase, we examined the effect of different concentrations of PLP on the GABA conversion. At 0 mM PLP, a GABA conversion of over 50% was observed, and at higher concentrations of PLP (0.05 mM to 0.5 mM), more than 90% of GABA conversion was observed (Fig. 2(b)).

To detect the substrate inhibition effect, the effect of the MSG concentration on GABA production was investigated. As the main

substrate, increasing concentrations of MSG (0.1, 0.25, 0.5, 0.75, and 1 M) revealed that the highest GABA conversion was achieved with 500 mM MSG, and over 90% yield of GABA was achieved from 250 mM to 500 mM of MSG in the same conditions (Fig. 2(c)). On increasing the MSG concentration to more than 500 mM, the GABA conversion decreased. The effect of cell concentration on GABA was investigated for maximum conversion using minimal cell concentration. The cells were examined at an OD<sub>600</sub> of 1, 3, 5, and 10. At an OD<sub>600</sub> of 1, the GABA conversion was about 53%. As the cell concentration increased, the GABA conversion increased sharply. At an OD<sub>600</sub> of over 5, the GABA conversion was more than 90% and we chose an OD<sub>600</sub> of 5 for maximum GABA production in this study (Fig. 2(d)).

For the whole-cell reaction with MSG, conversion was examined at different temperatures: 25 °C, 30 °C, and 37 °C (Fig. 3(a)). Whole-cell conversion of MSG to GABA experiments showed that the highest GABA conversion occurred at 30 °C for 6 h at an OD<sub>600</sub> of 5 (cell density) in 500 mM MSG, 50 mM citrate buffer, and 0.1 mM PLP. Besides, a GABA conversion yield of over 80% was achieved at 25 °C and 37 °C. When the effect of pH between 3 and 5 was examined, a conversion more than 90% was obtained up to pH 4.75 (Fig. 3(b)). The increase in pH after the reaction was

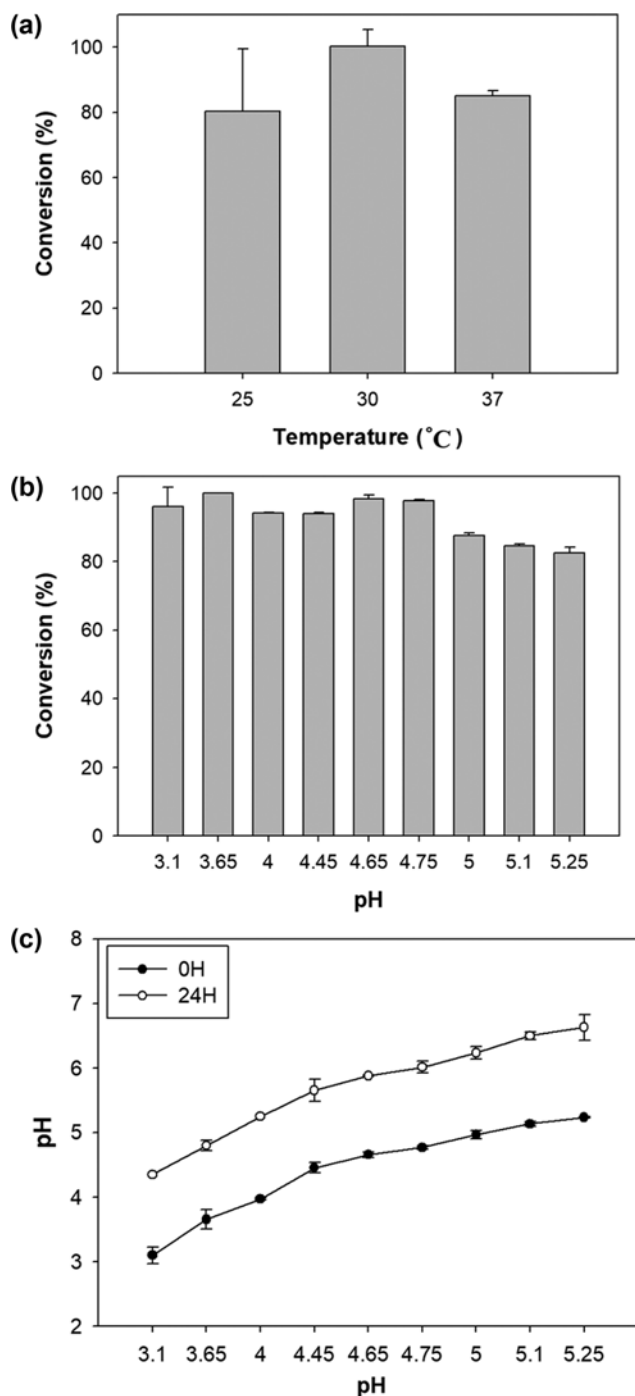


Fig. 3. Effect of temperature and pH on the whole-cell reaction of MSG conversion to GABA. Comparison of conversion at different temperatures (a), different pH (b), and pH change before (0 h) and after the reaction at 24 h (c). The reactions were performed with 500 mM MSG, 0.1 mM PLP, OD<sub>600</sub> of 5 (cell density), and 50 mM citrate buffer within 6 h except for (b) and (c).

monitored and GABA production increased the pH of the buffer by a value of 2 (Fig. 3(c)). Overall, the optimization of the reaction showed that an OD<sub>600</sub> of 5 with 500 mM MSG, 50 mM citrate buffer, and 0.1 mM PLP at 30°C resulted in the highest MSG to

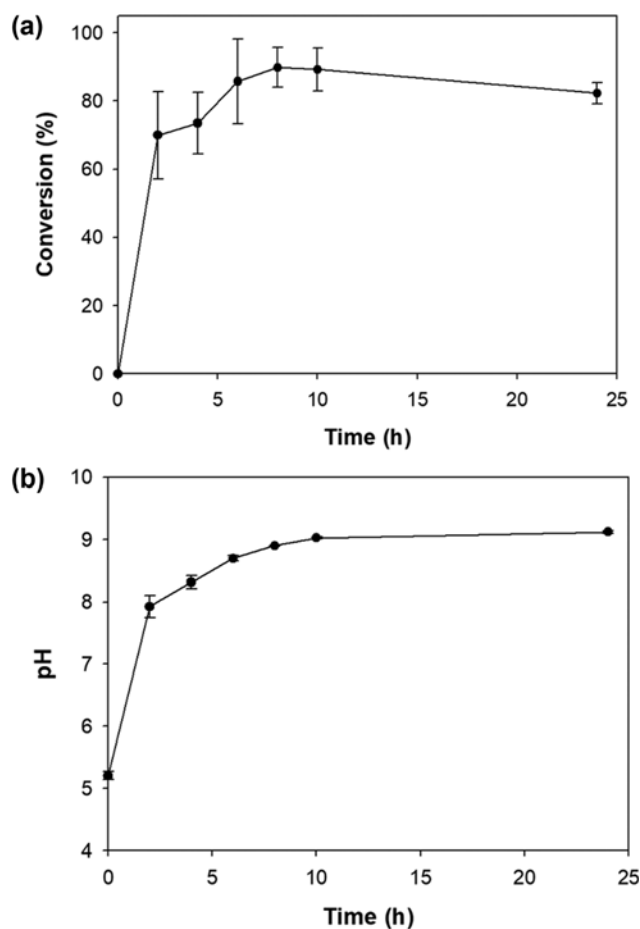


Fig. 4. Time-dependent monitoring of GABA production from MSG for 24 h (a) and pH profile of the medium pH (b). Samples were collected at 0, 2, 4, 6, 8, 10, 24 h and reactions were performed at 30°C with 500 mM MSG, 0.1 mM PLP, cells (OD<sub>600</sub> of 5), and 50 mM citrate buffer.

GABA conversion. When 500 mM MSG was used, the final pH of the medium increased over 7.5 after 6 h (data not shown).

### 3. Time Profile and a Repeated Batch Process

To monitor GABA production in a time-dependent manner, GABA conversion was examined for 24 h. GABA conversion from 500 mM MSG was investigated using an OD<sub>600</sub> of 5 at 30°C with 50 mM citrate buffer, 0.1 mM PLP, and a medium pH 4.87. The reaction showed the maximum yield at 6 h and then decreased to 82.3% at 24 h (Fig. 4(a)). Although the maximum yield was little less due to scale up, the GABA reaction could be completed within 6 h, which was less than the time required by wild type *L. brevis* to complete the reaction. The pH of the buffer showed a drastic increase to 9.1 (Fig. 4(b)).

The reusability of the whole-cell system for GABA production was examined by repeated use of whole cells for conversion of MSG to GABA. The results were investigated seven times every 6 h (Fig. 5). During the first four cycles under optimized reactions, the GABA conversion rate was over 89%. On assessing the seven repeated runs, the average GABA yield partly declined and was over 86%. These results show that the recombinant strain was capable of efficiently producing GABA at a productivity of 71.7 mM/h (Fig. 5).

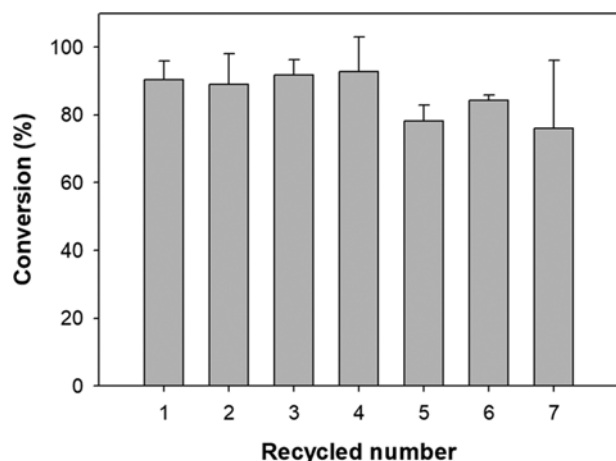


Fig. 5. GABA conversion from MSG in seven sequential repetitive batch reactions using the whole-cell system. Each reaction was repeated with 500 mM MSG, 0.05 mM PLP,  $OD_{600}$  of 5 (cell density), and 50 mM citrate buffer at 30 °C for 6 h.

## CONCLUSIONS

Naturally produced GABA is one of the well-known products in the pharmacotherapy and food industries [37–39]. Its application in the nylon industry is feasible; therefore, the mass production of GABA is important [5,40]. To utilize these advantages and achieve efficient production of GABA, we used the novel GAD gene from *L. brevis* and constructed a whole-cell system with *E. coli* BL21(DE3) for production of GABA from MSG. By optimizing important factors, such as the buffer concentration, PLP concentration, temperature, MSG concentration, and cell concentration, we could achieve more than 90% conversion. We ran repeated reactions resulting in an average of 86% conversion and a productivity of 71.7 mM/h. Although the process still needs to be scaled-up and the recovery process needs to be optimized [41], our approach demonstrated easy and successful GABA production by overcoming the low conversion of MSG by utilizing its high solubility. More process improvements, such as immobilization and a continuous process, will further increase the GABA yield.

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## SUPPORTING INFORMATION

Additional information as noted in the text. This information is available via the Internet at <http://www.springer.com/chemistry/journal/11814>.

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## Supporting Information

### Production of $\gamma$ -aminobutyric acid from monosodium glutamate using *Escherichia coli* whole-cell biocatalysis with glutamate decarboxylase from *Lactobacillus brevis* KCTC 3498

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Table S1. Nucleotide sequences and amino acids of *gad* from *Lactobacillus brevis* ATCC 14869

Nucleotide sequences
ATGAATAAAACGATCAGGAAACACAGCAGATGATTAATAATGTGGATTAGAAAAACGTTTTAGGCAGTGTCTGAAG CCGGGCAATCCTTACCCACCAATACATTACCAGATGATCCCATGGCACCGGATGTTGCCGCTCAATTGGTGGAACT ATCGTTTAAATGAAGCCAAGGCTAATCAAAACCTAGCGACCTTCTGTACCACGCAAATGGAACCACAAGCCGATGAAT TAATGAAGAACGCGTTGAATACCAATGCGATTGATAAATCGGAATACCCTAAGACCGCGGCAATGGAAAATTACTGTGT CAGCATGATTGCTCACCTATGGGGAATTCCTGACAATGAAAAGATTACGATGATTTCATTGGGACCTCAACGGTAGG TTCTTCTGAAGGATGTATGTTAGGCGGCTTGGCGCTACTACATAGTTGGAAGCACCGGGCCAAGGCAGCTGGTTTTG ATATTGAAGACCTGCATAGCCACAAGCCCAACTTGGTCATCATGTCAGGTTACCAAGTTGTTTGGGAAAAGTTCTGTA CCTACTGGAATGTCGAGATGCGCCAAGTGCCAATTAATGGTGACCAAGTTTCCTTAGATATGGATCATGTGATGGATTA CGTTGATGAAAATACGATTGGGATTATCGGAATTGAGGGCATTACGTACACGGGCTCCGTTGATGATATCAAACGCTA GATAACCTCGTGAGCGAATATAACAAGACCGCGACGATGCCGGTACGGATTACGTTGATGCTGCCTTTGGTGGCCT GTTGCGCGCGTTGTCGATGGCTTTAACCCGTGGGACTTCCGTTGAAGAACGTGGTTTCCATTAACTTTGGGGCC ATAAGTACGGGATGGTTTACCCTGGGTTGGGGTGGATTGTTTGGCGGCACAACACGGCTGATATTTACCCGCAGAA ATGCGTTTTCAAGTGCCATATCTAGGTAAGACCGTTGATTCAATCGCCATTAACCTTCTCACACAGTGGTGCCCATATCA GTGCGCAATACTACAATTTCAATCGATTTGGATTATCAGGTTATAAGACGATCATGCAAAATGTTGGAAGGTGTCATTG AAGCTGACGGCAGCTCTGAAAACGTATGGGATTTTCGATATTTAGTTGATGGGTACAGCTACCAATTAAGTTGGA AACTAGCGGACGATGCGCCGTTGGTTGGACGTTGTATGATTGGAGTCCGAGCTGGCTAAGTATGGTTGGCAAGTT CCGGCATATCCACTGCCAAAGAATCGCGACGATGTGACAATTAGCCGGATCGTGGTACGCCCATCCATGACCATGAC GATTGCCGATGATTTCTTGGATGATTTGAAATTAGCGATTGATGGATTAAATCACACATTTGGCGTGACGACCACCGTT GATCAAGATAACAAGACCACCGTTGGAAGTTAA
Amino acid sequences
MNKNDQETQQMINNVLDLEKTFGLSVEAGQSLPTNTLPDDPMAPDVAAQLVEHYRLNEAKANQNLFCTTQMEPQADEL MKNALNTNAIDKSEYPKTAAMENYCVSMIAHLWGIPDNEKIYDDFIGTSTVGSSEGCMLGGLALLHWSKHKRAKAAGFDIED LHSHKPNLVIMSGYQVWWEKFCTYWNVEMRQVPINGDQVSLDMDHVMDYVDENTIGIIEGITYTGSVDDIQTLDNLVTE YNKTATMPVRIHVDAAFGGLFAPFVDGFNPWFRLKNVVSINVSCHKYGMVYPGLGWIVWRHNTADILPAEMRFQVPYL GKTVDSIAINFSGAHISAQYYNFIREFLSGYKTIMQNVKVSLLKTAALKTYGIFDILVDGSQLPINCWKLADDAPVGWTL YDLESELAKYGWQVPAYPLPKNRDDVTISRIVVRPSMTMTIADDFLDDLKLAIDGLNHTFGVTTTVDQDNKTTVRS



