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Characterization of the Recombinant Glutamate Decarboxylase of *Lactobacillus brevis* G144 Isolated from Galchi Jeotgal, a Korean Salted and Fermented Seafood

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A γ-aminobutyric acid (GABA)-producing microorganism was isolated from galchi (hairtail fish, *Trichiurus lepturus*) jeotgal, a Korean salted and fermented seafood. The G144 isolate produced GABA excessively when incubated in MRS broth containing monosodium glutamate (MSG, 3%, w/v). G144 was identified as *Lactobacillus brevis* through 16S rRNA and *recA* gene sequencing. *gadB* and *gadC* encoding glutamate decarboxylase (GAD) and glutamate/GABA antiporter, respectively, were cloned and *gadB* was located downstream of *gadC*. The operon structure of *gadCB* was confirmed by reverse transcription (RT)-polymerase chain reaction. *gadB* was overexpressed in *Escherichia coli* and recombinant GAD was purified and its size was 54.4 kDa as evidenced by SDS-PAGE results. Maximum GAD activity was observed at pH 5.0 and 40℃ and the activity was dependent on pyridoxal 5'-phophate. The K_m and V_{max} of GAD were 8.6 mM and 0.01 mM/min, respectively.

Keywords: **GABA, *Lactobacillus brevis*, glutamate decarboxylase, galchi jeotgal**

Introduction

γ-Aminobutyric acid (GABA) is a non-protein amino acid that is widely distributed among microorganisms, animals, and plants [1, 2]. It is a major neurotransmitter inhibitor in animals and has various physiological functions including hypotensive, anti-anxiety, tranquilizing, analgesic, and diuretic effects [1]. GABA was also shown to improve visual function of old animals [3]. GABA is produced from L-glutamate by the action of glutamate decarboxylase (GAD, E.C. 4.1.1.15), a pyridoxal 5'-phophate (PLP)-dependent enzyme encoded by *gadB* gene [2]. Although many different microorganisms produce GABA, lactic acid bacteria (LAB) are the most well-known, and currently used for the production of various GABA-containing fermented foods [4–7]. LAB produce organic acids such as lactic acid and acetic acid as major metabolites during growth on various sugars, lowering pH of surrounding environments. Conversion of glutamate into GABA is one of the responses which prevent rapid decrease in cellular pH [8]. Previously, we isolated LAB producing GABA from Korean fermented foods including *Lactobacillus zymae* GU240 from kimchi [9], *Lb. sakei* A156 and *Enterococcus avium* M5 from myeolchi (anchovy) jeotgals [10, 11]. In this study, a new GABA producing *Lactobacillus brevis* G144 was isolated from galchi (hairtail fish) jeotgal, a Korean traditional salted and fermented sea food. *Lb. brevis* G144 possesses high GABA producing capacity, even higher than *Lb.*

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zymae GU240 and *Lb. sakei* A156 which have been used as positive controls for screening isolates. Thus *Lb. brevis* G144 seems promising as a starter for the production of fermented foods with high GABA contents. The *gadB* gene of *Lb.brevis* G144 was cloned and overexpressed in *Escherichia coli* BL21(DE3). Recombinant GAD was purified and the properties of GAD were examined.

Materials and Methods

Isolation of GABA Producing LAB from Galchi Jeotgal

Galchi (hairtail fish, *Trichiurus lepturus*) jeotgal was purchased at a local market in Jinju (Gyeongnam, South Korea) in the spring of 2019. Jeotgal was homogenized with 0.1% peptone water by using a stomacher®80 (Seward, USA), and serially diluted. Diluted samples were spreaded on lactobacilli MRS (Acumedia, USA) agar plates with 1% $CaCO_3$ and 0.006% bromocresol purple. Yellow colonies with clear zones were selected as putative LAB after 48 h incubation at 30℃. LAB producing GABA were screened by thin layer chromatography (TLC). Three isolates were co-inoculated into 1 ml of MRS broth with 3% (w/v) MSG (Sigma-Aldrich, USA), and the inoculated cultures were incubated for 48 h at 30℃. Cultures were centrifuged at 12,000 ×*g*, 4℃ for 5 min, and 1 μl of supernatant was spotted on an activated silica gel plate (Silica gel 60 F254; Merck Co., Germany). After separated in *n*-butanol: acetic acid: water (4:1:1, v/v/v), the plate was treated with 2% ninhydrin solution and developed at 70℃ for 10 min. For samples showing strong GABA production, each isolate was individually inoculated into 1 ml of MRS broth and GABA production was tested again.

Identification of GABA-Producing LAB

GABA-producing isolates were identified by 16S rRNA gene and *recA* gene sequencing. 16S rRNA genes were amplified using primer 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3'). *recA* gene was amplified using brevisF (5'-ATGGCTGACGAACGACAAGCGG-3') and brevisR (5'-GGCTGATTTGTCTGGTGCTAACTC-3'). Amplification was done in a volume of 50 μl consisting of 5 μl of DNA (100 ng), 0.5 μl (1 U) of EX *Taq* DNA polymerase (Takara, Japan), 5 μl of 10 × EX *Taq* buffer, 5 μl dNTP mixture (2.5 mM each), 10 pmol of each primer, and 33.5 μl of distilled water. The amplification conditions for 16S rRNA genes were as follows; 94℃ for 5 min followed by 30 cycles of 94℃ for 30 s, 58℃ for 45 s, 72℃ for 1 min, and a final extension at 72℃ for 4 min. Conditions for *recA* gene amplification were as follows; 94℃ for 5 min followed by 30 cycles of 94℃ for 30 s, 61℃ for 45 s, 72℃ for 1 min, and a final extension at 72℃ for 4 min. Amplified fragments were ligated with pGEM-T easy vector (Promega, USA) and sequenced. Nucleotide sequences were analyzed using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) at NCBI (National Center for Biotechnology Information).

Measurement of GABA using GC/MS

Isolates were grown in MRS broth with 3% (w/v) MSG for 48 h at 30℃. Culture supernatant was obtained by centrifugation at 12,000 ×*g* for 10 min. Two μl of supernatant was completely dried by vacuum CentriVao concentrator (Labconco, USA) at 40℃. For derivatization, the dried extract was methoximated with 70 μl of methoxyamine hydrochloride in pyridine (20 mg/ml) at 37℃ for 90 min and silylated by 70 μl of *N,O*-bis (trimethylsilyl) trifluoroacetamide (BSTFA) at 70℃ for 30 min. The derivatized sample was analyzed by GC/MS using a Shimazu GC-2010 plus (Shimazu, Japan) equipped with DB-5 MS column (30 m × 0.25 mm id, 0.25 μm film thickness, J & W Scientific, USA). The injection temperature was set at 200℃ and the oven temperature was maintained at 70℃ for 2 min, increased to 320℃ at 10℃/min, and then held at 320℃ for 5 min. The GC column effluent was analyzed by a Shimadzu GC/MS-TQ 8030 (Japan) with the electron impact (EI) ionization mode. The ion source temperature was 230℃. The interface temperature was 280℃ and ions were generated by a 70 eV. Effluents were monitored in the full scan mode in the range of 45–500 m/z with 0.3 sec of scan event time and 2000 u/sec of scan speed. Detector voltage was 0.1 kV and threshold was 100.

Growth Characteristics of *Lb. brevis* G144

Lb. brevis G144 was grown in MRS broth for 24 h at 30℃ and the culture was used to inoculate 50 ml of MRS broth (1%, v/v). Inoculated culture was incubated under different conditions for 120 h at 30℃: different temperature (4, 10, 25, 30, 37, and 45℃), different initial pH (pH

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3-10), and different NaCl concentration (1, 3, 5, 8, and 10%, w/v). Growth was monitored by measuring OD_{600} value at time intervals.

Cloning of *gadB* and *gadC* genes

gadB was amplified from genome of *Lb. brevis* G144 by using the primer set used for cloning of *gadB* from *Lb. zymae* GU240: *gadB*F (5'-CCCCTGCAGTTAAGGAGGCAAGCCATAT-3') and *gadB*R (5'-GGGAAGCTTTAACTCACCATTACTCGC-3') [9]. *gadC* was amplified by using a primer set based on *gadC* from *Lb. brevis* ATCC 367: *gadC*F (5'-GTTGTGAAGGGATTGTGTAG-3') and *gadC*R (5'-CACCAAAGGCAGCATCAA-3') [9]. Amplified fragments were ligated with pGEM T-Easy vector (Promega). *E. coli* DH5α cells were transformed with the ligation mixture by a standard protocol. DNA sequence analysis was done by using BLAST.

Reverse Transcription (RT)-PCR

RNA was isolated from *Lb. brevis* G144 by the Trizol-bead method [10]. RT-PCR was done after DNase (RQ1, RNase free DNase, Promega) treatment. One step RT-PCR kit (Intron, Korea) was used and the reaction mixture consisted of 8 μl of RT-PCR premixture, 1 μl of forward primer, 1 μl of reverse primer, 1 μl of RNase inhibitor, and 9 μl of RNase free water. Primers used for *gadC* are A (5'-ATTGTTTACGCCTATGGGGCCT-3') and B (5'-GAT CCACATTGACACCTAGCTGCA-3'). Primers used for *gadB* are C (5'-GGGCAATCCT TACCCACCAATACA-3') and D (5'-ACCAAAGGCAGCA TCAACGTGA-3'). The reaction was started by 30 min incubation at 45℃, followed by initial PCR activation for 5 min at 94℃. PCR cycles consisted of denaturation at 95℃ for 30 s, annealing at 60℃ for 30 s, and extension at 72℃ for 1 min. A total of 29 cycles were repeated, and the final extension was done at 72℃ for 5 min.

Overexpression of *gadB* in *E. coli* and Purification of GAD

gadB was amplified from chromosome of *Lb. brevis* G144 by using a primer pair: F (5'-GGGCATATGAATAAAACGATCAGG-3', *Nde*I site underlined) and R (5'-GGGCTCGAGACTTCGAACGGTGGT-3', *Xho*I site underlined). Amplification conditions were as follows: 94℃ for 5 min; 30 cycles of 94℃ for 30 s, 60℃ for 30 s, 72℃ for 2 min; and the final extension at 72℃ for 10 min. The amplified fragment was ligated into pET26b(+) (Novagen, 5.36 kb, Kan^R) and the ligation mixture was introduced into *E. coli* BL21(DE3). *E. coli* cells harboring pETG144 (pET26b(+) with *gadB* at *Nde*I and *Xho*I sites) were grown in LB broth (100 ml) containing kanamycin (60 μg/ml) at 37℃ until the OD_{600} value reached 0.6. Then isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to 1 mM and growth continued for 14 h at 20℃. Cells were harvested by centrifugation at 12,000 ×*g* for 15 min at 4℃, washed three times with phosphate buffered saline (PBS, pH 7.4), and resuspended in lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, and 10 mM imidazole, pH 7.0). Cells were disrupted by using an ultrasonicator (Bandelin Electronic, Germany). The disrupted cells were centrifuged at 12,000 ×*g* for 15 min, and the pellet (insoluble fraction) and supernatant (soluble fraction) were obtained. The soluble fraction was loaded onto a Ni-NTA column (GE Healthcare, Sweden). Bound recombinant GAD was eluted by buffer containing imidazole (40–500 mM). Protein concentration was determined by Bradford method using a Bio-Rad protein assay kit [12]. SDS-PAGE was done using a 12% (w/v) acrylamide gel.

Enzyme Assay and Properties of Recombinant GAD

The activity of recombinant GAD was measured by a GABase method [13]. The enzyme solution (1 μg GAD in 0.1 ml of lysis buffer) and 0.1 ml of 4 M ammonium sulfate were mixed. After 30 min of pre-incubation, the enzyme solution was mixed with 1.3 ml of substrate (20 mM MSG, 0.2 mM PLP, and, 0.2 M pyridine-HCl, pH 4.5), and incubated for 1 h. The reaction was stopped by boiling for 5 min, and the amount GABA was analyzed by GABase assay as described previously [9]. One unit of GAD activity was defined as the amount of enzyme producing 1 μmol GABA per minute under the experimental conditions.

Purified recombinant GAD (4 μg) was incubated in substrate buffer (20 mM MSG, 0.2 mM PLP) at different pH (pH 3–10) for 1 h at 37℃, and then the remaining GAD activity was measured by GABase assay. Purified GAD (4 μg) was incubated at temperature ranging from 25℃ to 65℃ for 30 min (pH 5.0) and the remaining GAD activity was measured by GABase assay. The effect of PLP concentration (0 to 1.8 mM) and chemicals (2 mM) on the GAD activity were also determined by incubating GAD (4 μg) for 1 h (PLP) and 30 min (chemicals) at 40℃

and pH 5.0. For testing stability, 10 μg of recombinant GAD was incubated at 40℃ and pH 5.0, and the remaining GAD activity was measured at 1, 3 and 6 h.

Kinetic measurements of Recombinant GAD

The effect of MSG concentration on the GAD activity was determined in total 1.5 ml mixture. Purified GAD (25.49 μl, 10 μg) was mixed with 74.51 μl of enzyme solution (10 mM imidazole, 300 mM NaCl, 50 mM Sodium phosphate buffer, pH 5.0) and 100 μl of 4 M ammonium sulfate. After 30 min of pre-incubation, the enzyme solution was mixed with 1.3 ml of substrate (0–100 mM MSG, 0.2 M pyridine-HCl, 0.2 mM PLP, pH 5.0), and incubated for 1 h. The reaction was stopped by boiling for 5 min, and the amount of GABA was analyzed by GABase assay. The Michaelis constant (Km) and maximum velocity (Vmax) were calculated using a Lineweaver-Burk plot.

Results and Discussion

Isolation and Identification of GABA-Producing LAB from Galchi Jeotgal

A total of 1,000 putative LAB were isolated from galchi jeotgal and screened for GABA production by TLC. Among the several positive isolates, G144 was the best producer as determined by TLC (Fig. 1). G144 was a non-spore forming, Gram-positive, facultative anaerobic, and short-rod shaped organism without flagella. API kit test results indicated that G144 was *Lactobacillus brevis* (results not shown). For more accurate identification, 16S rRNA and *recA* genes were amplified and sequenced. BLAST search for the 16S rRNA gene (1,387 nucleotides, MN065577) showed that the sequence showed 100% identity with those of *Lb. brevis* MG4563, *Lb. brevis* KT38-3, *Lb. brevis* SDCM, and *Lb. brevis* LMT1-73 (data not shown). BLAST search for the *recA* gene (1,013 nucleotides, MN065580) also indicated that the sequence was 100% identical with those of *Lb. brevis* UCCLBBS124 and LMT1-73 (data not shown). From these results, G144 was identified as *Lb. brevis,* and named *Lb. brevis* G144.

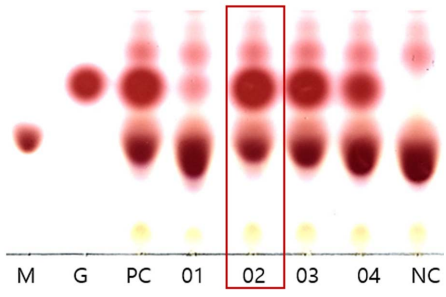

Fig. 1. Thin-layer chromatogram showing GABA production by *Lb. brevis* G144. M, 0.8 μl of 100 mM MSG; G, 0.8 μl of 100 mM GABA; PC, *Lb. zymae* GU240 (positive control); 01, S41; 02, G144; 03, G145; 04, G167; NC, *Leuconostoc mesenteroides* ATCC10830 (negative control).

GABA content of culture supernatant of *Lb. brevis* G144 was 14.58 ± 1.74 mM as determined by GC/MS (results not shown). Meanwhile, the GABA content of *Lb. zymae* GU240 was 12.16 ± 1.05 mM under the same conditions. *Lb. zymae* GU240, previously isolated from kimchi and a profuse GABA producer, has been used as a positive control [9]. Both cultures were grown under the same conditions and samples prepared by the same way. The result indicated that G144 is better than *L. zymae* GU240 in terms of GABA production.

Growth Characteristics of *Lb. brevis* G144

Lb. brevis G144 grew rapidly at 25, 30 and 37℃ in MRS broth, reaching OD_{600} value of 1.32, 1.47 and 1.52, respectively in 24 h, and 1.49, 1.56 and 1.49, respectively in 96 h (Fig. 2A). *Lb. brevis* G144 grew at 45℃, and reached OD_{600} value of 1.17 and 1.34 in 24 h and 48 h, respectively. But it did not grow at 4℃ and -1℃ in 120 h. At 15℃, growth was delayed until 24 h and then grew rapidly, reaching OD_{600} value of 1.45 in 120 h. *Lb. brevis* G144 grew rapidly at 3% (w/v) NaCl and reached OD_{600} value of 1.56 at 48 h, the same degree of growth of control (0% NaCl) (Fig. 2B). At 5% NaCl, growth was delayed until 24 h and then growth regained, reaching OD_{600} value of 1.44 at 120 h. *Lb. brevis* G144 grew very slowly at 8% NaCl, and did not grow at 10% NaCl in 120 h. *Lb. brevis* G144 possesses weak salt tolerance, and thus could be used as a starter for low-salt jeotgals or other fermented foods where the NaCl concentration does not exceed 8%.

Lb. brevis G144 grew well at the initial pH of 4.0 to 8.0, reaching OD_{600} values of 1.41–1.60 at 24 h (Fig. 2C). *Lb. brevis* G144 grew slowly at the initial pH of 3 and 9, reaching OD_{600} values of 1.2 at 120 h. But *Lb. brevis* G144 grew very slowly at the initial pH of 10, reaching 0.69 at 120 h.

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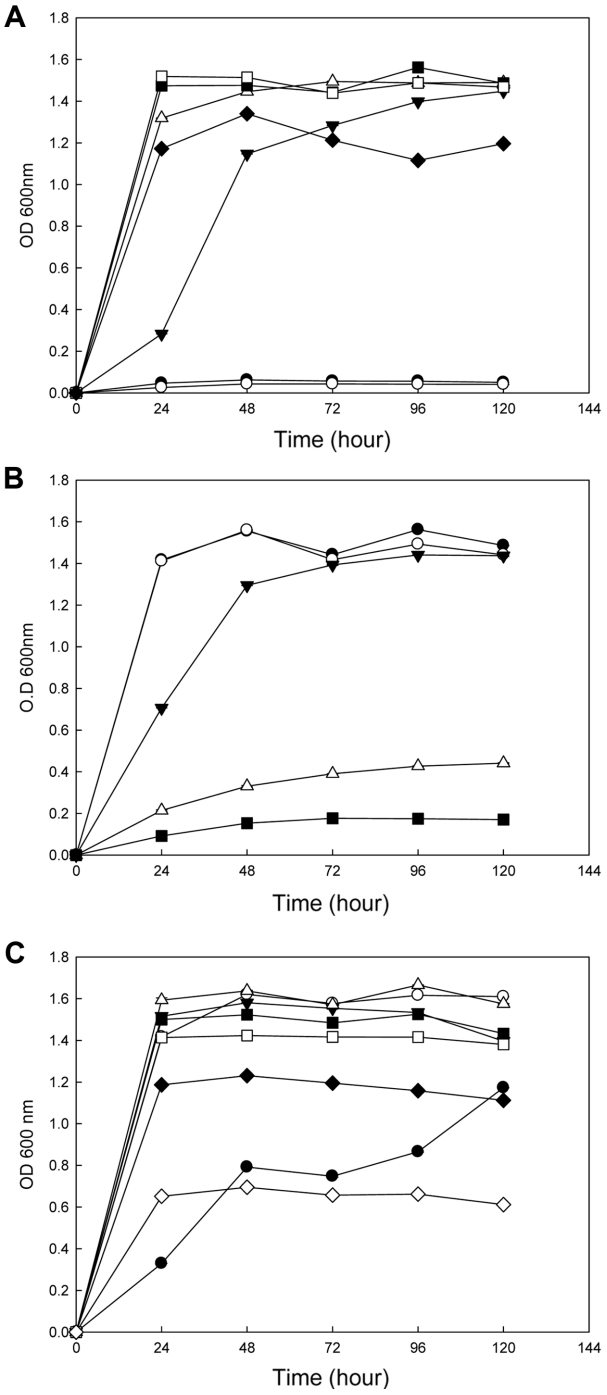

Fig. 2. Growth of *Lb. brevis* G144 in MRS broth under different conditions. The absorbance of each culture was measured at 600 nm and each value represents the mean value from three independent measurements. (A) Temperature: ●, -1 ℃; ○, 10 ℃; ▼, 15 ℃; △, 25 ℃; ■, 30 ℃; □, 37; ◆, 45 ℃. (B) NaCl concentration: ●, 0%; ○, 3%; ▼, 5%; △, 8%; ■, 10%. (C) initial pH of MRS broth: ●, pH 3; ○, pH 4; ▼, pH 5; △, pH 6; ■, pH 7; □, pH 8; ◆, pH 9; ◇, pH 10.

Cloning of *gadB* and *gadC*

A 1.5 kb fragment containing *gadB* was amplified and the nucleotide sequence was determined (accession number MN102361). An ORF consisting of 1,440 nucleotides was located, and the amino acids translated from the nucleotide sequence showed high homologies to GADs in the database. *gadB* could encode a protein of 479 amino acids with the calculated size of 53.50 kDa and isoelectric point (pI) of 4.95. The GAD from *Lb. brevis* G144 contained a highly conserved catalytic domain (T224, D255, H287, K288 in Fig. 4A) that belongs to the PLP-dependent decarboxylase superfamily [13]. The amino acid sequence of GAD from *Lb. brevis* G144 was aligned with those of GADs from other LAB. *Lb. brevis* G144 showed 99% identity with the GAD from *Lb. zymae* GU240 (AHF72525), *Lb. brevis* ATCC367 (ABJ63253), and *Lb. brevis* BH2 (AIC75925), differing only at two amino acids. The GAD from *Lb. brevis* G144 differed from those from *Lb. sakei* A156 (KM982734),

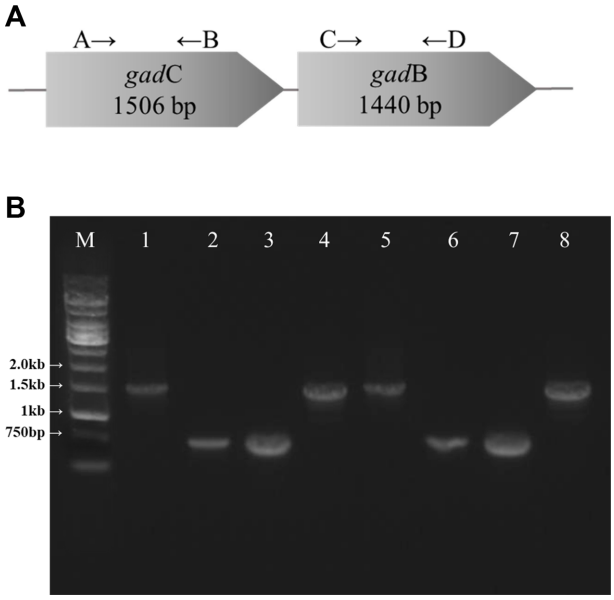

Fig. 3. Reverse transcription (RT)-PCR results showing the operon structure of *gadCB*. (A) Operon structure of *gadCB* genes in *Lb. brevis* G144. The arrows indicate the binding sites for primers used for RT-PCR. (B) Agarose gel electrophoresis for RT-PCR products. Lane M, GeneRuler 1kb DNA ladder (Thermo scientific, USA); 1-4, RNA preps; 5-8, RNA preps treated with DNase I. Lanes 1 and 5, RT-PCR using universal primers (27F and 1492R) for 16S rRNA genes; 2 and 6, RT-PCR using primers C and D for *gadB*; 3 and 7, RT-PCR using primers A and B for *gadC*; 4 and 8, RT-PCR using primers A and D for *gadCB*.

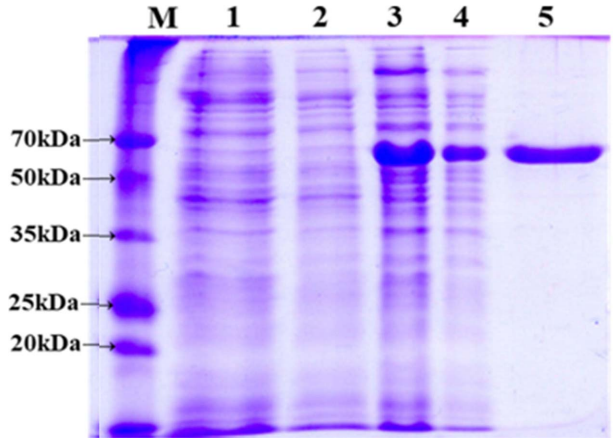

Fig. 4. SDS-PAGE of recombinant GAD. M, size marker (Dokdo-mark, Eplis Biotech, Korea); 1, insoluble fraction from *E. coli* BL21 [pET26(b)+] (negative control); 2, soluble fraction from *E. coli* BL21 [pET26(b)+] (negative control); 3, insoluble fraction from *E. coli* BL21 [pETG144]; 4, soluble fraction from *E. coli* BL21 [pETG144]; 5, GAD eluted from a HiTrap affinity column at 300 mM imidazole concentration.

Lb. brevis CGMCC1306 (AEY81112.1), and *Lb. brevis* IFO12005 (BAF99137.2) at three amino acids.

The nucleotide sequence of *gadC* was determined (MN102362). An ORF of 1,506 nucleotides was located, which could encode a protein of 501 amino acids with the calculated molecular mass of 55.15 kDa and isoelectric point (pI) of 9. *gadC* from *Lb. brevis* G144 showed 99.9% identity with the *gad*C gene from *Lb. zymae* GU240 (AHF72525), *Lb. brevis* ATCC367 (ABJ63253), *Lb. sakei* A156 (KM982734), *Lb. brevis* CGMCC1306 (AEY81112.1), *Lb. brevis* IFO12005 (BAF99137.2), and *Lb. brevis* BH2 (AIC75925).

It was found that *gadC* was located in the immediate upstream of *gadB* with just 55 nucleotides apart (data not shown). This strongly indicated the operon structure of *gadCB* in *Lb. brevis* G144 (Fig. 3A). RT-PCR was done for RNA sample prepared from *Lb. brevis* G144. A transcript, 1.5 kb, covering both *gadC* and *gadB* was detected (Fig. 3B), confirming the *gadCB* operon structure in *Lb. brevis* G144. The same operon structure was reported for *Lb. brevis* IFO12005 [14], *Lactococcus lactis* subsp. *lactis* 01-7 [15], and *Lb. zymae* GU240 [9]. However, except for *Lb. zymae*, the operon structure was just suspected from the close locations of two genes, but not proven experimentally by methods such as RT-PCR used in this work.

Overexpression of *gadB* in *E. coli* BL21(DE3) and Purification of Recombinant GAD

The *gadB* gene from *Lb. brevis* G144 was overexpressed in *E. coli* BL21(DE3) and recombinant GAD was produced in IPTG induced cells. A control, *E. coli* BL21(DE3) harboring intact pET26b(+), did not produce the protein (Fig. 4, lane 1 and 2). Recombinant GAD was observed from both soluble and insoluble fractions of cell extract. The soluble fraction was loaded onto a Ni-NTA column, and bound GAD was eluted by imidazole at 300 mM concentration (Fig. 4). Sufficiently purified GAD was obtained, and the size was 54.4 kDa on a SDS gel, matching well with the expected size of GAD with additional His tag (Fig. 4).

Properties of Recombinant GAD

The optimum temperature for recombinant GAD was 40℃ (Fig. 5A, and Table 1). It was lower than that of GAD from *Enterococcu avium* M5 (45℃) [11]. The optimum pH for recombinant GAD was 5.0 (Fig. 5B). Most GADs are active at pH 4.0–5.0 as reported for GAD from *Lactococcus lactis* (pH 4.7) [16], *Lb. brevis* CGMCC1306 (pH 4.8) [17], *E. coli* (pH 4.4) [18] *Lb. sakei* A156 (pH 5.0) [10], *Lb. zymae* GU240 (pH 4.5) [9], and *S. thermophiles* Y2 (pH 4.2) [19]. GAD is believed to be involved in maintaining cellular pH near neutral pH under acidic conditions and this role is important for LAB [20]. The GAD of *Lb. brevis* G144 depends on PLP for its activity (Fig. 5C) and the highest activity was obtained around 0.6 mM, but the degree of increase was small at PLP concentrations above 0.02 mM. The effect of chemicals (2 mM) on GAD activity was examined (Fig. 5D). The activity was increased by $CaCl_2$ (128%), $FeCl_3$ (112%), KCl (117%), and $MnCl_2$ (114%), whereas it was decreased by $CoCl_2$ (78%), $CuSO_4$ (12%), $ZnCl_2$ (95%), and $AgNO_3$ (20%). For testing stability in optimum pH and temperature (pH 5.0 and 40℃), purified GAD was incubated up to 6 h, and GAD remained active at 6 h (data not shown).

The K_m of GAD from *Lb. brevis* G144 was 8.6 ± 0.06 mM and V_{max} was 0.010 ± 0.0002 mM/min when MSG was used as the substrate (Table 1). The Km value was higher than those of GADs from *Lb. zymae* GU240 [9], *E. avium* M5 [11], *Lb. brevis* 877G [21], *L. lactis* ssp. *lactis* 01-7 [15], *Pyrococcus horikoshii* [22], *E. coli* [18], and *St. salivalivarius* ssp. *thermophilus* Y2 [19], but lower than those from *Lb. sakei* A156 [10], *Lb. brevis*

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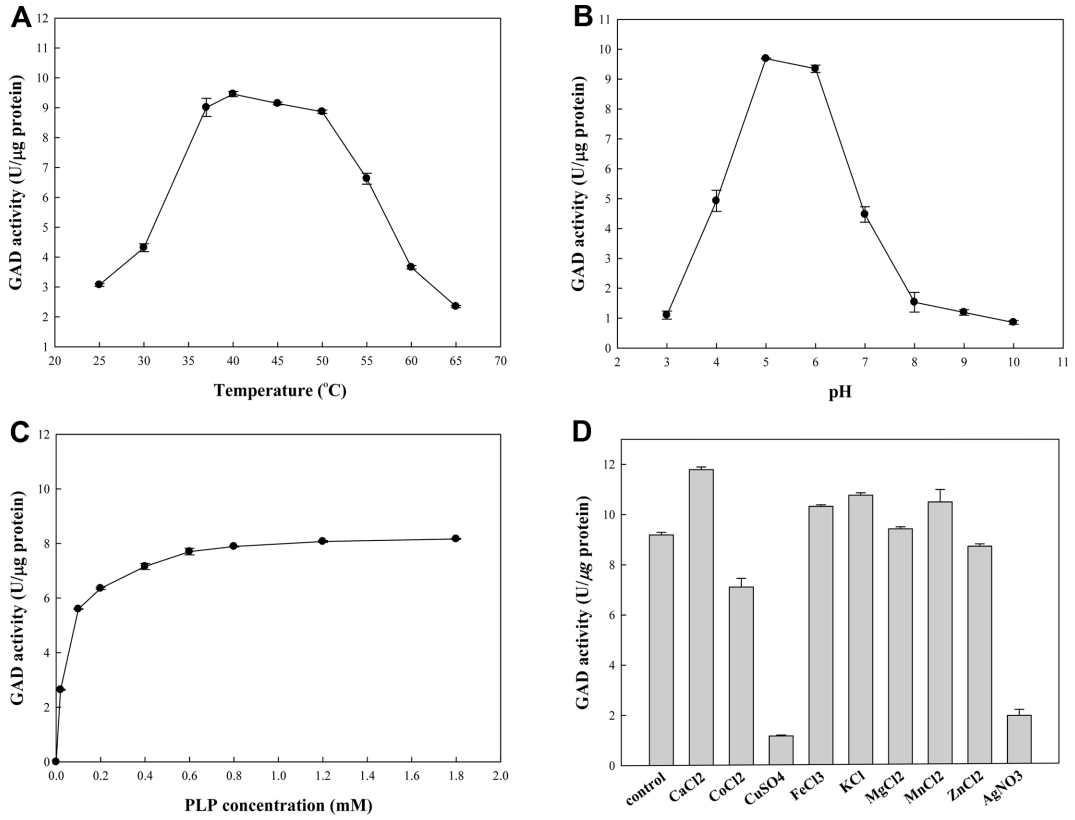

Fig. 5. Properties of recombinant GAD under different conditions. Activities of recombinant GAD were measured at different conditions. (A) temperature, 25-65 ℃, (B) pH, 3-10, (C) PLP concentration, 0-1.8 mM, and (D) chemicals, 2 mM.

Table 1. Characteristics of recombinant GAD and other GADs.

Organism	Optimum temperature (℃)	Optimum pH	Km (mM)	Reference
Aspergillus oryzae	60	-	13.3	[23]
Enterococcu avium M5*	55	4.5	3.26	[11]
Escherichia coli	-	4.4	1.4	[18]
Lactobacillus brevis G144*	40	5.0	8.6	This study
Lactobacillus brevis 877G*	45	5.2	3.6	[21]
Lactobacillus brevis IFO12005*	30	4.2	9.3	[14]
Lactobacillus brevis CGMCC 1306*	48	4.8	10.3	[17]
Lactobacillus sakei A156*	55	5.0	16.0	[10]
Lactobacillus sakei OPK2-59	30	5.0	-	[24]
Lactobacillus paracasei NFRI 7451	50	5.0	-	[25]
Lactobacillus zymae GU240*	41	4.5	1.7	[9]
Lactococcus lactis ssp. *lactis* 01-7	-	4.7	0.51	[15]
Streptococcus salivarius ssp. *thermophilus* Y2*	55	4.0	2.3	[19]
*Pyrococcus horikoshii**	-	8.0	3.9	[22]

*Recombinant GAD was used for testing properties.

IFO12005 [14], *LB. brevis* CGMCC 1306 [17], and *A. oryzae* [23].

A GABA-producing *Lb. brevis* G144 was isolated form galchi jeotgal and its *gadB* gene was cloned and overexpressed in *E. coli*. *Lb. brevis* G144 and its *gadB* gene might be useful for the production of GABA-enriched foods including kimchi, jeotgals, and fermented soy milk where the NaCl concentration does not exceed 8% (w/v). But more studies are necessary on the growth and GABA production by *Lb. brevis* G144 in such foods. Questions to be answered are how to supply precursor of GABA and PLP, a required cofactor for GAD for food fermentations. These questions must be answered before *Lb. brevis* G144 is used as a starter for a specific food type.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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