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Methods

Acid tolerance assay

Lactobacillus isolated from kimchi was cultivated in acidic MRS medium (pH 2.5, supplemented with 1000 U/ml pepsin) at 37°C for 2 h [1]. Then, bacteria were plated in MRS agar and enumerated after 24 h of incubation at 37°C in anaerobic jar with steel wool as previous described [2]. *Lactobacillus* strains that exhibited acid tolerance were used for subsequent analyses.

Monosodium glutamate tolerance

Lactobacillus strains exhibiting acid tolerance were cultivated in MRS media supplemented with different concentrations of monosodium glutamate (MSG) [1%, 2%, or 5% (w/v); Sigma-Aldrich, St. Louis, MO, USA] for 18 h at 37°C under anaerobic conditions. MSG tolerance of these *Lactobacillus* strains was determined by measuring the bacterial growth in the presence of MSG at an optical density of 600nm.

GABA determination

The amount of GABA produced by the selected *Lactobacillus* strains was determined as previously described [3] with slight modification. The selected *Lactobacillus* strains were grown in MRS medium supplemented with 1% (w/v) MSG for 48 h at 37°C under anaerobic conditions. Upon incubation, the supernatant was collected and filtered through a 0.22-μm filter membrane prior GABA determination. The supernatant (100 μl) was added to 90 μl of Tris-HCl buffer (100 mM, pH 8.9) containing 10 mM α-ketoglutarate, 2 mM 2-

mercaptoethanol, 0.50 mM NADP, and 0.25 U/mL of GABAse and incubated at 30°C for 15 min. Then, 10 µl of 1 M sulfuric acid was added to the mixture, followed by the addition of 5 µl of 1-MeO-PMS:WST-8 reagent, and the reagent mixture was mixed well. The concentration of GABA in the mixture was determined by measuring the absorbance at 470 nm. MRS medium without MSG was used as a blank. The concentration of GABA in samples was calculated from the calibration curve of the GABA standard solution.

GABA determination with high-performance liquid chromatography (HPLC)

The supernatant was first diluted with water, followed by reaction with 2% 5-sulfosalicylic acid in 1:1 ratio. The mixture was then filtered through 0.22 µm filter (Sartorius, Gottingen, Germany) prior to derivatized using *o*-phthalaldehyde/9-fluorenylmethyl chloroformate (OPA/FMOC) for HPLC analysis [4]. The Agilent 1200 series HPLC system used was equipped with a Water 600 pump, a Water Symmetry reversed phase C18 column (3.9 x 150 mm, 5 µm particle diameter; Water, Milford, MA, USA), and a fluorescence detector (Jusco FP-920; Jusco Inc., Tokyo). The A, B, and C mobile phases consisted of 0.05% (v/v) trifluoroacetic acid, methanol, and acetonitrile, respectively [5]. The *Lactobacillus* strain with the highest GABA production was identified by 16S rDNA sequencing as previously described [6]. *L. brevis* KCTC 13094 obtained from Korean Collection for Type Culture (KCTC) was used as a control.

Growth characteristic of *L. brevis* Bmb5 in MSG-MRS medium

L. brevis Bmb5 was grown in MRS medium supplemented with 1% (w/v) MSG for 72 h at 37°C under anaerobic conditions. The number of the viable cells was determined at every 3 h intervals using MRS agar plate.

Gene expression of the GAD system

L. brevis Bmb5 and *L. brevis* KCTC 13094 were grown in MRS medium supplemented with 1% (w/v) MSG for 72 h at 37°C. Total RNA was extracted from the strains using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instruction. Complimentary DNA (cDNA) was synthesized from one μg of the extracted RNA using Maxime RT Premix Oligo(dT) (Intron, Seongnam, Korea), according to the manufacturer's instruction. The primers used in this study (Table 1) were designed based on the genome sequence of *L. brevis* ATCC 367 (GenBank accession number: NC_008497.1), using Primer3plus software (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi). Quantitative polymerase chain reaction (qPCR) was performed using KAPA SYBR FAST qPCR kit (KapaBiosystems, Boston, MA, USA) in a thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA). PCR reaction conditions were initial denaturation at 94°C for 5 min, 40 cycles of 95°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec, followed by a final elongation step at 72°C for 15 min. The *pheS* gene was used as an internal control [7, 8]. The expression of the GAD system gene was determined relative to *pheS* gene expression based on threshold value (Ct) values as follows:

Relative expression = $2^{-\Delta Ct}$ with $C_t = C_{t\ gene} - C_{t\ pheS}$

Where C_t is the threshold cycle, $C_{t\ gene}$ is the threshold cycle of the GAD system gene, and $C_{t\ pheS}$ is the threshold cycle of the *pheS* gene.

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Table S1. Oligonucleotide primers used in the gene expression study of *Lactobacillus* GABA producing genes.

Gene	Sequence of PCR primers (5' to 3')	Target locus	Product	Reference
gadB	Forward : GCTGATGTTACTGCTGATGC Reverse : ATCCGTATTTTGAACGGGTA	LVIS_1847	glutamic acid decarboxylase	*Lactobacillus brevis* ATCC 367 (GenBank accession number NC_008497.1)
gadC	Forward : CAACTTGATGGGACAACGTA Reverse : TCTGGTGCTTTTGTTTCTGA	LVIS_0078	glutamate/GABA antiporter	
pheS	Forward : GATTATCGGGTTGATTCTGG Reverse : AAATCGCCTTCGTACATCTC	LVIS_1010	phenylalanine-tRNA ligase alpha subunit	

Table S2. Acid tolerance of *Lactobacillus* strain isolated from kimchi.

***Lactobacillus* strains**	**MRS media at pH 2.5 with 1000 units/ml pepsin (log_{10} CFU/ml)**	
	0 h	2 h
1	5.84 ± 0.01	5.84 ± 0.00 ^{ab}
2	5.84 ± 0.00	5.84 ± 0.00 ^{ab}
3	6.84 ± 0.00	6.84 ± 0.00 ^{ab}
4	6.84 ± 0.00	5.84 ± 0.00 ^{bcde}
5	6.85 ± 0.00	4.69 ± 0.00 ^{fg}
6	5.85 ± 0.00	4.69 ± 0.00 ^{ef}
7	4.75 ± 0.00	4.69 ± 0.03 ^{bcd}
8	5.69 ± 0.01	5.67 ± 0.01 ^{abc}
9	6.83 ± 0.01	5.76 ± 0.00 ^{cde}
10	6.78 ± 0.00	6.76 ± 0.01 ^{ab}
11	6.81 ± 0.00	5.79 ± 0.02 ^{bcde}
12	5.76 ± 0.00	5.10 ± 1.15 ^{bcde}
13	6.80 ± 0.01	5.77 ± 0.01 ^{bcde}
14	5.77 ± 0.00	5.77 ± 0.00 ^{ab}
15	6.79 ± 0.01	5.69 ± 0.07 ^{def}
16	5.83 ± 0.00	6.83 ± 0.01 ^{a}
17	6.78 ± 0.00	6.64 ± 0.09 ^{bcde}

Lactobacillus strains	MRS media at pH 2.5 with 1000 units/ml pepsin (log_{10} CFU/ml)	
	0 h	2 h
18	6.84 ± 0.00	5.82 ± 0.00 ^{bcde}
Bmb4	7.48 ± 1.15	4.57 ± 0.01 ^{g}
Bmb5	6.84 ± 0.01	6.81 ± 0.01 ^{abc}
Bmb6	6.85 ± 0.00	5.74 ± 0.01 ^{def}
Bmb7	6.83 ± 0.09	0.18 ± 0.32 ^{h}
Bmb10	4.83 ± 0.01	4.33 ± 0.20 ^{bcde}
Bmb15	5.80 ± 0.09	0.17 ± 0.29 ^{h}
Sa2	5.82 ± 0.16	0.13 ± 0.23 ^{h}
J1	5.80 ± 0.00	4.76 ± 0.00 ^{bcde}

The data are expressed as the mean ± standard deviation from three independent experiments (n = 3). Tukey's multiple range test was performed with difference lowercase letters (a, b, c, d, e, and h) demonstrated the significant difference ($p < 0.05$) between the *Lactobacillus* strains after 2 h incubation in acidic MRS media (pH 2.5).

Figure S1

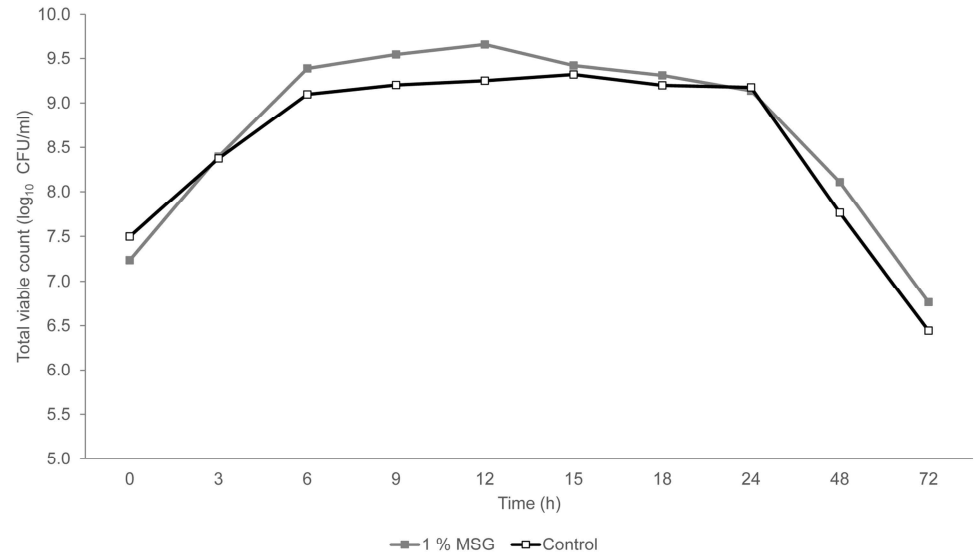

Fig. S1 Growth curve of *Lactobacillus brevis* Bmb5 in the presence (filled-square with grey line) or absence (open-square with black line) of 1% (w/v) monosodium glutamate (MSG). Results are expressed as the mean from three independent experiments (n =3).