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RESEARCH ARTICLE

Optimization of culture conditions for and assessment of kimchi-originated lactic acid bacterial isolates toward their extracellular GABA-producing ability

Jin-Sung Lee[1], Keun-Sung Kim[2]*

[1]Department of Life Sciences, Kyonggi University, Suwon 442-760, South Korea, [2]Department of Food Science and Technology, Chung-Ang University, Ansung 456-756, South Korea

ABSTRACT

The objectives of this study were to optimize the medium and culture conditions using a strong γ-aminobutyric acid (GABA) producer as a reference lactic acid bacterial strain, to screen and identify GABA-producing lactic acid bacterial isolates from kimchi, and to determine their extracellular GABA-producing abilities. Thin-layer chromatography was used to screen GABA-producing bacterial isolates and high-performance liquid chromatography was used to evaluate the bacterial GABA production abilities. Species-specific polymerase chain reaction analyses were used to identify GABA-producing bacterial isolates. The optimal medium and culture conditions were found to be the modified Man–Rogosa–Sharpe (MRS) broth (with an initial pH of 6.5) containing 4% sucrose, 5% glutamate, and 1% yeast extract at 37 °C for 5 days. After incubation under the optimized culture conditions, 217 kimchi bacterial isolates were screened to evaluate their respective GABA-producing abilities. Screening the 217 kimchi bacterial isolates identified 24 GABA-producing lactic acid bacterial isolates (11%): *Lactobacillus plantarum* (17), *Lactobacillus brevis* (six), and *Leuconostoc mesenteroides* (one), indicating that only a small proportion of the strains produce GABA in the culture broth. The extracellular GABA-producing abilities of the bacterial strains identified in this study varied even within the same species, ranging from 5.8 to 101.7 mM among the 17 GABA-producing *L. plantarum* isolates and from 8.5 to 88.6 mM among the six GABA-producing *L. brevis* isolates. In summary, three species of the 24 kimchi GABA-producing bacterial isolates were identified, including one rare species (*L. mesenteroides*) and the two most dominant species (*L. brevis* and *L. plantarum*).

Keywords: Lactobacillus brevis; Lactobacillus plantarum; Leuconostoc mesenteroides; Optimization; γ-aminobutyric acid (GABA)

INTRODUCTION

Lactic acid bacteria (LAB) catabolize carbohydrates into lactic acid as a major end metabolite during fermentation and possess distinct acid-resistance mechanisms. Among them, the glutamic acid decarboxylase (GAD) system serves as a fitness determinant in LAB for acid resistance (Wu and Shah, 2017). This bacterial group can decarboxylate L-glutamic acid into γ-aminobutyric acid (GABA), which is catalyzed by GAD. GABA is a free non-proteinaceous amino acid that is found in animals, plants, and microorganisms, including LAB (Somkuti et al., 2012).

In the vertebrate central nervous system, GABA is known to function as a major inhibitory neurotransmitter. In addition, clinical implications of GABA ingestion has led to positive correlations with various important physiological functions, including tranquilization and anti-anxiety functions, although whether the orally administered GABA can enter the blood-brain barrier is still a contradictory issue to be clarified (Somkuti et al., 2012).

Consequently, various functional food supplements containing GABA are commercially mass-produced. To produce such GABA-rich foods, food-grade bacteria, such as LAB, require utilization in various fermentations because chemically-synthesized GABA is a prohibited food additive in developed countries (Wu and Shah, 2017).

Therefore, most studies on GABA-producing LAB have been accomplished to isolate individual strains and determine their GABA-producing abilities under the specific fermentation conditions of the respective LAB (Li and Cao, 2010; Dhakal et al., 2012; Wu and Shah, 2017). In

***Corresponding author:**
Keun-Sung Kim, Department of Food Science and Technology, Chung-Ang University, Ansung, Kyungki-do 456-756, South Korea
E-mail: keunsung@cau.ac.kr

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addition, recent reviews report various GABA-producing LAB strains isolated from different food and environmental sources, while focusing on the high GABA producers (Dhakal et al., 2012; Wu and Shah, 2017; Luo et al., 2021). Moreover, most of the LAB isolates ranked as high GABA producers have been identified as *Lactobacillus plantarum* and *Lactobacillus brevis* (Wu and Shah, 2017).

Kimchi is a traditional Korean fermented vegetable-based dish made from Korean cabbage (*baechu*) and other minor ingredients. (Patra et al., 2016). High numbers of LAB are produced in kimchi during fermentation. Metagenomic analysis of kimchi showed that its fermentation was dominated by members of three genera: *Leuconostoc*, *Lactobacillus*, and *Weissella* (Jung et al., 2014). More recent metagenomic studies have suggested that five *Lactobacillus* species, including *L. plantarum, L. brevis*, and *L. sakei*, three *Leuconostoc* species, including *L. mesenteroides*, two *Weissella* species, and one *Pediococcus* species dominantly enriched kimchi fermentation (Jung et al., 2014; Swain et al., 2014).

Kimchi or other pickled vegetables are acid-based fermented foods and often the habitats of various GABA producers. LAB communities in kimchi are highly diverse, with *Leuconostoc*, *Lactobacillus*, and *Weissella* as the three most abundant genera (Jung et al., 2014). GABA-producing LAB strains have been screened in various fermented foods, and numerous LAB strains of the genus *Lactobacillus* have been isolated compared with other genera, such as *Leuconostoc* (Wu and Shah, 2017). Although many LAB isolates have been evaluated for their respective GABA-producing abilities, rare GABA-producing LAB strains from kimchi, an important LAB source, still require screening.

This study first aimed to find the optimal medium and culture conditions to augment GABA production in the culture broth by the GABA-producing LAB isolates of kimchi. Second, a total of 217 bacterial isolates from kimchi were screened to isolate both rare and dominant (such as the *Lactobacillus* species) GABA-producing LAB strains. Finally, GABA-producing LAB strains were identified, and their respective GABA-producing potentials were determined.

MATERIALS AND METHODS

Bacterial strains and their identification

Lactobacillus sakei subsp. *sakei* ATCC 15521^T was used as a reference strain to determine the optimal medium and culture conditions. A collection of 217 kimchi bacterial isolates were obtained from the Food Microbiology Laboratory (Chung-Ang University, Ansung, South Korea). Species-specific polymerase chain reaction (PCR) methods were used to identify GABA-producing LAB isolates, as described previously (Cho et al., 2009; Kim and Kim, 2014).

Medium and culture conditions for GABA production

The reference strain *L. sakei* subsp. *sakei* ATCC 15521^T was used to optimize the culture conditions. This strain was cultured in modified de Man–Rogosa–Sharpe (MRS) broth (Difco, Becton Dickinson Co., Sparks, MD, USA) containing 4% of various extra carbon sources (sucrose, maltose, galactose, glucose, fructose, or lactose), 0–5% monosodium glutamate (MSG), and 1% yeast extract, with initial pH values between 3.5 and 8.5 for 7 days at 37 °C with shaking. After the optimization step, 217 kimchi bacterial isolates were evaluated for their respective GABA-producing abilities using thin-layer chromatography (TLC), and the produced GABA was quantified by high-performance liquid chromatography (HPLC).

Qualitative determination of GABA produced in the culture broth

After incubation under the optimized culture conditions (in the modified MRS broth with an initial pH of 6.5 containing 4% sucrose, 5% glutamate, and 1% yeast extract at 37 °C for 5 days), the broth culture was clarified by centrifugation coupled with filtration through a 0.45-μm syringe filter. GABA production (in the filtered supernatant) was qualitatively evaluated on silica gel 60 F254 TLC plates (Merck, Darmstadt, Germany), as described previously (Thwe et al., 2011). GABA and glutamate were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). All chemical reagents used in this study were of analytical or HPLC grade.

Determination of GABA-producing capabilities

The glutamate and GABA in the culture supernatant were quantified using a reversed-phase HPLC system equipped with a Nova-Pak C18 analytical column (3.9 mm × 150 mm, 4 μm particle size; Waters, Milford, MA, USA), a Waters 474 scanning fluorescence detector (Waters), a Waters 600S controller (Waters), and Clarity Lite software (DataApex, Prague, Czech Republic), as described previously (Jin et al., 2013).

Data analysis

Glutamate and GABA quantification data were obtained from three independent measurements and presented as mean ± standard deviation. The quantitative data were statistically analyzed, as described previously (Jin et al., 2013).

RESULTS

Addition of 4% sucrose to increase GABA production

Various extra carbon sources (sucrose, maltose, galactose, glucose, fructose, and lactose) were added (4%) to the culture broth containing 5% glutamate to evaluate their

respective effects on GABA production by *L. sakei* subsp. *sakei* ATCC 15521^T (Fig. 1). GABA production increased maximally to 275.2 mM after 5 days of cultivation when 4% sucrose was added to the culture broth ($p < 0.05$). However, the reference strain yielded less GABA production (below 200.0 mM) when each of the other tested carbon sources was added.

Five-day cultivation to increase GABA production

The effect of incubation time on GABA production by *L. sakei* subsp. *sakei* ATCC 15521^T was monitored from the initial cultivation point to 7 days when grown in the modified MRS broth (Fig. 2). GABA rapidly increased from days 2 to 5 ($p < 0.05$) in the modified MRS broth, while glutamate gradually disappeared from initial cultivation until day 5 ($p < 0.05$). High GABA production (above 275.0 mM) was maintained between days 5 and 7; therefore, the optimal cultivation period for GABA production was found to be 5 days.

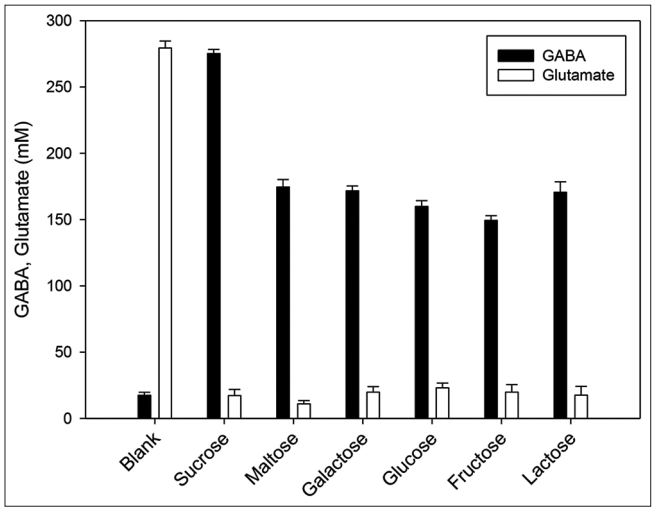

Fig 1. Extracellular production patterns of γ-aminobutyric acid (GABA) from glutamate by *Lactobacillus sakei* subsp. *sakei* ATCC 15521^T with various carbon sources. The strain was cultured at 37 °C for 5 days with shaking in 10 mL of de Man–Rogosa–Sharpe (MRS) broth containing 4% of each carbon source, 5% glutamate, and 1% yeast extract.

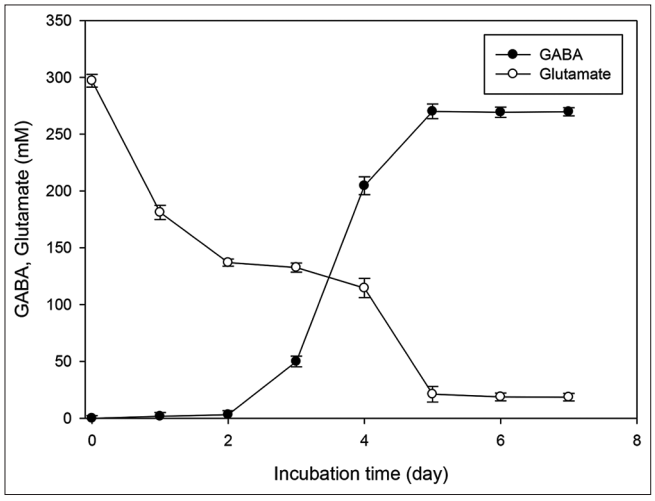

Fig 2. Extracellular production patterns of γ-aminobutyric acid (GABA) from glutamate by *Lactobacillus sakei* subsp. *sakei* ATCC 15521^T with increased cultivation time. The strain was cultured at 37 °C for 7 days with shaking in 10 mL of de Man–Rogosa–Sharpe (MRS) broth containing 4% sucrose, 5% glutamate, and 1% yeast extract.

Addition of 5% glutamate to increase GABA production

Glutamate was used as a substrate to produce GABA. To maximize GABA production by *L. sakei* subsp. *sakei* ATCC 15521^T, the effect of the initial substrate levels of 0 to 6% was monitored in the culture broth after 5 days of cultivation (Fig. 3). GABA production rapidly increased from 8.5 to 274.9 mM ($p < 0.05$) when the initial substrate content gradually increased from 3 to 5% ($p < 0.05$). However, high GABA production (above 270.0 mM) slightly decreased when the substrate level was increased to 6%. Therefore, GABA was maximally produced by the reference strain when 5% glutamate was added as the substrate for the GAD enzyme.

Initial pH of 6.5 to increase GABA production

To maximize GABA production by *L. sakei* subsp. *sakei* ATCC 15521^T, the effect of an initial pH of 3.5–8.5 on the GABA level was monitored in the culture broth (Fig. 4). GABA gradually increased as the initial pH was increased over the range of 3.5–6.5 ($p < 0.05$), while the unconverted substrate gradually decreased to 22.2 mM ($p < 0.05$). However, high GABA production (270.0 mM) rapidly decreased when the initial pH was increased from 6.5 to 8.5 ($p < 0.05$), while the unconverted substrate rapidly increased ($p < 0.05$). Therefore, GABA was maximally produced by the reference strain when the initial pH of the culture broth was 6.5.

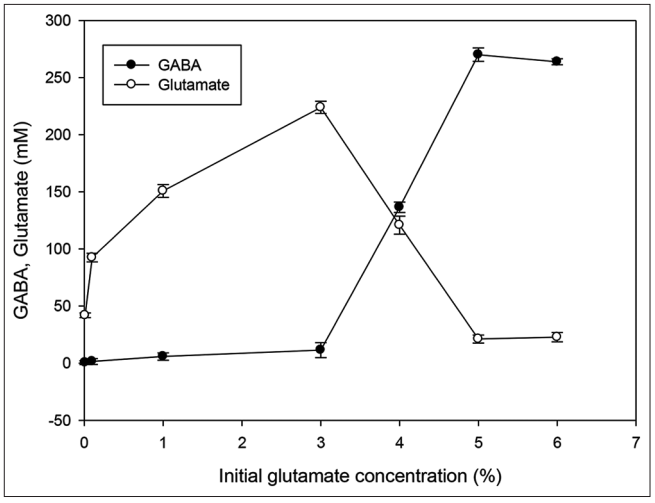

Fig 3. Extracellular production patterns of γ-aminobutyric acid (GABA) from glutamate by *Lactobacillus sakei* subsp. *sakei* ATCC 15521^T with increased initial glutamate concentration. The strain was cultured at 37 °C for 5 days with shaking in 10 mL of de Man–Rogosa–Sharpe (MRS) broth containing 4% sucrose and 1% yeast extract.

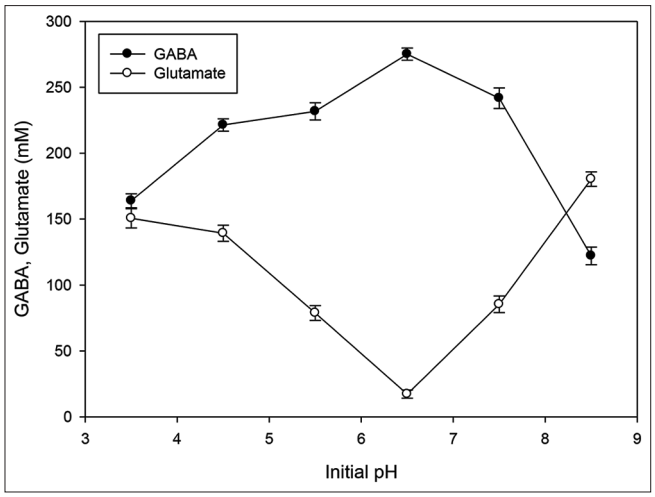

Fig 4. Extracellular production patterns of γ-aminobutyric acid (GABA) from glutamate by *Lactobacillus sakei* subsp. *sakei* ATCC 15521^T with increased initial pH value. The strain was cultured at an initial pH of 3.5 to 8.5 and 37 °C for 5 days with shaking in 10 mL of de Man–Rogosa–Sharpe (MRS) broth containing 4% sucrose, 5% glutamate, and 1% yeast extract with different initial pH values.

Screening and identifying extracellular GABA-producing bacterial isolates

Qualitative screening of 217 bacterial strains was performed to isolate the strains capable of producing extracellular GABA. The extracellular GABA-producing ability of *L. sakei* subsp. *sakei* ATCC 15521^T is shown in Fig. 5. Twenty-four (11%) isolates were determined to be putatively positive in the screening experiment.

For this study, species-specific PCR reactions using previously published primer sets for *L. plantarum*, *L. brevis*, and *L. mesenteroides* (Cho et al., 2009; Kim and Kim, 2014) could identify the 24 GABA-producing isolates. Representative species-specific PCR fragments amplified from each bacterial species are shown in Fig. 6. Each target PCR fragment could be species-specifically generated from the 24 GABA-producing isolates by the three species-specific PCR primer sets, and only one species-specific PCR fragment was amplified from each isolate. Therefore, each isolate was identified by PCR reactions using the three species-specific PCR primer sets. Among the 24 GABA producers, 17 were identified as *L. plantarum*, six as *L. brevis*, and one as *L. mesenteroides*.

Determining extracellular GABA-producing abilities

To determine their GABA-producing abilities, the 24 putatively-positive strains were subjected to a quantitative analysis of GABA production in the culture broth (Table 1). *Lactobacillus plantarum* KC-Q1 exhibited the highest extracellular GABA production capacity (102.0 mM) among the positive strains. Isolates KC-D13 (89.0 mM) and KC-I60 (71.0 mM) produced GABA in higher concentrations than the other isolates in this study

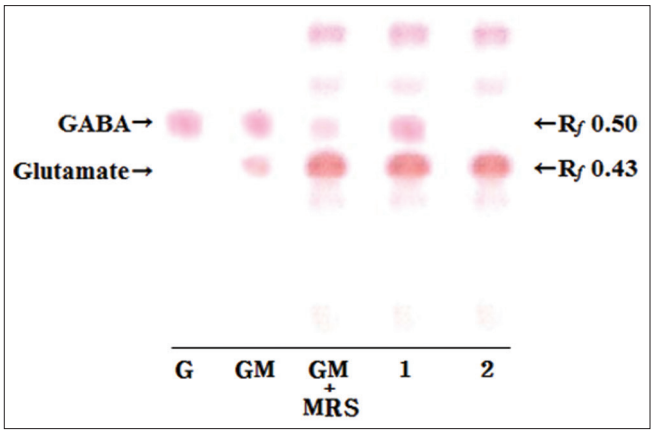

Fig 5. Thin-layer chromatogram displaying the extracellular production of γ-aminobutyric acid (GABA) by *Lactobacillus sakei* subsp. *sakei* ATCC 15521^T. Lane G, GABA standard; lane GM, GABA and glutamate standards; lane GM + MRS, GABA and glutamate standards in de Man–Rogosa–Sharpe (MRS) broth; lane 1, GABA-producing strain *L. sakei* subsp. *sakei* ATCC 15521^T; lane 2, GABA-nonproducing strain (negative control).

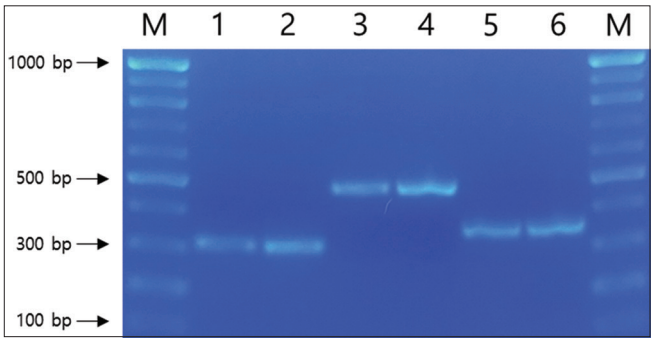

Fig 6. Representative species-specific polymerase chain reaction (PCR) fragments amplified from each species of *L. plantarum*, *L. brevis*, and *L. mesenteroides*. A target fragment (313 bp) was amplified using the primer pair LplF/LplR from *L. plantarum* NCIMB 6105 (lane 1) and another *L. plantarum* isolate (lane 2). A target fragment (482 bp) was amplified using the primer pair LbrF/LbrR from *L. brevis* ATCC 8287 (lane 3) and another *L. brevis* isolate (lane 4). A target fragment (358 bp) was amplified using the primer pair LmeF/LmeR from *L. mesenteroides* ATCC 10830 (lane 5) and another *L. mesenteroides* isolate (lane 6). A 100 bp DNA ladder was used as the DNA molecular weight marker (lane M).

and were identified as *L. brevis*. The extracellular GABA-producing abilities of the GABA-producers identified in this study varied even within the same species when they were grown in the culture broth containing 5% MSG, ranging from 5.8 to 101.7 mM among the 17 GABA-producing *L. plantarum* isolates and from 8.5 to 88.6 mM among the six GABA-producing *L. brevis* isolates.

DISCUSSION

Groups of LAB possessing specific physiological activities have long been extensively utilized in food industries (Wu and Shah, 2017). LAB produce organic

Table 1: γ-aminobutyric acid (GABA)-producing abilities quantitatively determined by high-performance liquid chromatography (HPLC) analysis[a]

Concentration range of GABA produced by each group (mM)	Numbers of GABA-producing isolates within each group		Average concentration of GABA produced within each group (mM)
	Species	Number	
100.0–124.9	*Lactobacillus plantarum*	1	101.7±2.2
75.0–99.9	*Lactobacillus brevis*	1	88.6±2.9
50.0–74.9	*Lactobacillus plantarum* *Lactobacillus brevis*	1 1	70.4±1.9
25.0–49.9	*Lactobacillus plantarum* *Lactobacillus brevis*	3 1	28.5±2.5
1.0–24.9	*Lactobacillus plantarum* *Lactobacillus brevis* *Leuconostoc mesenteroides*	12 3 1	10.7±2.5

[a]Mean±standard deviation among isolates within a group.

Table 2: List of 36 representative species of natural γ-aminobutyric acid (GABA)-producing lactic acid bacterial (LAB) isolates[a]

LAB species	Origin	GABA amount produced	Reference
Bifidobacterium adolescentis DPC6044	Infant feces	1.57 g/L	(Barrett et al., 2014)
Bifidobacterium dentium NCFB 2243^T	Infant feces	6.24 g/L	(Barrett et al., 2014)
Bifidobacterium infantis UCC 35624	Infant feces	2.84 g/L	(Barrett et al., 2014)
Enterococcus avium 9184	Fermented scallop	3.71 g/L	(Yang et al., 2016)
Enterococcus faecalis L3A1K4	Pico cheese	139 mg/L	(Ribeiro et al., 2018)
Enterococcus faecium CFR 3002	Not provided	11 mM	(Gangaraju et al., 2014)
Enterococcus lactis LSP6-3	Flower	100 mg/L	(Phuengjayaem et al., 2021)
Enterococcus raffinosus TCCC11660	Pickled vegetables	30.4 g/L	(Gao et al., 2013)
Lactobacillus brevis SL9-6	Silage	17.32 g/L	(Phuengjayaem et al., 2021)
Lactobacillus buchneri WPZ001	Fermented sausages	129 g/L	(Zhao et al., 2015)
Lactobacillus casei 2749	Cheeses	24.7 mg/kg	(Nejati et al., 2013)
Lactobacillus delbrueckii subsp. *bulgaricus* PR1	Cheeses	63 mg/kg	(Siragusa et al., 2007)
Lactobacillus farciminis D323	Fermented tinfoil barb	493.6 mM	(Thwe et al., 2011)
Lactobacillus fermentum HP3	Thai fermented foods	2.11 g/L	(Woraharn et al., 2016)
Lactobacillus futsaii 32d	Fermented mustard	4.68 mM	(Ly et al., 2019)
Lactobacillus helveticus ND01	Koumiss	165.11 mg/L	(Sun et al., 2009)
Lactobacillus namurensis NH2	Fermented pork	7.34 g/L	(Ratanaburee et al., 2013)
Lactobacillus otakiensis L3C1R1	Pico cheese	659 mg/L	(Ribeiro et al., 2018)
Lactobacillus paracasei NFRI 7415	Fermented fish	302 mM	(Komatsuzaki et al., 2005)
Lactobacillus paraplantarum L2B21R5	Pico cheese	48 mg/L	(Ribeiro et al., 2018)
Lactobacillus pentosus LPC1-1	Flower	36 mg/L	(Phuengjayaem et al., 2021)
Lactobacillus plantarum L2A21R1	Pico cheese	937 mg/L	(Ribeiro et al., 2018)
Lactobacillus rhamnosus GG	Adult feces	1.13 g/L	(Song and Yu, 2018)
Lactobacillus sakei subsp. *sakei* ATCC 15521^T	Sake starter	275.2 mM	This study
Lactobacillus senmaizukei L13^T	Pickles	Not provided	(Hiraga et al., 2008)
Lactobacillus zymae GU240	Kimchi	Not provided	(Lee et al., 2018)
Lactococcus garvieae L3B1M8	Pico cheese	39 mg/L	(Ribeiro et al., 2018)
Lactococcus lactis L-571	Artisanal Mexican Cheese	1.15 g/L	(Santos-Espinosa et al., 2020)
Leuconostoc citreum L3C1E7	Pico cheese	29 mg/L	(Ribeiro et al., 2018)
Leuconostoc mesenteroides KC-I39	Kimchi	24.2 mM	This study
Leuconostoc pseudomesenteroides LCH2-3	Fruit	266 mg/L	(Phuengjayaem et al., 2021)
Pediococcus pentosaceus MN12	Fermented fish sauce	27.9 mM	(Thuy et al., 2021)
Streptococcus salivarius subsp. *thermophilus* Y2	Not provided	6,000 mg/L	(Yang et al., 2008)
Streptococcus thermophilus ST110	Yogurt starter	655 mg/L	(Somkuti et al., 2012)
Weissella cibaria LCH1-6	Fruit	315 mg/L	(Phuengjayaem et al., 2021)
Weissella hellenica SB 105	Fermented fish	7.69 g/L	(Barla et al., 2016)

[a]Only one isolate representative of each GABA-producing LAB species has been listed in this table.

acids, including lactic acid and acetic acid, ethanol, aroma compounds, bacteriocins, exopolysaccharides, and several enzymes. These constituents may positively impact the sensory characteristics of the final products, improving texture, enhancing microbial safety, and extending shelf life.

LAB have been considered as generally-regarded-as-safe (GRAS) organisms in the food fermentation industry since the status of GRAS was introduced by the USA Food and Drug Administration (Wu and Shah, 2017). Besides generating a tremendous self-survival challenge, organic acids (mostly lactic acid) produced by these microorganisms lead to rapid acidification of the raw materials during fermentation (Leroy and De Vuyst, 2004; Wu and Shah, 2017). Therefore, self-protection of the LAB from the acidic environment is pivotal, and various kinds of acid-resistance mechanisms are used by these microorganisms to neutralize the acid stress (Liu et al., 2015). Among these mechanisms, the GAD system is one of the most crucial (Gong et al., 2019).

Various LAB strains with GABA-producing potential have been studied in numerous foods or environments over the past three decades (Wu and Shah, 2017; Gao et al., 2019; Luo et al., 2021). In this study, we focused on the LAB species that have been reported as GABA producers based on GABA yield confirmation, with only one isolate representative of each GABA-producing LAB species listed in Table 2. Table 2 shows GABA-producing LAB strains that have been identified in a variety of fermented foods and belong to 36 different LAB species, namely three *Bifidobacterium* species, five *Enterococcus* species, 18 *Lactobacillus* species, two *Lactococcus* species, three *Leuconostoc* species, one *Pediococcus* species, two *Streptococcus* species, and two *Weissella* species. It is evident that only a fraction of the strains in a reported LAB species can produce GABA from its substrate. In other words, only a fraction of the strains belonging to each of the 36 LAB species may possess the GAD system. This scenario implies that there may be interstrain variation rather than interspecies variation in their GABA-producing potential (Lyu et al., 2018; Zhuang et al., 2018; Gao et al., 2019).

Owing to the human and animal health benefits conferred by the genus *Lactobacillus,* over 200 species of *Lactobacillus* are extensively used in the food industry (Salvetti et al., 2018). Consistent with the recognition of *L. plantarum* and *L. brevis* as the main GABA-producing LAB (Wu and Shah, 2017), in this study, *L. brevis* KC-D13 and KC-I60, and *L. plantarum* KC-L10 and KC-Q1 (which secreted 88.6, 71.4, 69.5, and 101.7 mM of GABA into the culture broth, respectively) represented the highest GABA producers among the 217 kimchi bacterial isolates. The GABA-producing abilities of 36 GABA-producing LAB strains, each representing 36 different species, including the two main GABA-producing LAB species, are listed and compared in Table 2. In addition, 18 (50%) out of the 36 different species are members of *Lactobacillus* (Table 2). Therefore, *Lactobacilli* represents the most dominant group of LAB that possess GABA-producing ability, even in kimchi. In contrast, as mentioned above, the *Leuconostoc* species strains possessing GABA-producing potential have been rarely reported. In this study, numerous kimchi-originated bacterial strains, including LAB, were screened, and a rare GABA-producing LAB species, *L. mesenteroides*, was isolated. Here, we also revealed that the reference strain *L. sakei* subsp. *sakei* ATCC 15521^T can produce 275.2 mM of GABA in the culture broth under the optimized conditions. Although 36 LAB species possessing GABA-producing ability have been identified and characterized, further screening studies are necessary.

CONCLUSIONS

In this study, the culture conditions were optimized for the reference strain *L. sakei* subsp. *sakei* ATCC 15521^T to produce a high GABA concentration. Consequently, the optimal medium and culture conditions for the reference strain were found to be the modified MRS broth with an initial pH of 6.5 containing 4% sucrose, 5% glutamate, and 1% yeast extract at 37 °C for 5 days. Under these conditions, the reference strain produced up to 275.2 mM of GABA in the culture broth and was evaluated to be a strong GABA-producer. Under the optimal conditions, 24 LAB strains (11%) with GABA-producing ability were isolated after 217 kimchi-originated bacterial strains were screened, indicating that only a small proportion of the strains produce GABA in the culture broth. Among the 24 GABA-producers, 17 isolates belonged to *L. plantarum*, six to *L. brevis*, and one to *L. mesenteroides*, respectively. The extracellular GABA-producing abilities of the GABA-producers identified in this study varied even within the same species, ranging from 5.8 to 101.7 mM among the 17 GABA-producing *L. plantarum* isolates and from 8.5 to 88.6 mM among the six GABA-producing *L. brevis* isolates. Besides, the four best GABA producers belonged to *L. plantarum* (two) and *L. brevis* (two), the two most dominant GABA-producing LAB species. In addition, one GABA producer belonged to *L. mesenteroides*, a rare GABA-producing LAB species. Nonetheless, further screening studies are needed to identify and characterize more LAB species possessing GABA-producing ability.

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Conflict of interest

The authors declare no conflicts of interest.

Authors' contributions

Keun-Sung Kim contributed to the conception/design, coordination, and supervision of the research and to the drafting and revision of the manuscript. Jin-Sung Lee performed the analysis and interpretation of the results and revised the manuscript. Both authors critically reviewed and agreed on the final version of the manuscript before submission.

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