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RESEARCH ARTICLE

The Probiotic Characteristics and GABA Production of *Lactobacillus plantarum* K154 Isolated from Kimchi

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Abstract In order to identify strains with a high GABA production ability and glutamate decarboxylase activity, 273 bacteria were isolated from kimchi. K154 produced 154.86 μg/mL of GABA in an MRS broth containing 1% MSG, 170.42 μg/mL of GABA in an MRS broth containing 2% MSG, and 201.78 μg/mL of GABA in an MRS broth containing 3% MSG. K154 was identified as *Lactobacillus plantarum* based on API carbohydrate fermentation pattern testing. The 16s rDNA sequence was investigated in order to determine physiological characteristics. The optimum growth temperature of K154 was 37°C. K154 was more sensitive to novobiocin and bacitracin than to other antibiotics, and exhibited greater resistance to polymyxin B and vancomycin. K154 was comparatively tolerant to bile juice and acid, and displayed resistance to *Escherichia coli, Salmonella* Typhimurium, and *Staphylococcus aureus* at rates of 19.0, 18.9, and 13.6% respectively.

Keywords: *Lactobacillus plantarum*, physiological characteristic, γ-aminobutyric acid, functional product

Introduction

Kimchi is a traditional Korean fermented vegetable food prepared from Chinese cabbage, salt, green onion, red pepper powder, garlic, ginger, and fermented seafood (1). Kimchi is low in calories, carbohydrates and fat, and high in the nutrient components of dietary fiber, vitamins, chlorophyll, β-carotene, and minerals (2,3). Lactic acid bacteria (LAB) are produced in kimchi during the process of lactic acid fermentation. Bacteria isolated from kimchi have been investigated with respect to production and characterization of beneficial enzymes, such as dextransucrase and alcohol/acetaldehyde dehydrogenase (4,5). In addition, some strains of LAB are known to catalyze the α-decarboxylation of L-glutamic acid (MSG) using glutamic acid decarboxylase (GAD), resulting in release of the end products γ-aminobutyric acid (GABA) and CO_2 (6,7).

GABA is a non-protein amino acid that is broadly distributed among microorganisms, plants, and animals in the natural world (8). As the major inhibitory neurotransmitter in the mammalian central nervous system, GABA improves the function of growth hormones, increases the plasma concentration, and promotes protein synthesis in the brain (9). Additionally, GABA exhibits antihypertensive, tranquilizing, anti-diabetic and anti-stress effects in humans (10,11). Furthermore, consumption of GABA-enriched foods can improve memory and learning abilities (12).

This study was performed to investigate the physiological characteristics of *Lactobacillus plantarum* K154 with excellent GABA production selected from 273 lactic acid bacteria isolated from kimchi, and to determine the potential of *L. plantarum* K154 as a starter for functional food products.

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Materials and Methods

Isolation of lactic acid bacteria After collecting 48 different homemade and domestic kimchi products, strain K154 was isolated from kimchi using a modified MRS medium (13). Bacteria were incubated in Lactobacilli MRS broth as a growth medium at 37°C for 18 h.

Measurement of GABA concentrations
Sample preparation: Measurement of GABA concentrations

was carried out as described by Zhang and Bown (14). Bacteria were incubated in an MRS broth containing MSG at 37ºC for 18 h. To measure the concentrations of GABA, 100 μL of a sample was mixed with 400 μL of methanol in an Eppendorf tube, which was then thoroughly dried using methanol in a water bath at 70ºC. After drying in the water bath, 1 mL of 70 mM $LaCl_3$ was added, followed by mixing with vortex mixer. After centrifugation for 5 min at 10,000×*g* in a Sorvall Biofuge pico centrifuge (Kendro, Hanau, Germany), 700 μL of the resulting supernatant was mixed with 160 μL of 0.1M KOH in an Eppendorf tube and agitated with vortex mixer for 5 min. Then, 200 μL of the supernatant (centrifuged in a Sorvall Biofuge pico centrifuge at 10,000×*g* for 5 min) was diluted 5× using a 0.5 M potassium pyrophosphate buffer, and 550 μL of the diluted solution was then put in a cuvette.

***Standard preparation and measurement of GABA*:** Each volume of 1 mM GABA, 0.5 M $K_4P_2O_7$, 4 mM NADP, and 2.0 units/mL Gabase in Table 1 were mixed with vortex mixer and the absorbance value was measured at 340 nm using an Optizen UV/visible spectrophotometer (Mecasys, Daejeon, Korea) (initial A). After measurement, 50 μL of 20 mM α-ketoglutaric acid (KG) was added to each cuvette and left for an hour at room temperature. Then, absorbance values were measured again at 340 nm using an UV/visible spectrophotometer (final A). The concentrations of GABA were determined as:

$$\text{GABA } (\mu\text{g/mL}) = \left\{ \left(\frac{\text{Final A} - \text{Initial A}}{\text{Standard curve}} \times \frac{100}{70} \times \frac{86}{55} \right) \right\} \times 100$$

Identification of strain K154 Bacteria of strain K154 were cultivated twice in MRS broth for 18 h at 37ºC. Bergey's Manual of Systematic Bacteriology by Buchanan and Gibbons (15) was used to examine bacterial morphological and physiological properties. Bacteria were tested using Gram staining and microscopic observation with an Olympus BH2 microscope (Olympus, Tokyo, Japan) after cultivation on tryptic soy agar for 24 h at 37ºC. Spore forming, aerobic and anaerobic growth, catalase reaction, growth at 15 and 45ºC, gas formation from glucose, and ammonia production from arginine were also investigated. K154 was identified using API 50 CH strips (Api bio-Mérieux, Marcy l'Etoile, France) with API 50 CHL medium (Api bio-Mérieux). The API 50 CHL kit test (Api bio-Mérieux) was used to analyze fermentation of 49 sugars following the instructions of the manufacturer, and results were read after 48 h of incubation at 37ºC. Identification was based on use of the software package API LAB (Api bio-Mérieux) provided by the manufacturer. K154 was also identified by using the 16S rDNA sequencing method (16). Bacterial chromosomal DNA was separated using a Genomic DNA prep kit (SolGent, Daejeon, Korea). DNA extracts were used for polymerase chain reaction (PCR) with the universal primers [27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-GGT TAC CTT GTT ACG ACT T-3')]. PCR was performed using a programmable therma cycler (Solgent EF-Taq) with 1 cycle of denaturation at 95ºC for 15 min; 30 cycles at 95ºC for 20 s, at 50ºC for 40 s, and at 72ºC for 90 s. Final extension was carried out at 72ºC for 5 min. The PCR product was purified using a PCR purification kit (SolGent) and used for sequencing with an ABI 3730XL DNA analyzer (Applied Biosystems, Foster City, CA, USA).

Growth of bacteria The number of viable *L. plantarum* K154 cells was determined based on a serial 10× dilution in 0.1% peptone water. First, 10 μL (9.6×10^5 CFU/mL) of *L. plantarum* K154 was inoculated into 150 mL of MRS broth then the cultures were incubated at 3 h intervals for a total of 24 h at 30, 34, 37, and 40ºC, respectively. All pour plates were incubated aerobically at 37ºC for 48 h using BCP plate count agar. GABA concentrations of *L. plantarum* K154, depending on the incubation temperature and time, were measured. *L. plantarum* K154 was inoculated (1%) into 10 mL of sterile MRS broth containing 2% MSG, then the culture was incubated at 3 h intervals for a total of 24 h at 30, 34, 37 and 40ºC, respectively.

Antibiotic tolerance *L. plantarum* K154 was grown at 37ºC for 18 h in MRS broth and inoculated (1%, v/v) into Tryptic soy broth (Difco, Detroit, MI, USA) supplemented with the antibiotics amikacin, gentamicin, kanamycin, neomycin, streptomycin, penicillin-G, methicillin, oxacillin, ampicillin, bacitracin, rifampicin, novobiocin, lincomycin, polymyxin B, chloramphenicol, and vancomycin, all obtained from Sigma Chemical Co. (St. Louis, MO, USA) at various concentrations (the maximum concentrations of antibiotics

Table 1. Standard preparation and measurement of GABA concentrations

GABA (mM)	0	0.005	0.01	0.02	0.05	0.1	Sample	Blank
① 1 mM GABA	0 (mL)	0.005	0.01	0.02	0.05	0.1	0.55	0
② 0.5 M $K_2P_2O_7$	1.75	0.745	0.74	0.73	0.7	0.65	0.2	0.75
③ 4 mM NADP	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
④ 2.0 units Gabase/mL	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
⑤ 20 mM α-KG	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05

were as follows: amikacin=320 μg/mL, gentamicin=1280 μg/mL, kanamycin=3200 μg/mL, neomycin=3200 unit/mL, streptomycin=1600 unit/mL, penicillin-G=160 unit/mL, methicillin=320 μg/mL, oxacillin=240 μg/mL, ampicillin=1280 μg/mL, bacitracin=120 unit/mL, rifampicin=120 μg/mL, novobiocin=120 μg/mL, lincomycin=800 unit/mL, polymyxin B=2,400 unit/mL, chloramphenicol=320 μg/mL, and vancomycin 3,200 μg/mL) in a 2× dilution step. The minimal inhibitory concentration (MIC) was determined by noting the concentration at which bacteria ceased growth after incubation at 37°C for 48 h.

Enzyme activity An API ZYM kit (Apibio Merieux) was used to determine the enzyme activity. *L. plantarum* K154 was grown at 37°C for 18 h in MRS broth. Sediment from a centrifuged (centrifuged for 15 min at 1,500×*g* in a Sigma 3-16K centrifuge; Sigma) broth culture was used to prepare a suspension of 10^5-10^6 CFU/mL. 65 μL of the suspension were added to each cupule of the API-ZYM strip and incubated for 5 h at 37°C. After incubation, 1 drop each of reagent ZYM A and ZYM B were added to each cupule. Inclusion of a surface active agent (ZYM A reagent) in the cupules facilitated solubilization of the ZYM B reagent in the medium. The color was allowed to develop for at least 5 min, and values ranging from 0-5 corresponding to developed colors were assigned as 0=a negative reaction, 1=5 nmol, 2=10 nmol, 3=20 nmol, 4=30 nmol, and 5=40 nmol or more. The approximate number of free nmol of hydrolyzed substrate was determined based on the color strength.

Bile tolerance The bile tolerance was tested as described by Gilliland and Walker (17). *L. plantarum* K154 was grown at 37°C for 18 h in MRS broth. *L. plantarum* K154 cultures (1%) were inoculated into sterilized MRS broth containing 0.05% L-cysteine with and without 0.3% ox gall, then cultures were anaerobically incubated at 1 h intervals up to 7 h at 37°C. Intermittent plating onto petri dishes followed after 1, 2, 3, 4, 5, 6, and 7 h in order to compare growth potentials in the presence of bile. The cultures were taken at each time after resuspension of samples, followed by serial 10× dilutions in 0.1% peptone water. All pour plates were incubated anaerobically at 37°C for 48 h using BCP plate count agar.

pH tolerance The pH tolerance was tested as described by Clark *et al*. (18). Solutions of 37% HCl in double-distilled water were adjusted to pH levels of 2.0, 3.0, and 4.0 by diluting with double-distilled water. Sterile double-distilled water (pH 6.4) served as a control. An amount of 10 mL of each pH solution was transferred to sterile test tubes. Then 1 mL of a stock culture containing approximately 10^9 CFU/mL of *L. plantarum* K154, using MRS agar containing 0.05% cysteine, was then transferred into each of the 4 pH solutions, which were then incubated anaerobically at 37°C. Intermittent plating onto petri dishes followed after 1, 2, and 3 h to stimulate the survival of *L. plantarum* K154 cells under pH conditions common to the human stomach. Samples of pH solutions were taken at 1, 2, and 3 h after resuspension of samples, followed by serial 10× dilutions in 0.1% peptone water. All pour plates were then incubated anaerobically at 37°C for 48 h using BCP plate count agar.

Antimicrobial activity The antimicrobial activity was determined as described by Gilliland and Speck (19). *Escherichia coli* KFRI 174, *Salmonella* Typhimurium KFRI 250, and *Staphylococcus aureus* KFRI 219 were obtained from the culture collection of the Korea Food Research Institute (Sungnam, Korea). *E. coli* cells were enumerated on EMB agar, *S.* Typhimurium on Bismuth sulfite agar, and *S. aureus* on Baird parker agar. All plates were incubated for 48 h at 37°C. Pathogen cultures (control) and associative cultures with *L. plantarum* K154 and each pathogen were incubated for 6 h at 37°C. At the end of the incubation time, samples were removed and placed in an ice bath until analysis. The number of pathogenic CFUs/mL was determined using the appropriate selective medium, and the pH of the samples was also measured to show influence of acid on pathogen growth. Percentages of inhibition were determined as:

$$\text{Inhibition (\%)} = \frac{\text{(CFU/mL in control)} - \text{(CFU/mL in associative culture)}}{\text{(CFU/mL in control)}} \times 100$$

Statistical analysis Results were expressed as a mean± standard deviation (SD). Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS; SPSS Inc., Chicago, IL, USA). Differences were analyzed using a one-way analysis of variance (ANOVA) with Duncan's multiple range test. Values of $p<0.05$ were considered statistically significant.

Results and Discussion

Isolation of lactic acid bacteria A total of 48 different home-made and domestic kimchi products were collected, and a total of 273 lactic acid bacterial strains were isolated from kimchi using a modified MRS medium.

Selection of lactic acid bacteria producing GABA After incubation in an MRS broth containing 2% MSG at 37°C for 18 h, 75 bacterial strains that produced over 50 μg/mL of GABA each were identified based on repeated triplicate measurements. A total of 7 strains that produced over 90

Table 2. pH and GABA contents after incubation at 37°C for 18 h in an MRS broth containing added monosodium glutamate and 1% lactic acid bacteria

Strains	1% MSG		2% MSG		3% MSG	
	GABA (μg/mL)	pH	GABA (μg/mL)	pH	GABA (μg/mL)	pH
K4	70.39±8.12[de1)]	4.09	106.13±2.82[c]	4.16	150.16±4.69[d]	4.37
K66	114.19±7.18[b]	4.04	122.46±4.89[b]	4.23	183.01±4.69[bc]	4.43
K154	154.86±8.12[a]	4.04	170.42±2.86[a]	4.19	201.78±0.00[ab]	4.42
K174	32.84±0.00[f]	4.42	92.65±4.96[d]	4.30	168.93±5.41[cd]	4.60
K177	78.21±2.70[d]	4.13	104.24±4.96[c]	4.45	209.61±27.09[a]	4.47
K200	98.54±0.00[c]	4.04	92.65±1.03[e]	4.21	181.45±2.70[c]	4.42
K256	62.57±7.16[e]	4.06	110.89±2.86[c]	4.25	118.88±5.41[e]	4.48

[1)]All values are reported as a mean±standard deviation (SD) of 3 replicates; [a-f]mean values with a different superscript within the same proportion of MSG were significantly different (p<0.05).

μg/mL of GABA were then selected and incubated in an MRS broth containing 1, 2, and 3% MSG at 37°C for 18 h (Table 2). Strain K154 produced 154.86 μg/mL of GABA in an MRS broth containing 1% MSG, 170.42 μg/mL of GABA in an MRS broth containing 2% MSG, and 201.78 μg/mL of GABA in an MRS broth containing 3% MSG.

According to a report of Lim *et al.* (20), almost all commercial bacterial strains produced 5-30 μg/mL of GABA in 10% skimmed milk containing 1% MSG. Tung *et al*. (21) reported that *L. plantarum* KTU 102 isolated from homemade Korean-style cabbage pickles produced 33 μg/mL of GABA in 8% skimmed milk containing 0.6% MSG after fermentation for 24 h. Thus, K154 was superior to other strains for production of GABA.

Identification and DNA sequencing of strain K154 Physiological and biochemical testing was conducted to identify K154. The bacterium was non-spore, rod-type, hetero fermentative, and Gram positive and was negative for catalase and motility. Furthermore, K154 could grow in the temperature range of 15 and 45°C. K154 did not produce gas and ammonia from glucose and arginine and was, thus, identified as a *Lactobacillus*. API 50 CHL testing allowed specific identification as *L. plantarum*. Identification based on the 16s rDNA sequencing method using PCR and a universal primer resulted in identification as *L. plantarum* with a probability of 99% (data not shown). Based on the results of a previous study (22), the bacterium was named *L. plantarum* K154.

Bacterial growth The number of viable *L. plantarum* K154 cells was determined based on a serial 10× dilution in 0.1% peptone water. An amount of 10 μL (9.6×10^5 CFU/mL) of *L. plantarum* K154 was inoculated into 150 mL of MRS broth, then the culture was incubated at 30, 34, 37, and 40°C for 24 h with checking every 3 h. The highest growth rate was observed at 37°C and 13 h was required to reach a pH of 4.4 under these conditions (Fig. 1). The concentration of GABA increased during incubation

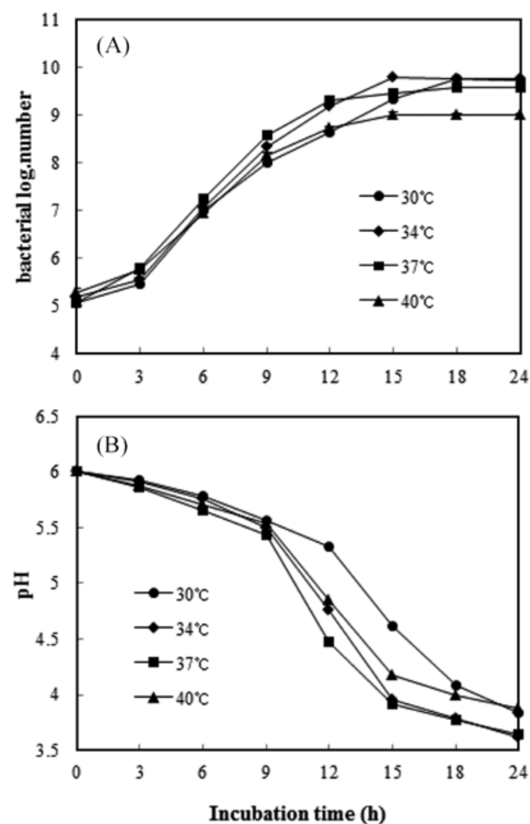

Fig. 1. Growth curve (A), and pH changes (B) of *Lactobacillus plantarum* K154 in MRS broth at different temperatures. All values are reported as a mean±SD of 3 replicates.

for 24 h, and the highest concentration value was also observed at 37°C (Fig. 2).

Antibiotic tolerance It is essential for a probiotic strain to survive in the presence of antibiotics. If probiotics are eliminated due to ingestion of antibiotics present in medicines and foods, prebiotics cannot function (23). The tolerance of *L. plantarum* K154 against 16 antibiotics is shown in Table 3. *L. plantarum* K154 was most sensitive to novobiocin and bacitracin and exhibited greatest resistance

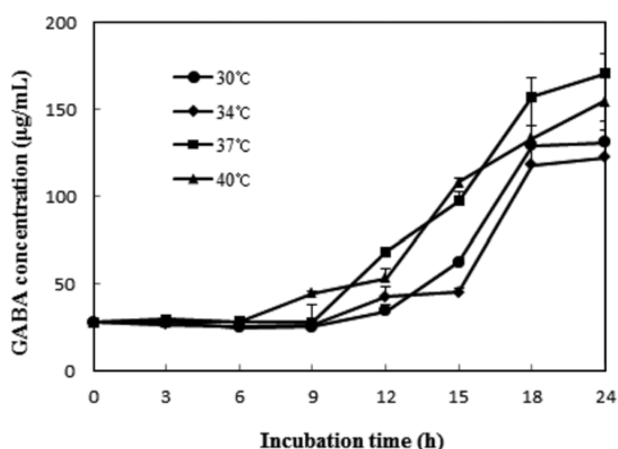

Fig. 2. GABA concentration of *L. plantarum* K154 in MRS broth containing 2% MSG at different temperatures. All values are reported a mean±SD of 3 replicates.

Table 3. Antibiotic susceptibility of *Lactobacillus plantarum* K154

Antimicrobial agents	Minimal inhibitory concentrations (μg/mL)
Aminoglycosides	
Amikacin	160±0[1)]
Gentamycin	80±0
Kanamycin	200±0
Neomycin*[2)]	200±0
Streptomycin	200±0
β-Lactams	
Penicillin-G*	160±0
Methicillin	320±0
Oxacillin	60±0
Ampicillin	160±0
Gram-positive spectrum	
Bacitracin*	30±0
Rifampicin	480±0
Novobiocin	7.5±0
Lincomycin*	50±0
Gram-negative spectrum	
Polymyxin B*	2400±0
Broad spectrum	
Chloramphenicol	40±0
Vancomycin	3200±0

[1)]All values are reported as a mean±SD of 3 replicates.
[2)]*, units/mL

to polymyxin B and vancomycin. Moon *et al.* (24) reported that 10 strains isolated from 93 taxa of *Bifidobacterium* and *Lactobacillus* showed greater sensitivity to chloramphenicol and rifampicin and exhibited greater resistance to vancomycin and the aminoglycoside group than to other antibiotics According to a review by Mathur and Singh (25), different strains exhibit different degrees of antibiotic resistance. However, because *L. plantarum* K154 showed a comparatively high antibiotic resistance value, the strain presents a potential hazard for dissemination of an antibiotic resistance gene. Cloning and expression of the glutamate decarboxylase gene from *L. plantarum* K154 could be a method of resolution to this problem. Kim *et al.* (26) reported that a full-length glutamate decarboxylase gene from *L. brevis* BH2, which was found to produce a large amount of GABA and was also isolated from kimchi, was cloned using rapid amplification of cDNA ends in PCR and was expressed in *E. coli*.

Table 4. Enzyme patterns of *L. plantarum* K154[1)]

Enzyme	*L. plantarum* K154
Alkaline phosphatase	0
Esterase (C4)	0
Esterase Lipase (C8)	0
Lipase (C14)	0
Leucine arylamidase	5
Valine arylamidase	4
Cystinearylamidase	1
Trypsin	0
α-Chymotrypsin	0
Acid phosphatase	1
Naphtol-AS-BI-phosphohydrolase	3
α-Galactosidase	0
β-Galactosidase	5
β-Glucuronidase	0
α-Glucosidase	4
β-Glucosidase	5
N-Acetyl-β-glucosaminidase	3
α-Mannosidase	0
α-Fucosidase	0

[1)]A value ranging from 0 to 2 was assigned to the standard color, where 0 represented a no reaction, and 5 represented a reaction of maximum intensity. Values 1 through 4 represented intermediate reactions depending on the level of intensity. The approximate activity was estimated based on the color strength; 1 corresponded to liberation of 5 nmol, 2 corresponded to 10 nmol, 3 corresponded to 20 nmol, 4 corresponded to 30 nmol, and 5 corresponded to 40 nmol or more.

Enzyme activity Enzyme activity is important for selection of probiotics because microorganisms can produce carcinogenic enzymes, such as β-glucuronidase (27). However, *L. plantarum* K154 did not produce β-glucuronidase, but did produce leucine arylamidase, valine arylamidase, crystine arylamidase, acid phosphatase, naphtol-AS-BIphospho-hydrolase, β-galactosidase, α-glucosidase, β-galactosidase, and N-acetyl-β-glucosaminidase (Table 4). The leucine arylamidase, β-galactosidase, and β-glucosidase activities of *L. plantarum* K154 were 5 value which is a maximum intensity. *L. plantarum* PH04 isolated from infant feces (28) and 8 amylolytic lactic acid bacteria isolated from Nigerian fermented products (29) were comparable in β-glucuronidase activities with *L. plantarum* K154. Enzyme profiles of *L. plantarum* K154 were similar to profiles of *L.*

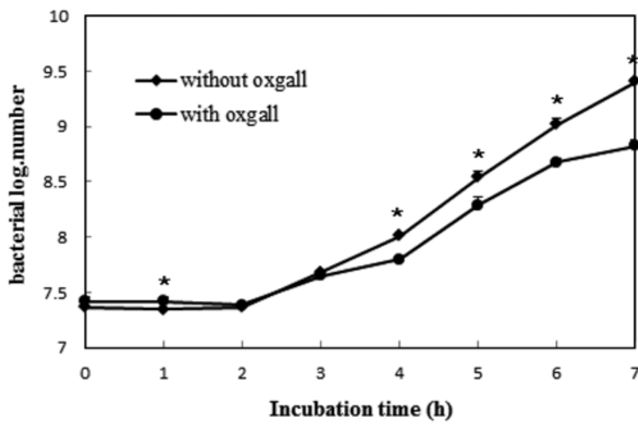

Fig. 3. Growth of *L. plantarum* K154 in MRS broth containing 0.05% L-cysteine with and without 0.3% ox gall. All values are reported as a mean±SD of 3 replicates; *$p<0.05$ between with ox gall and without ox gall (*t*-test)

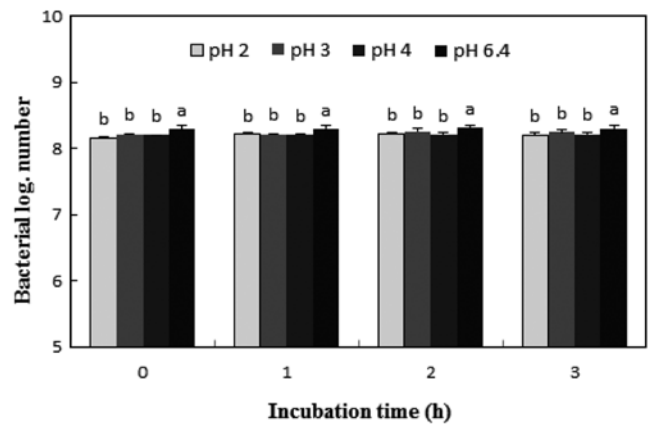

Fig. 4. Survival of *L. plantarum* K154 after 3 h in an HCl solution (pH 2.0, 3.0, 4.0, and 6.4). All values are reported as a mean±SD of 3 replicates; [a-b]mean values with different superscripts within the same time were significantly different ($p<0.05$).

plantarum C5 isolated from *fufu*, a Nigerian fermented food, with the exception of crystine arylamidase (29).

Bile tolerance Bile salt disorganizes the structure of cell membranes and is, therefore, toxic to living cells. Bile tolerance is a vital requirement for lactic acid bacteria for survival and growth in the small intestine (30). Gilliland *et al.* (31) reported that a 0.3% rate of bile salt is considered the critical index for lactic acid bacteria in the human gastrointestinal tract.

Bacterial growth curves in MRS broth and MRS broth containing 0.3% bile are shown in Fig. 3. The log value of the bacterial population after 7 h of incubation without 0.3% ox gall was 9.4 log CFU/mL and was 8.8 log CFU/mL with addition of 0.3% bile. Therefore, the survival rate of *L. plantarum* K154 in an MRS broth containing 0.3% bile was 93.6%. This rate was high in comparison with a report of Lee *et al*. (32) on the bile tolerance of *L. johnsonii* IDCC 9203 isolated from infant feces. The survival rate of *L. johnsonii* IDCC 9203 after 3 h of incubation in an MRS broth containing 0.3% bile salt was 57%. Consequently, *L. plantarum* K154 has a strong bile tolerance.

Acid tolerance To benefit human health, a probiotic must have both bile and acid tolerances (33). The pH value of the stomach will rise to pH 3 if food is present, although the pH of secreted HCl in the stomach is 0.9 (34). Therefore, it is necessary for probiotics to survive at a pH value lower than 3 for probiotics to reach the small intestine through the stomach (35). The pH tolerance of *L. plantarum* K154 is shown in Fig. 4. The log value of the bacterial population after 3 h of incubation in a control solution with the pH value adjusted to pH 6.4 was 8.3, and it was 8.2 in an acidic solution with the pH adjusted to 2.0. Therefore, the survival rate of *L. plantarum* K154 in a solution of pH 2.0 was 98.8%. *L. plantarum* K154 exhibited a probiotic ability because a comparatively high percentage of cells survived under highly acidic conditions of pH 2.0.

Antimicrobial activity Daeshcel (36) and Havinnar *et al*. (37) reported that the antimicrobial activity of lactic acid bacteria can be attributed to a low pH, reduction of the redox potential, competitive consumption of nutrients, generation of hydrogen peroxide, and the bactericidal activities of organic acids and bacteriocins produced by lactic acid bacteria. The antimicrobial activity of *L. plantarum* K154 against pathogenic strains is shown in Table 5. *L. plantarum* K154 showed resistance against *E. coli*, *S.* Typhimurium, and *S. aureus* at rates of 19.0, 18.9, and 13.6%, respectively. The pH value of pathogens after

Table 5. Inhibition of pathogens by *L. plantarum* K154 in MRS broth[1)]

Pathogens	Pathogens[a2)]		*L. plantarum* K154[a]+Pathogens		Inhibition (%)
	CFU/mL	pH	CFU/mL	pH	
Escherichia coli	$5.8\pm0.6\times10^{4}$[3)]	6.6	$4.7\pm0.3\times10^4$	5.5	19.0±3.6
Salmonella Typhimurium	$3.7\pm0.7\times10^5$	6.5	$3.0\pm0.2\times10^5$	5.6	18.9±5.7
Staphylococcus aureus	$2.2\pm0.5\times10^6$	6.4	$1.9\pm0.2\times10^6$	5.6	13.6±12.6

[1)]Initial count of *L. plantarum* K154: $2.4\pm0.5\times10^6$ CFU/mL
[2)a]Determined after 6 h of incubation at 37°C
[3)]All values are reported as a mean±SD of 3 replicates.

incubation for 7 h was in the range of 6.6-6.7, but the pH value of a mixed culture of *L. plantarum* K154 and pathogens was 5.5-5.6. *L. plantarum* K154 did not exhibit a high antimicrobial activity.

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Disclosure The authors declare no conflict of interest.

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