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ARTICLE

Physiological Characteristics and GABA Production of *Lactobacillus plantarum* K255 Isolated from Kimchi

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Abstract

As a major inhibitory neurotransmitter of the central nervous system in animals, γ-aminobutyric acid (GABA) has several physiological functions, such as anti-hypertensive, diuretic, tranquilizer and anti-stress effects in human. In order to determine strains with high GABA producing ability and glutamate decarboxylase (GAD) activity, 273 bacteria were isolated from various types of Kimchi. Strain K255 contained 386.37 μg/mL of GABA in MRS broth containing 1% MSG, 600.63 μg/mL of GABA in MRS broth containing 2% MSG and 821.24 μg/mL of GABA in MRS broth containing 3% MSG. It showed that K255 had the highest GABA production ability compared to other commercial lactic acid bacteria. K255 was identified as *Lactobacillus plantarum* based on its API carbohydrate fermentation pattern and 16S rDNA sequence. K255 was investigated for its physiological characteristics. The optimum growth temperature of K255 was 37°C and cultures took 13 h to reach the pH 4.4. K255 showed more sensitive to bacitracin in a comparison of fifteen different antibiotics, and showed most resistance to kanamycin and vancomycin. Moreover, it was comparatively tolerant to bile juice and acid and displayed resistance to *Escherichia coli, Salmonella* Typhimurium*, Staphylococcus aureus,* with rates of 30.8%, 29.7%, and 23.4% respectively. These results demonstrate that K255 could be an excellent strain for the production of functional products.

Key words: *Lactobacillus plantarum*, physiological characteristics, γ-aminobutyric acid, functional product

Introduction

Kimchi is a Korean typical and traditional fermented food. Also, it has been in the top five healthy food in the world. Kimchi has abundant nutrient such as vitamin A, B and C, minerals, high numbers of lactic acid bacteria (LAB) (Kim *et al.*, 2007). Especially, LAB number could be extended during lactic acid fermentation in Kimchi. In Kimchi fermentation, the major anaerobic bacteria are *Lactobacillus sakei, Leuconostoc mesenteroides, Lactobacillus brevis*, *Lactobacillus plantarum*, *Lactobacillus buchneri*, *Pediococcus pentasaceus,* and *Enterococcus faecalis* (Cheigh and Park, 1994; Cho *et al.*, 2006). Kimchi has various tastes and functionalities by LAB which release biologically active substances such as organic acid including lactic acid and γ-aminobutyric acid (GABA) (Hur *et al.*, 2006; Kim *et al.*, 2002; Oh *et al.*, 2008).

GABA is a non-protein amino acid that is broadly distributed in nature (Manyam *et al.*, 1981), and exists in brain and spinal cord of mammal. As a major inhibitory neurotransmitter of the central nervous system in animal, GABA has several physiological functions such as anti-hypertensive, diuretic, tranqulizer and anti-stress effects in human (Jakobs *et al.*, 1993; Vaiva *et al.*, 2004; Wong *et al.*, 2003). Owing to numerous physiological functions of GABA, development of functional products containing GABA at high concentration has been actively performed (Saikusa *et al.*, 1994; Tsushida and Murai, 1987).

GABA is mainly produced from irreversible α-decarboxylation of L-glutamic acid (MSG), catalyzed by glutamic acid decarboxylase (GAD), which has been found in LAB (Ueno, 2000). It is expected that the ability to GABA production depends on the degree of activation of GAD which LAB has (Oh and Yu, 2011).

The aim of this study was to isolate and screen specific LAB which has great ability of GABA production from Kimchi for applying it to functional food products.

Materials and Methods

Isolation of lactic acid bacteria

Forty eight types of home-made and domestic Kimchi

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products were collected. Strain K255 was isolated from Kimchi in modified MRS medium (Lim *et al.,* 2011). The strain was incubated in *Lactobacilli* MRS broth as the 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 (1997). The strain was incubated in MRS broth containing L-glutamic acid (MSG) at 37°C for 18 h. To measure the GABA concentrations, 100 μL of the sample was mixed with 400 μL of methanol at eppendorf tube, which was then thoroughly dried methanol at water bath set 70°C. After methanol dried, 1 mL of 70 mM $LaCl_3$ was added and mixed well. After centrifugation (10,000 rpm/5 min), 700 μL of the supernatant was mixed with 160 μL of 0.1 M KOH in eppendorf tube and was agitated for 5 min. 200 μL of the supernatant which was centrifuged (10,000 rpm/5 min) was diluted 5-fold with 0.5 M potassium pyrophosphate buffer. 550 μL of diluted solution was put in a cuvette.

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 $K_4P_2O_7$	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

①, ②, ③ and ④ were mixed and measured its absorbance at 340 nm (initial A). After measurement, 50 μL of 20 mM α-KG was added each cuvette and stayed for an hour at room temperature. And then measured final absorbance at 340 nm (final A). Concentrations of GABA were determined using the following formula:

$$\text{GABA}(\mu\text{g/mL}) = \left(\frac{\text{Final A} - \text{Initial A}}{\text{Standard curve}} \times \frac{100}{70} \times \frac{86}{55}\right) \times 5 \times 10$$

Identification of strain K255

The properties of the strain K255 was investigated by testing the Gram staining and microscopic observation after cultivation on tryptic soy agar for 24 h at 37°C. Bergey's Manual of Systematic Bacteriology by Buchanan and Gibbons (1974) was used to examine the morphological and physiological properties of the isolated strains. K255 strain was identified by using the 16S rDNA sequencing method. Chromosomal DNA of isolated strain was separated by using SolGent Genomic DNA prep kit (SolGent, Korea). The 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 were carried out in a programmable thermo cycler (Solgent EF-Taq, Korea), with the following steps: one cycle of denaturation at 95°C for 15 min, 30 cycles of 95°C for 20 s, 50°C for 40 s and 72°C for 90 s were performed. Final extension was carried out at 72°C for 5 min. PCR product purified by using SolGent PCR purification kit (SolGent, Korea) was used for sequencing with ABI 3730XL DNA analyzer (Applied Biosystems, USA).

Growth of strain

The number of viable *L. plantarum* K255 was determined by serial 10-fold dilution in 0.1% peptone water. *L. plantarum* K255 was inoculated 50 μL (9.6×10^5 CFU/mL) into 150 mL of MRS broth. And then culture was incubated to 3 h interval for 24 h at 34°C, 37°C and 40°C. All pour plates were incubated aerobically at 37°C for 48 h using BCP plate count agar.

Antibiotic tolerance

L. plantarum K255 was grown at 37°C for 18 h in MRS broth and inoculated (1%, v/v) in Tryptic soy broth (Difco, USA) supplemented with antibiotics (amikacin, gentamicin, kanamycin, neomycin, streptomycin, penicillin-G, methicillin, oxacillin, ampicillin, bacitracin, rifampicin, novobiocin, lincomycin, polymyxin B, and chloramphenico; Sigma) at various concentrations of 2-fold dilution step. Minimal inhibitory concentration (MIC) was determined by the checking the moment of the strain stop growing after incubation at 37°C for 48 h.

Enzyme activity

The API ZYM kit (bioMerieux, France) was used to study enzyme activity. *L. plantarum* K255 was grown at 37°C for 18 h on MRS broth. Sediment from centrifuged broth culture was used to prepare the suspension at 10^5-10^6 CFU/mL. After inoculation, cultures were incubated for 5 h at 37°C. Placing a surface active agent (ZYM A

reagent) in the cupules facilitated solubilization of the ZYM B reagent in the medium. Color was allowed to develop for at least 5 min, and values from 0-5 corresponding to the colors developed, were assigned. The approximate number of free nmol hydrolyzed substrate was determined based on the color strength: 0, negative reaction; 1, 5 nmol; 2, 10 nmol; 3, 20 nmol; 4, 30 nmol; 5, 40 or higher.

Bile tolerance

Bile tolerance was carried out as described by Gilliland and Walker (1990). *L. plantarum* K255 was grown at 37°C for 18 h on the MRS broth. Culture of *L. plantarum* K255 was compared for their ability to grow in the presence of bile by individual inoculation (1%) into sterile MRS broth containing 0.05% L-cysteine with and without 0.3% oxgall. After plating for initial counts, mixtures were incubated anaerobically for 7 h at 37°C. *L. plantarum* K255 was then enumerated again to test the survival rates after 7 h incubation. All pour plates were incubated anaerobically for 48 h at 37°C.

pH tolerance

pH tolerance was carried out as described by Clark *et al*. (1993). Solutions of 37% HCl in double-distilled water were adjusted to pH level to 2.0, 3.0, and 4.0. Sterile double-distilled water (pH 6.4) served as the control. 10 mL of each pH solution were transferred into sterile test tubes. 1 mL of stock culture containing approximately 10^9 CFU/mL of *L. plantarum* K255 using MRS agar containing 0.05% cysteine was then transferred into each of the four pH solutions. The pH solutions containing *L. plantarum* K255 were then incubated anaerobically at 37°C, followed by intermittent plating after 1, 2, and 3 h to stimulate survival of *L. plantarum* K255 under pH conditions common to the human stomach. Samples from the pH solution were taken at 1, 2, and 3 h after the samples was suspended again and subjected to serial dilutions. About 100 μL above sample solution was spread onto the surface of BCP plate count agar plates and incubated anaerobically at 37°C for 48 h.

Antimicrobial activity

Antimicrobial activity test was carried out as described by Gilliland and Speck (1977). *Escherichia coli* KFRI 174, *Salmonella* Typhimurium KFRI 250, *Staphylococcus aureus* KFRI 219 were from the culture collection of the Korea Food Research Institute. *Escherichia coli* was enumerated on EMB agar, *Salmonella* Typhimurium on Bismuth sulfite agar, and *Staphylococcus aureus* on Baird parker agar. All plate were incubated 48 h at 37°C. The control and associative culture were incubated for 6 h in a water bath at 37°C. At the end of the incubation time, samples were removed and placed in an ice bath until analyzed. The number of CFU of pathogens per mL was determined using the appropriate selective medium and in some experiments the pH of the samples was also measured. Percentages of inhibition were determined by the following formula:

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

Statistical analysis

The results were expressed as the mean±standard deviation (SD). The statistical analysis was performed with the Statistical Package for Social Sciences (SPSS, SPSS Inc., Chicago, IL, USA). The significance of the differences was analyzed by using one-way analysis of variance (ANOVA) with Duncan's multiple range tests. Value of $p<0.05$ were considered as statistically significant.

Results and Discussion

Isolation of lactic acid bacteria

Forty eight kinds of home-made and domestic Kimchi products were collected. Total 273 strains were isolated as lactic acid bacteria from Kimchi in the modified MRS medium.

Selection of lactic acid bacteria producing GABA

After incubation of MRS broth containing 2% MSG at 37°C for 18 h, Seventy five strains over 50 μg/mL of GABA containing were selected from 273 strains by the measurement of GABA concentrations. GABA concentrations of Seventy five strains were repeatedly measured in triplicate. Nine strains over 90 μg/mL of GABA containing were selected and incubated in MRS broth containing 1%, 2% and 3% MSG at 37°C for 18 h (Table 1). As a result, K255 was selected which contained 386.37 μg/mL of GABA in MRS broth containing 1% MSG, 600.63 μg/mL of GABA in MRS broth containing 2% MSG and 821.24 μg/mL of GABA in MRS broth containing 3% MSG. It was higher level compared to the commercial strains which producing 5-30 μg/g D.W of GABA. Bae *et al.* (2009) has reported that *Lactobacillus* sp. OPK2-59 strain isolated from Kimchi produced 390

Table 1. pH and GABA content produced after incubation at 37°C for 18 hr in the MRS broth 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
K13	86.70±12.41^{d}	4.03	117.56±8.48^{def}	4.18	187.71±4.69^{cd}	4.4
K61	64.13±7.16^{e}	4.07	107.76±12.66^{f}	4.18	150.16±16.25^{e}	4.42
K63	101.67±5.41^{c}	4.05	140.42±2.82^{c}	4.22	192.40±4.69^{c}	4.37
K67	82.90±2.70^{d}	4.05	128.99±7.48^{cd}	4.43	154.86±4.69^{e}	4.47
K71	89.16±4.69^{d}	4.01	109.40±2.82^{ef}	4.14	186.14±2.70^{c}	4.44
K74	134.52±10.83^{b}	4.13	212.27±7.48^{b}	4.30	234.63±24.38^{b}	4.43
K170	76.64±2.70^{d}	4.04	94.31±4.96^{g}	4.18	153.29±5.41^{e}	4.44
K173	62.57±2.70^{e}	4.15	120.78±7.58^{de}	4.25	159.49±2.70^{de}	4.55
K255	386.37±15.08^{a}	4.13	600.63±4.96^{a}	4.24	821.24±12.41^{a}	4.49

All values are mean±standard deviation of three replicates.
$^{a-g}$Means values with different superscript within same proportion of MSG are significantly different (p<0.05).

μg/g GABA in MRS media containing 1% MSG. K255 strain was similar in GABA production ability to the *Lactobacillus* sp. OPK2-59 strain.

Identification and DNA sequencing of selected strain K255

The physiological and biochemical test was done to determine the genus and species of selected K255 strain. Selected K255 strain was non-spore, rod type, hetero fermentative, gram positive bacteria and exhibited negative properties on catalase and motility. Also, K255 strain can grow at 15°C and 45°C. It does not produce gas and ammonia from glucose and arginine so that it was identified as a genus Lactobacillus (Table 2). Identification using the 16S rDNA sequencing method by the PCR of universal primer was result in the *Lactobacillus plantarum* with possibility of 99% (data not shown). Based upon the result of previous study, it has named as a *Lactobacillus plantarum* K255.

Growth of strain

The number of viable *L. plantarum* K255 was determined by serial 10-fold dilution in 0.1% peptone water. 50 μL (9.6×10^5 CFU/mL) of *L. plantarum* K255 was inoculated in 150 mL of MRS broth. And then culture was incubated at 34°C, 37°C and 40°C for 24 h by checking 3 h and highest growth rate was found at 34°C. The optimum growth temperature of *L. plantarum* K255 was 37°C and it has taken 13 h to reach the pH 4.4 under this condition (Fig. 1, Fig. 2).

Antibiotic tolerance

It is very important for the probiotic strain can survive in the antibiotic circumstance. Table 3 shows the tolerance of *L. plantarum* K255 strain on the sixteen kinds of antibiotics. *L. plantarum* K255 showed more sensitive to bac-

Table 2. Physiological characteristics of *L. plantarum* K255

Gram reaction	+		
Cell type	rod		
Spore forming	−		
Motility	−		
Aerobic growth	+		
Anaerobic growth	+		
Catalase reaction	−		
Growth at 15°C	+		
Growth at 45°C	+		
Gas forming from glucose	−		
Ammonia production from alginin	−		
Acid production from			
Glycerol	−	Salicin	+
Erythritol	−	D-Celiobiose	+
D-Arabinose	−	D-Maltose	+
L-Arabinose	−	D-Lactose	+
D-Ribose	+	D-Melibiose	+
D-Xylose	−	D-Saccharose	+
L-Xylose	−	D-Trehalose	+
D-Adonitol	−	Inulin	−
Methyl-βD-Xylopyranoside	−	D-Melezitose	−
D-Galactose	+	D-Raffinose	+
D-Glucose	+	Amidon(starch)	−
D-Fructose	+	Glycogen	−
D-Mannose	+	Xylitol	−
L-Sorbose	−	Gentiobiose	+
L-Rhamnose	−	D-Turanose	−
Dulcitol	−	D-Lyxose	−
Inositol	−	D-Tagatose	−
D-Mannitol	+	D-Fucose	−
D-Sorbitol	+	L-Fucose	−
Methyl-aD-Mannopyranoside	+	D-Arabitol	−
Methyl-aD-Glucopyranoside	−	L-Arabitol	−
N-AcetylGlucosamine	+	Potassium Gluconate	−
Amygdalin	+	Potassium 2-KetoGluconate	−
Arbutin	+	Potassium 5-KetoGluconate	−
Esculin	+		

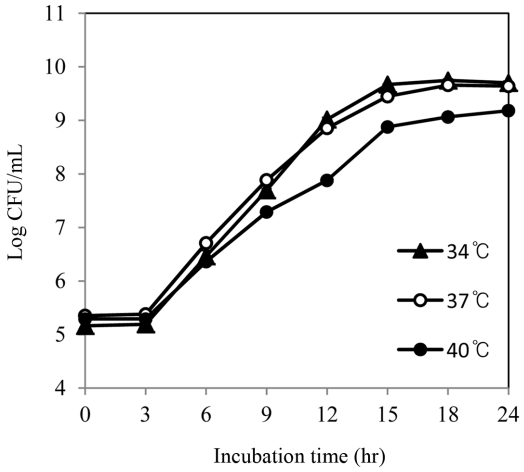

Fig. 1. Growth of *Lactobacillus plantarum* K255 in MRS broth at various temperature.

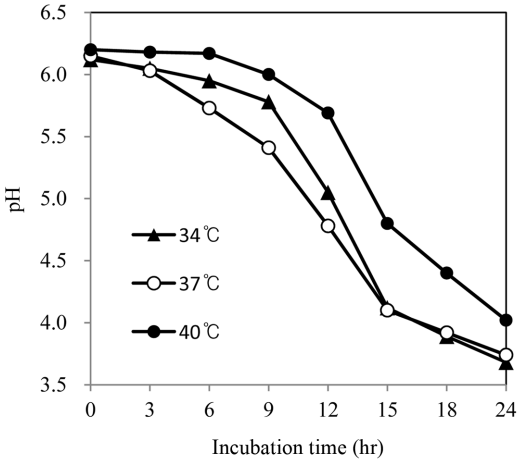

Fig. 2. pH changes of MRS broth during the growth of *Lactobacillus plantarum* K255 in MRS broth at various temperature.

itracin in a comparison of fifteen different antibiotics, and showed most resistance to kanamycin and vancomycin.

Kim *et al.* (2010) has reported that *L. plantarum* TJ-LP-002 isolated from *phakimchi* was sensitive to streptomycin sulfate but it shown high resistance to neomycin sulfate, spectinomycin dihydrochloride and lincomycin hydrochloride. According to Rojo-Bezares *et al.* (2006), the minimal inhibitory concentrations of antibiotics against *L. plantarum* strains isolated from wine were, respectively, the following ones: penicillin (0.5-4 μg/mL), chloramphenicol (4-16 μg/mL), vancomycin (128 μg/mL), streptomycin (16-512 μg/mL), gentamicin (32-128 μg/mL), kanamycin (128-1024 μg/mL). These results showed higher susceptibility against antibiotics than *L. plantarum* K255 strain.

Table 3. Antibiotics susceptibility of *Lactobacillus plantarum* K255

Antimicrobal agents	minimal inhibitory concentrations (μg/mL)
Aminoglycosides	
Amikacin	160±0
Gentamycin	1280±0
Kanamycin	3200±0
Neomycin*	800±0
Streptomycin	1600±0
β-lactams	
Penicillin-G*	160±0
Methicillin	320±0
Oxacillin	240±0
Ampicillin	320±0
Gram-positive spectrum	
Bacitracin*	15±0
Rifampicin	480±0
Novobiocin	120±0
Lincomycin*	800±0
Gram-negative spectrum	
Polymyxin B*	2400±0
Broad spectrum	
Chloramphenicol	320±0
Vancomycin	3200±0

*units/mL.
All values are mean±standard deviation of three replicates.

Enzyme activity

The enzyme activity is important for the selection of probiotic strain, because microorganisms can produce carcinogenic enzyme such as β-glucuronidase (Beaud *et al.*, 2005). *L. plantarum* K255 did not produce the β-glucuronidase. The enzymes produced by *L. plantarum* K255 were esterase, esterase lipase, leucine arylamidase, valine arylamidase, crystine arylamidase, acid phosphatase, naphtol-AS-BIphosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-galactosidase, and N-acetyl-β-glucosaminidase. Especially, leucine arylamidase, β-galactosidase, β-glucosidase, N-acetyl-β-glucosaminidase activity was 5 degree.

This result is comparable trends to the report of Lee *et al.* (2008) on the β-glucuronidase activity of *L. acidophilus* A12. Also, the enzyme profiles of *L. plantarum* K255 strain were similar to the *L. plantarum* isolated from fermented ewe's milk (Medina *et al.* 2001) except alkaline phosphatase and N-acetyl-β-glucosaminidase.

Bile tolerance

Bile salts are toxic effect for living cells, since they dis-

Table 4. Enzyme patterns of *Lactobacillus plantarum* K255

Enzyme	*L. plantarum* K255
Alkaline phosphatase	0
Esterase (C4)	1
Esterase Lipase (C8)	1
Lipase (C14)	0
Leucine arylamidase	5
Valine arylamidase	4
Cystinearylamidase	1
Trypsin	0
α-chymotrypsin	0
Acid phosphatase	2
Naphtol-AS-BI-phosphohydrolase	2
α-galactosidase	1
β-galactosidase	5
β-glucuronidase	0
α-glucosidase	4
β-glucosidase	5
N-acetyl-β-glucosaminidase	5
α-mannosidase	0
α-fucosidase	0

*A value ranging from 0 to 2 is assigned to the standard color, Zero represents a negative ; 5 represent a reaction of maximum intensity. Values 1 through 4 represent intermediate reactions depending on the level of intensity. The approximate activity may be estimated from the color strenght ; 1 corresponds to the liberation of 5 nanomoles, 2 to 10 nanomoles, 3 to 20 nanomoles, 4 to 30 nanomoles and 5 to 40 nanomoles or more.

organize the structure of the cell membrane and bile salt tolerance is considered one of the essential properties required for lactic acid bacteria to survive in the small intestine (Succi *et al.*, 2005).

Fig. 3 shows growth curves in MRS broth or MRS broth containing 0.3% bile. The log value of population after 7 h incubation without 0.3% oxgall was 9.1 but 8.6 with the addition of 0.3% bile. Therefore, the survival rate of *L. plantarum* K255 in MRS broth containing 0.3% bile was 90%. This survival rate is relatively high compared with the report of Kim *et al.* (2010) on the bile tolerance of *L. plantarum* TJ-LP-002 isolated from *phakimchi*. The survival rate of *L. plantarum* TJ-LP-002 in MRS broth containing 0.3% bile was 54.76%.

Acid tolerance

In order to survive in the gastrointestinal tract, the strain should express high tolerance to acid (Kirjavainen *et al.*, 1998; Lee and Salminen, 1995). To be a good probiotic, it is necessary to survive in the pH lower than 3 so that it could reach to the small intestine through the stomach (Booth, 1985; Mcdonald *et al.*, 1990). The acid tolerance of lactic acid bacteria has been linked to the induction of H^+-ATPase activity (Matsumoto *et al.*, 2004; Ventura *et al.*, 2004). Therefore, the variation in the acid tolerance of the selected probiotics might be related to the difference in H^+-ATPase activity in the probiotics. Fig. 4 shows the pH tolerance of *L. plantarum* K255. At the pH 2, The log value of population had increased from 7.68 to 8.18 after 3 hours incubation. Bacterial growth of *L. plantarum* K255 at pH 2-4 was not increased significantly compared to pH 6.4. And this result is comparable to the result of Jeon *et al.* (2007) that the cell density of 5.0×10^6 CFU/mL at pH 3 was decreased to 1.7×10^1 CFU/mL after 3 hours incubation.

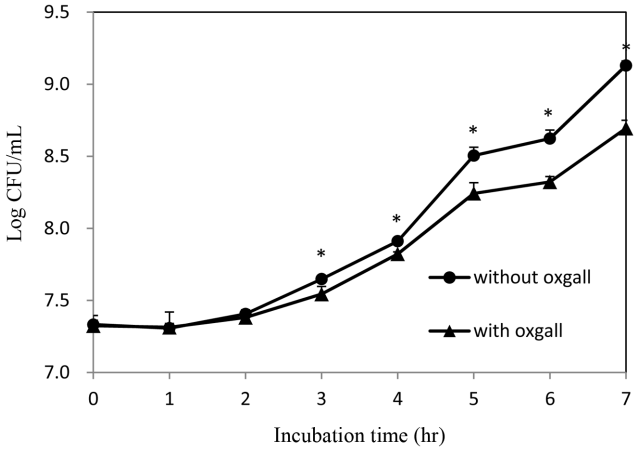

Fig. 3. Growth of *Lactobacillus plantarum* K255 in MRS broth containing 0.05% L-cysteine with/without 0.3% oxgall. *p<0.05 between with oxgall and without oxgall.

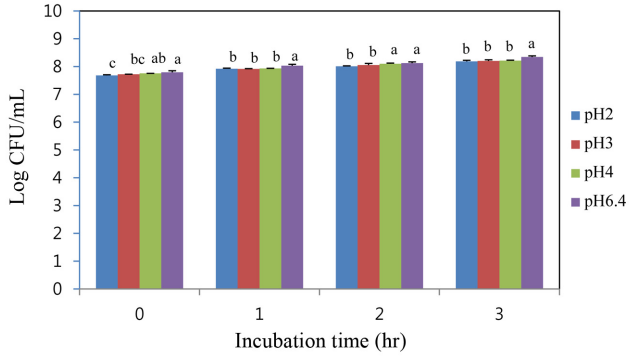

Fig. 4. Survival of *Lactobacillus plantarum* K255 after three hours in HCl solution (pH 2.0, 3.0, 4.0 and 6.4). [a-c]Means values with different superscript within same time are significantly different (p<0.05).

Antimicrobial activity

The antimicrobial ability is one of important property for probiotics. The antimicrobial activity of lactic acid bacteria may be due to a number of factors. Table 5 shows

Table 5. Inhibition of pathogens by *Lactobacillus plantarum* K255 in MRS broth

pathogens	pathogens[a]	*L. plantarum* K255[a] + Pathogens	Inhibition (%)
	CFU/mL	CFU/mL	
Escherichia coli	$5.8\times10^4\pm0.6\times10^4$	$4.0\times10^4\pm0.3\times10^4$	30.8±3.6
Salmonella Typhimurium	$4.8\times10^5\pm0.7\times10^5$	$3.4\times10^5\pm0.2\times10^5$	29.7±5.7
Staphyloccous aureus	$2.2\times10^6\pm0.5\times10^6$	$1.7\times10^6\pm0.2\times10^6$	22.7±12.6

*Initial count of *L. plantarum* K255 : $3.4\times10^6\pm0.5\times10^6$ CFU/mL
[a]Determined after 6 h of incubation at 37°C
All values are mean±standard deviation of three replicates.

the antimicrobial activity of *L. plantarum* K255 against the pathogenic strains. *L. plantarum* K255 showed resistence against *E. coli*, *S.* Typhimurium and *S. aureus* with the rate of 30.8%, 29.7%, and 23.4% respectively.

Kim *et al.* (2010) has reported that *L. casei* CU2064 has antimicrobial activity on the *E. coli, S.* Typhimurium and *S. aureus* with the rate of 86.89%, 85.00% and 82.50% respectively. *L. plantarum* K255 has relatively low antimicrobial activity.

Conclusion

LAB with great GABA production ability was isolated from Kimchi. MRS medium containing MSG as a source of glutamic acid was used in order to select the strains with GABA-producing ability from the isolated strains. Selected K255 strain was identified as *Lactobacillus plantarum* by the result of API carbohydrate fermentation test and 16S rDNA sequence. The optimum growth temperature of K255 was 37°C and cultures took 13 h to reach the pH 4.4. *L. plantarum* K255 could survive in the antibiotic circumstance with high concentration and didn't produce carcinogenic enzyme such as β-glucuronidase. Moreover, it was comparatively tolerant to bile juice, acid and displayed resistance to the pathogenic strains. These results demonstrate that *L. plantarum* K255 could be an excellent strain for the production of functional products with GABA production.

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References

1. Bae, M. O., Kim, H. J., Cha, Y. S., Lee, M. K., and Oh, S. H. (2009) Effects of Kimchi lactic acid bacteria *Lactobacillus* sp. OPK2-59 with high GABA producing capacity on liver function improvement. *J. Korean Soc. Food Sci. Nutri*. **38**, 1499-1505.
2. Beaud, D., Tailliez, P., and Anba-Mondoloni, J. (2005) Genetic characterization of the β-glucuronidase enzyme from a human intestinal bacterium, *Ruminococcus gnavus*. *Microbiology* **151**, 2323-2330.
3. Booth, I. R. (1985) Regulation of cytoplasmic pH in bacteria. *Microbiol. Rev.* **49**, 359-378.
4. Buchanan, R. E. and Gibbons, N. E. (1974) Bergey's manual of determinative bacteriology. 8th ed, Waverly Press, Inc., Baltimore, pp. 576-593.
5. Cheigh, H. S. and Park, K. Y. (1994) Biochemical, microbiological, and nutritional aspects of Kimchi (Korean fermented vegetable products). *Crit. Rev. Food Sci*. **34**, 175-203.
6. Cho, J. H., Lee, D. Y., Yang, C. N., Jeon, J. I., Kim, J. H., and Han, H. U. (2006) Microbial population dynamics of Kimchi, a fermented cabbage product. *FEMS Microbiol. Lett.* **257**, 262-267.
7. Clark, P. A., Cotton, L. N., and Martin, J. H. (1993) Selection of bifidobacteria for use as dietary adjuncts in cultured dairy foods: II. Tolerance to simulated pH of human stomachs. *Cul. Dairy Prod. J.* **28**, 11-14.
8. Gilliland, S. E. and Speck, M. L. (1977) Antagonistic action of *Lactobacillus acidophilus* toward intestinal and foodborne pathogens in associative cultures. *J. Food Prot.* **40**, 820-823.
9. Gilliland, S. E. and Walker D. K. (1990) Factors to consider when selecting a culture of *Lactobacillus acidophilus* as a dietary adjunct to produce a hypocholesterolemic effect in humans. *J. Dairy Sci.* **73**, 905-911.
10. Hur, H. J., Lee, K. W., Kim, H. Y., Chung, D. K., and Lee, H. J. (2006) *In vitro* immunopotentiating activities of cellular fractions of lactic acid bacteria isolated from Kimchi and bifidobacteria. *J. Microbiol. Biotechnol.* **16**, 661-666.
11. Jakobs, C., Jaeken, J., and Gibson, K. M. (1993) Inherited disorders of GABA metabolism. *J. Inherit. Metab. Dis*. **16**, 704-715.
12. Jeon, S. R., Song, T. S., Kim, J. Y., Shin, W. C., Her, S. W., and Yoon, S. S. (2007) Identification and characterization of lactic acid bacteria starters isolated from the commercial drink-yogurt products. *Korean J. Food Sci. An.* **27,** 509-516.
13. Kim, J. H., Kwon, M. J., Lee, S. Y., Rye, J. D., Moon, G. S., Cheigh, H. S., and Song, Y. O. (2002) The effect of Kimchi intake on production of free radicals and anti-oxidative enzyme activities in the liver of SAM. *J. Korean Soc. Food Sci. Nutr.* **31**, 109-116.
14. Kim, S. H., Yang, J. Y., Kang, S. A., Chun, H. K., and Park, K. Y. (2007) Current state and improvement for Korean Kim-

chi industry. *Food Indus. Nutr.* **12**, 7-13.
15. Kim, Y. J., Jang, S. J., Park, J. M., Kim, C. U., and Park, Y. S. (2010) Culture conditions of garlic resistant lactic acid bacteria for feed additives. *Food Eng. Progress* **14**, 65-74.
16. Kirjavainen, P. V., Ouwehand, A. C., Isolauri, E., and Salminen, S. J. (1998) The ability of probiotic bacteria to bind to human intestinal mucus. *FEMS Microbiol. Lett*. **167,** 185-189.
17. Lee, N. K., Yun, C. W., Kim, S. W., Chang, H. I., Kang, C. W., and Paik, H. D. (2008) Screening of lactobacilli derived from chicken feces and partial characterization of *Lactobacillus acidophilus* A12 as animal probiotics. *J. Microbial. Biotechnol.* **18**, 338-342.
18. Lee, Y. K. and Salminen, S. (1995). The coming age of probiotics. *Trends Food Sci. Technol*. **6**, 241–245.
19. Lim, S. D., Kim, K. S., and Do, J. R. (2011) Physiological characteristics and production of vitamin K_2 by *Lactobacillus fermentum* LC272 isolated from raw milk. *Korean J. Food Sci. An.* **31**, 513-520.
20. Manyam, B. V., Katz, L., Hare, T. A., Kaniefski, K., and Tremblay, R. D. (1981) Isoniazid induced elevation of cerebrospinal fluid (CSF) GABA levels and effects on chorea in huntington's disease. *Ann. Neurol*. **10**, 7-35.
21. Matsumoto, M., Ohishi, H., and Benno, Y. (2004) H^+-ATPase activity in bifidobacterium with special reference to acid tolerance. *Int. J. Food Microbiol.* **93**, 109-113.
22. Mcdonald, L. C., Fleming, H. P., and Hassan, H. M. (1990) Acid tolerance of *Leuconostoc mesenteroides* and *Lactobacillus casei*. *Appl. Environ. Microbial.* **53**, 2124-2128.
23. Medina, R., Katz, M., Gonzalez, S., and Oliver, G. (2001) Characterization of the lactic acid bacteria in ewe's milk and cheese from Northwest Argentina. *J. Food Prot.* **64**, 559-563.
24. Oh, S. H., Kim, H. J., Kim, Y. H., Yu, J. J., Park, K. B., and Jeon, J. I. (2008) Changes in some physico-chemical properties and γ-aminobutyric acid content of Kimchi during fermentation and storage. *J. Food Sci. Nutr.* **13**, 219-224.
25. Oh, S. H. and Yu, J. J. (2011) γ-Aminobutyric acid production and glutamate decarboxylase activity of *Lactobacillus sakei* OPK2-59 isolated from Kimchi. *Korean J. Microbiol*. **47**, 316-322.
26. Rojo-Bezares, B., Saenz, Y., Poeta, P., Zarazaga, M., Ruiz-Larrea, F., and Torres, C. (2006) Assessment of antibiotic susceptibility within lactic acid bacteria strains isolated from wine. *Int. J. Food Microbiol.* **111,** 234-240.
27. Saikusa, T., Horino, T., and Mori, Y. (1994) Accumulation of γ-aminobutyric acid (GABA) in the rice germ during water soaking. *Biosci. Biotech. Biochem*. **58**, 292-2291.
28. Succi, M., Tremonte, P., Reale, A., Sorrentino, E., Grazia, L., and Pacifico, S. (2005) Bile salt and acid tolerance of *Lactobacillus rhamnosus* strains isolated from Parmigiano Reggiano cheese. *FEMS Microbiol. Lett.* **244**, 129-137.
29. Tsushida, T. and Murai, T. (1987) Conversion of glutamic acid to g-aminobutyric acid in tea leaves under anaerobic conditions. *Agric. Biol. Chem*. **51**, 2865-2871.
30. Ueno, H. (2000) Enzymatic and structural aspects on glutamate decarboxylase. *J. Mol. Catal. B-Enzym*. **10,** 67-79.
31. Vaiva, G., Thomas, P., Ducrocq, F., Fontaine, M., Boss, V., Devos, P., Rascle, C., Cottencin, O., Brunet, A., Laffargue, P., and Coudemand, M. (2004) Low posttrauma GABA plasma levels as a predictive factor in the development of acute posttraumatic stress disorder. *Biol. Psychiat.* **55**, 250-254.
32. Ventura, M., Canchaya, C., van Sinderen, D., Fitzgerald, G. F., and Zink, R. (2004) *Bifidobacterium lactis* DSM 10140: identification of the atp (atpBEFHAGDC) operon and analysis of its genetic structure, characteristics, and phylogeny. *Appl. Environ. Microbiol.* **70**, 3110-3121.
33. Wong, C. G. T., Bottiglieri, T., and Snead, O. C. (2003) GABA, γ-hydroxybutyric acid, and neurological disease. *Ann. Neurol*. **54.** S3-S12.
34. Zhang, G. and Bown, A.W. (1997) The rapid determination of gamma aminobutyric acid. *Phytochem*. **44**, 1007-1009.

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