Food Science of Animal Resources

Food Sci. Anim. Resour. 2022 November 42(6):996~1008
DOI https://doi.org/10.5851/kosfa.2022.e43

pISSN : 2636-0772 eISSN : 2636-0780
http://www.kosfaj.org

ARTICLE

Probiotic Property and Anti-Obesity Effect of *Lactiplantibacillus plantarum* KC3

Seulki Kim[1], Eunchong Huang[2], Yosep Ji[2], Wilhelm Helnrich Holzapfel[2], and Sang-Dong Lim[1,*]

[1]Korea Food Research Institute, Wanju 55365, Korea
[2]Advanced Green Energy and Environment, Handong Global University, Pohang 37673, Korea

OPEN ACCESS

Received June 21, 2022
Revised July 22, 2022
Accepted August 11, 2022

***Corresponding author** : Sang-Dong Lim
Korea Food Research Institute, Wanju 55365, Korea
Tel: +82-63-219-9082
Fax: +82-63-219-9288
E-mail: limsd@kfri.re.kr

***ORCID**
Seulki Kim
https://orcid.org/0000-0002-0064-9366
Eunchong Huang
https://orcid.org/0000-0003-3760-6164
Yosep Ji
https://orcid.org/0000-0001-5349-7901
Wilhelm Helnrich Holzapfel
https://orcid.org/0000-0002-3669-1419
Sang-Dong Lim
https://orcid.org/0000-0002-1500-4413

Abstract Lactic acid bacteria are representative probiotics that have beneficial effects on humans. Nineteen strains among the 167 single strains from kimchi was selected and their physiological features were investigated. The selection of a strain was based on strong enzyme (lipase, α-amylase, and α-glucosidase) inhibitory activities and anti-obesity effects in the adipocytes. For the final selection, the strain *Lactiplantibacillus plantarum* KC3 was tested for its potential as a starter. To assess its functionality, a freeze-dried culture of *L. plantarum* KC3 was administered to a diet-induced obese mouse model receiving a high-fat diet. The animal group administered with *L. plantarum* KC3 showed significant body weight loss during the 12-week feeding period compared to the high-fat control group. This study investigated the physiological characteristics of selected strain and evaluated its potential as an anti-obesity probiotic in mice.

Keywords lactic acid bacteria, *Lactiplantibacillus plantarum*, probiotics, anti-obesity, probiotic property

Introduction

The history of probiotics can probably be traced back to the first use of fermented food products, such as cheese and yogurt, which were recommended for daily consumption. Over time, numerous fermented foods with health-promoting properties based on the functional microbial strains involved in fermentation have entered the market. Meanwhile, traditional fermented foods such as kefir, kombucha, sauerkraut and kimchi have been shown to contain microbial strains with probiotic features (Marco et al., 2017). Kimchi is a Korean traditional fermented food prepared at low temperature by mixing vegetables such as radish, Chinese cabbage or other similar vegetables, cucumber, pepper, garlic, persimmon, and a low concentration of salt. In addition to beneficial lactic acid bacteria (LAB), it contains various minerals and vitamins. Taxonomic studies on the microbiota typical of kimchi fermentation have

revealed a succession pattern typically initiated by *Leuconostoc* spp. and *Weissella* spp., and generally followed by *Lactobacillus* spp. (Rhee et al., 2011). There are *Lactobacillus* (11 strains), *Lactococcus* (1 strain), *Enterococcus* (2 strains), *Streptococcus* (1 strains), and *Bifidoacterium* (4 strains) among 19 Strains of probiotics authorized from Korean Food Standards Codex. Therefore, representative strains of *Lactobacillus* spp. are known to be the most promising probiotic candidates.

Recently, numerous studies aimed at identifying probiotic strains have shown that fermented dairy products can also be a good source of probiotics (Heller, 2001). Probiotics are defined as "living microorganisms which, when, administered in adequate quantities, confer a health benefit on the host" (FAO and WHO, 2002). Many cultivable and predominantly probiotic candidates in fermented dairy products have been widely isolated. The FAO/WHO guidelines on the development and application of probiotics constitute a set of parameters for strains to be called 'probiotics' and to prove health benefits for a particular condition or disease. The initial screening and selection of probiotics includes the inhibitory activities of lipase, α-amylase and α-glucosidase. In addition, selected probiotics should further be tested for their functional health characteristics. One method of investigating anti-adipogenicity in 3T3-L1 pre-adipocytes is a simple *in vitro* technique for selecting appropriate stains for *in vivo* studies conducted to support claims about probiotic. Likewise, each important strain property and its influence on health should ultimately be supported by clinical effects.

One assessment of safety and potential functionality of probiotics included antibiotic resistance testing (FEEDAP, 2012), bile salt and low pH tolerance, biogenic amine (BA) formation (BIOHAZ, 2011), enzymatic activity, and intestinal epithelial adhesion properties (Sanders et al., 2010) was used in the selection of an appropriate strain for an *in vivo* study in a diet induced murine model.

In this study, we isolated 167 different single strains from homemade kimchi, and carried out *in vitro* test and anti-adipogenic activity in 3T3-L1 cell to select functional strain. We investigated the physiological characteristics of selected strain and evaluated its potential as an anti-obesity probiotic in mice.

Materials and Methods

Bacterial strains

Two well-known and widely studied probiotic strains, *Lactiplantibacillus plantarum* 299V and *Lactobacillus rhamnosus* GG (LGG), have served as positive controls in various studies.

Isolation of lactic acid bacteria (LAB)

LAB were isolated from 40 kinds of homemade kimchi by using a modified man rogosa sharpe (MRS) medium (Lim et al., 2011). The strain was cultured for 18 h at 37°C in *Lactobacillus* MRS broth (BD Difco, Fraklin Lakes, NJ, USA) and stored at –80°C. Before use, the stock cultures were grown twice at 37°C for 18 h in MRS broth.

Enzyme assay

According to method described by Kim et al. (2018), lipase inhibitory activity, α-amylase inhibitory activity and α-glucosidase inhibitory activity was determined.

Anti-adipogenic activity

Cell culture

According to method described by Kim et al. (2018), 3T3-L1 cells (American Type Culture Collection, Manassas, VA,

USA) were cultured in DMEM supplemented with 10% FBS and 1% P/S under 5% CO_2 condition.

Sample preparation and treatment

The strain was cultured in the MRS medium at 37℃ for 18 h. After cultivation, all strains were harvested in a centrifuge at 1,500×g at 4℃ for 15 min and washed three times with distilled water to remove the remaining MRS medium. The washed strain was lyophilized, re-suspended in distilled water at a concentration of 10 mg/mL, homogenized for 50 seconds using a sonicator (Branson 8800, Branson Ultrasonics, Danbury, CT, USA), and then rested for 3 minutes (repeated three times). The 3T3-L1 cells were treated with 100 μg/mL of strain (10^9 CFU/mL).

Oil red O staining of 3T3-L1 adipocyte

The amount of lipids accumulated in the cells was measured using Oil Red O (Sigma-Aldrich, St. Louis, MO, USA), which reacts specifically with intracellular lipids. 3T3-L1 adipocyte was measured according to method by Huang et al. (2021). The differentiated cells were washed three times with PBS and fixed with 10% formaldehyde, followed by oil red O solution (stock solution: 3.5 mg/mL in isopropanol; working solution: 60% oil red O stock solution and 40% distilled water) for 30 min at room temperature. After staining, the solution was removed and the sample washed three times with distilled water. The amount of lipid accumulation was determined by adding 2 mL of iso-propyl alcohol to the completely dried well, re-eluting the oil red O, and measuring the absorbance at 520 nm.

Identification of lactic acid bacteria (LAB)

The isolated strain was identified by using the 16S rDNA sequencing method as described previously (Kim et al., 2018). Bacterial genomic DNA samples were extracted using the InstaGene™ Matrix (Bio-Rad Laboratories, Hercules, CA, USA). The primers 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1492R (5'-TAC GGY TAC CTT GTT ACG ACT T-3') were used for the PCR.

Probiotics property

The antibiotic susceptibility of *L. plantarum* KC3 was tested using the broth micro-dilution procedure according the method described by Phillips et al. (1991). The LAB susceptibility test medium with cysteine (LSM-C), which consists of a mixture of Iso-Sensitest broth (90%) and MRS broth (10%), supplemented with 0.3 g/L L-cysteine (Klare et al., 2007), was used as the medium. The enzyme activity of strain was determined using an API ZYM kit (bioMérieux, Lyon, France). Acid tolerance was measured according the method described by Clark et al. (1993). Bile tolerance was tested by the method of Gilliland and Walker (1990). The *L. plantarum* KC3 strain culture was inoculated into MRS broth containing 0.05% L-cysteine (Sigma-Aldrich) with or without 0.3% ox gall (Sigma-Aldrich). Antimicrobial activity was tested according to method of Gilliland and Speck (1977). *Escherichia coli* KCCM 11587 and *Staphylococcus aureus* KCCM 11335, antimicrobial indicator bacteria used in this study were purchased from the Korean Culture Center of Microorganisms (KCCM, Seoul, Korea), and *Salmonella* Typhimurium ATCC 14028 and *Listeria monocytogenes* ATCC 15313 were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). BA formation was tested with LB agar (pH 5.0; BD Difco) containing 0.25% glycerol, 0.006% BCP, and 0.1% precursor amino acid, as described by Chang and Chang (2012). According to the method of Kim et al. (2018), the intestinal adhesion ability of the strain was performed using HT-29 cells.

Animal experiments

The Committee on the Ethics of Animal Experiments of Handong Global University approved the animal experiments (20160615-002). Five-week-old C57BL/6J male mice were provided by Koatec (Gyeonggi, Korea) and housed in a controlled environment (at 23±1℃ and 55±10% humidity, in a 12 h light/dark cycle) and given free access to filtered water and food. All of the mice were acclimated with normal diet during the first week. After this period, the mice were randomly assigned to groups (n=6/group) with different diets for 12 weeks. The customized (IF) high-fat diet was composed of 40% carbohydrate, 45% fat and 15% protein. The freeze-dried probiotic strains in the laboratory were incorporated into 3 grams of the IF diet to provide 5.0×10^9 CFU/mouse/day. The weight of each animal and its feed consumption was measured once a week. At the end of the experimental period, the animals were anesthetized by diethyl ether inhalation, samples collected, and their weight measured. Blood serum samples were extracted by centrifugation of the whole blood at 2,000×g for 20 min. Adipose tissues and serum samples were stored at –80℃ without repeated freeze-and-thaw steps.

Statistical analysis

The statistical analysis was performed with a statistical analysis system (XLSTAT version 2015, Addinsoft, Paris, France). The significance of the differences was analyzed by conducting a one-way analysis of variance (ANOVA) using Duncan's multiple range tests. Significance was considered to be $p<0.05$. Student's t-test was performed with data from in probiotic characteristic test. In the animal study, the data were analyzed with ANOVA using Dunnett's multiple range test compared to different groups. Significance was accepted at $p<0.05$. The statistical analysis was performed using a GraphPad Prism 7 Program (version 7.03, GraphPad Software, San Diego, CA, USA).

Results and Discussion

Isolation and screening of lactic acid bacteria (LAB)

Using the modified MRS medium, 167 single strains were isolated from 40 kinds of homemade Korean kimchi. Among the 167 single strains, 19 strains of *L. plantarum* were selected for their strong inhibitory activity against pancreatic lipase of over 80%, and were tested for their inhibitory activities against α-amylase and α-glucosidase. Six of these strains (KC3, K40, K42, K58, K112, and K134) showed strong inhibitory activity against α-amylase and α-glucosidase of over 90% (Table 1). Natural and synthetic pancreatic lipase inhibitors are effective in preventing obesity because they inhibit intestinal lipid absorption (Hirose et al., 2013). Since Asian diets generally contain considerably more carbohydrates than Western diets, a combined mechanism may be required to inhibit carbohydrate absorption and to improve obesity by inhibiting fat absorption (Jang and Jeong, 2010).

Anti-adipogenic activity

Obesity is also related to the degree of differentiation of pre-adipocytes into adipocytes, and to the enlargement of adipocytes in the adipose tissues (Wang and Jones, 2004). After the enzyme assay test of isolated strains, 6 single strains (KC3, K40, K42, K58, K112, and K134) were selected for the anti-adipogenic activity test. Fig. 1 shows the effect of these 6 single strains on 3T3-L1 adipocyte stained with Oil red O. The cells treated with KC3 resulted in a reduction of lipid accumulation of about 38%, compared with the untreated control ($p<0.001$, Fig. 1A). Among the strains, the greatest reduction in Oil red O staining was observed in KC3. As shown in Fig. 1B, KC3 also caused a greater reduction in lipid

Table 1. Inhibitory activity of 19 selected strains against pancreatic lipase, α-amylase, and α-glucosidase

Strain (%)	Pancreatic lipase inhibitory activity	α-Amylase inhibitory activity	α-Glucosidase inhibitory activity
KC2	82.16±0.99	93.22±3.93	99.81±0.36
KC3	90.97±1.80	95.52±5.712	97.97±1.08
K14	87.51±5.00	92.70±3.92	99.92±0.18
K17	91.09±2.21	79.16±1.39	79.31±0.45
K28	89.92±0.87	94.73±5.32	60.03±1.26
K29	91.32±1.93	96.82±2.08	69.45±0.56
K40	85.17±0.79	96.78±3.29	92.55±9.62
K42	87.40±1.41	94.66±4.34	99.78±0.28
K58	89.39±3.48	97.01±4.88	99.99±0.38
K61	85.30±2.39	96.13±4.37	61.63±1.46
K66	93.01±2.90	76.55±1.81	28.19±2.39
K87	90.13±3.22	94.50±6.11	55.92±0.16
K98	92.38±1.87	95.26±1.46	73.63±1.92
K109	91.10±1.51	94.46±0.85	73.67±2.49
K112	87.59±2.46	90.77±5.69	96.33±5.23
K123	88.00±1.27	80.82±8.24	14.89±0.19
K134	91.52±1.82	96.20±4.23	98.62±0.4
K146	82.98±0.08	81.83±5.23	87.4±2.16
K158	88.46±1.93	89.23±3.13	89.66±0.22

accumulation in rounded cells compared with the untreated control cells when visualized by staining. KC3 was then selected as the final experimental strain according to the results of the anti-adipogenesis test.

Identification of lactic acid bacteria (LAB)

The total nucleotide sequence of 1508 bp was determined from the 16S rDNA gene of KC3. After PCR amplification using universal primers targeting 16S rDNA, and the subsequent sequence analysis, the alignment of this sequence showed a strong similarity (of around 99%) with the *L. plantarum* type strain. Based on the nucleotide sequence of the 16S rDNA gene, it was confirmed that it was identical to *L. plantarum*, and it was named *L. plantarum* KC3.

Antibiotic susceptibility of *Lactiplantibacillus plantarum* KC3

The tolerance of the *L. plantarum* KC3 to 16 types of antibiotics is shown in Table 2. According to Klarin et al. (2019), the ampicillin minimum inhibitory concentration (MIC) values of *L. plantarum* 299v and *L. plantarum* 299 were both 0.094 μg/mL. The MIC of *L. plantarum* KC3 showed a high resistance to ampicillin (MIC>256 μg/mL); while its resistance to penicillin, vancomycin, gentamicin, streptomycin, erythromycin, clindamycin, and chloramphenicol was found to be similar to the 46 *L. plantarum* strains reported by Klare et al. (2007). KC3 was more sensitive to clindamycin and erythromycin than to other antibiotics, but showed the highest resistance to ampicillin and vancomycin. The resistance of KC3 to kanamycin, streptomycin, clindamycin, rifampicin, and chloramphenicol was within the range accepted by the European Food Safety Authority (EFSA,

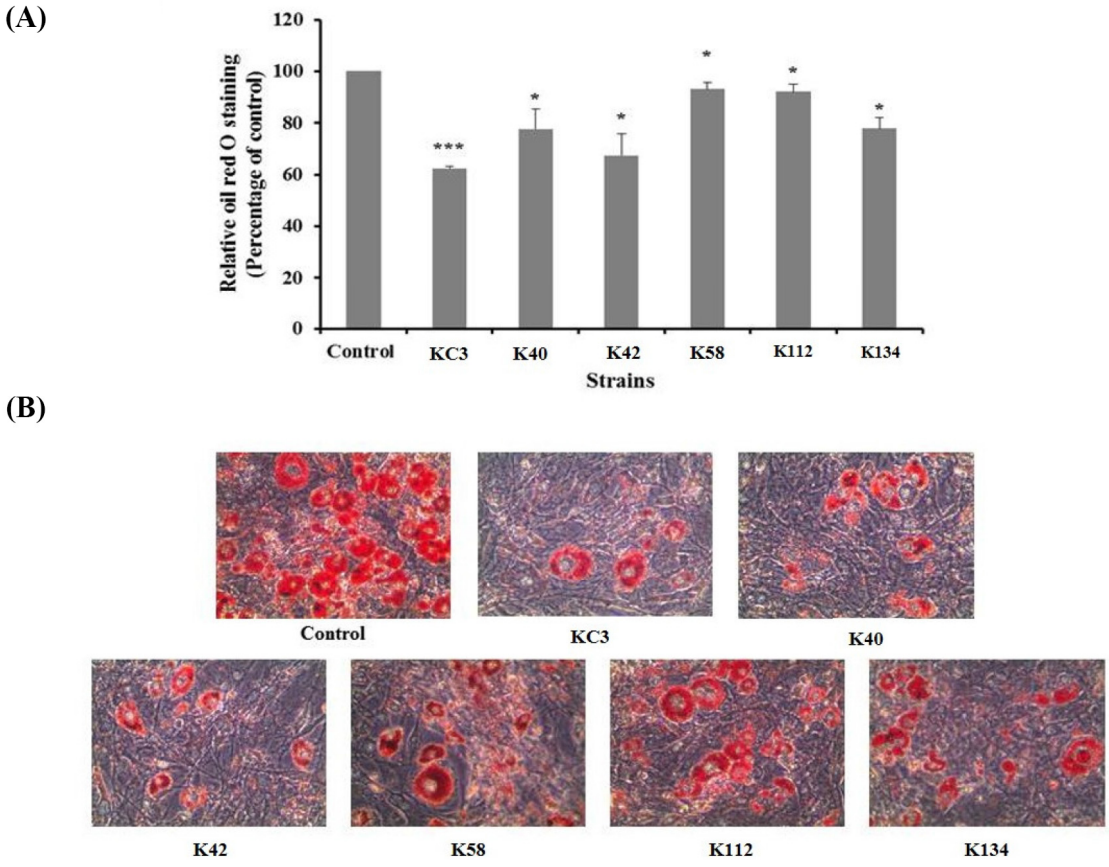

Fig. 1. The effect of the 6 selected strains on adipocytes revealed by oil red O staining in 3T3-L1. (A) Quantification of oil red O staining. (B) Photograph of oil red O staining. The cells were stained with oil red O and observed with a microscope (original magnification ×200). * $p<0.05$ and *** $p<0.001$ compared with the control (t-test).

2008) and the Scientific Committee for Animal Nutrition (European Commission, 2001). However, KC3 was resistant to gentamycin, ampicillin, ciprofloxacin, tetracycline, and vancomycin, and had an equal or higher MICs according to the European Food Safety Authority (EFSA, 2008) and the Scientific Committee for Animal Nutrition (European Commission, 2001).

Enzyme activity of *Lactiplantibacillus plantarum* KC3

Unlike *Bacillus* spp. and fungi, *Lactobacillus* is known for producing intracellular enzymes (Jeon, 1998). The results of the enzyme activity of *L. plantarum* KC3 are shown in Table 2. *L. plantarum* KC3 produced enzymes such as esterase, lipase, leucine arylamidase, valine arylamidase, cystimearylamidase, acid phosphatase, naphtol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase, and N-acetyl-β-glucosaminidase. In particular, it produced large amounts of such enzymes as leucine arylamidase, valine arylamidase, β-galactosidase, and β-glucosidase. However, no activity associated with β-glucuronidase, a pro-carcinogenic enzyme that converts benzopyrene to a carcinogenic substance (Rhee et al., 1998) was detected in this particular strain.

Enzymes secreted by probiotics can improve the utilization of nutrients such as starch, protein and fat when consumed by humans or animals, thereby increasing the energy value of food or feed (Walsh et al., 1993). In particular, β-galactosidase enzymes could alleviate the symptoms of lactose intolerance by converting lactose into galactose and glucose in milk (de Vrese et al., 2001). The β-galactosidase activity in *L. plantarum* KC3 was determined at 5 degree.

Table 2. **Susceptibility to antibiotics and enzyme activity of *Lactobacillus plantarum* KC3**

Anti-microbial agents	Minimal inhibitory concentration (μg/mL)	EFSA suggested values	Enzyme	KC3
Amikacin	4	-	Alkaline phosphatase	0[1)]
Gentamycin	1	16	Esterase (C4)	0
Kanamycin	16	64	Esterase lipase (C8)	1
Streptomycin	8	-	Lipase (C14)	0
Ampicillin	256	2	Leucine arylamidase	5
Penicillin-G	1	-	Valine arylamidase	4
Oxacillin	8	-	Cystinearylamidase	2
Bacitracin	32	-	Trypsin	0
Polymyxin B	128	-	α-Chymotrypsin	0
Ciprofloxacin	8	-	Acid phosphatase	2
Tetracycline	16	32	Naphtol-AS-BI-phosphohydrolase	2
Clindamycin	0.0156	2	α-Galactosidase	0
Erythromycin	0.125	1	β-Galactosidase	5
Rifampicin	1	-	β-Glucuronidase	0
Vancomycin	2,048	-	α-Glucosidase	3
Choloramphenicol	4	8	β-Glucosidase	4
			N-Acetyl-β-glucosaminidase	3
			α-Mannosidase	0
			α-Fucosidase	0

[1)] A value ranging from 0 to 5 is assigned to the standard color: Zero represents a negative; 5 represents a reaction of maximum intensity. Values 1 to 5 represent intermediate reactions depending on the level of intensity. The approximate activity may be estimated from the color strength: 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.
EFSA, European Food Safety Authority.

Bile acid and acid tolerance of *Lactiplantibacillus plantarum* KC3

After oral ingestion, bacteria encounter several hurdles erected by the human defense system, such as mucins in the gut, gastric acid and bile acid. The bile acid secreted into the duodenum destroys the membrane of bacterial cells and inhibits their growth. Therefore, in order to function as probiotics, resistance to physiological (and at least 0.3%) bile concentration is essential (Saarela et al., 2000).

Subsequent to the antibiotic susceptibility test, bile acid and acid tolerance were tested againt *L. plantarum* KC3. The growth curves of the KC3 strain in MRS broth and in MRS broth with 0.3% ox gall are shown Fig. 2A. After incubation for 7 h, the number of viable cells was counted in both the MRS broth and the MRS broth with ox gall. Compared to other strains, *L. plantarum* KC3 was not affected by the addition of 0.3% ox gall up to 5 h, after which a slight decrease was detected. After incubation for 7 h, the number of the viable bacteria was 9.37 Log CFU/mL without ox gall (bile acid) and 8.96 Log CFU/mL with ox gall. Strain KC3 showed a high survival rate of 95.62% in the MRS broth with 0.3% ox gall, compared to the control without bile acid.

To function effectively as a probiotic, survival at a pH value of pH 3 or lower would be necessary to survive passage through the upper gastrointestinal tract. The pH of gastric juice is pH 0.9, but when food is ingested its pH rises to pH 3

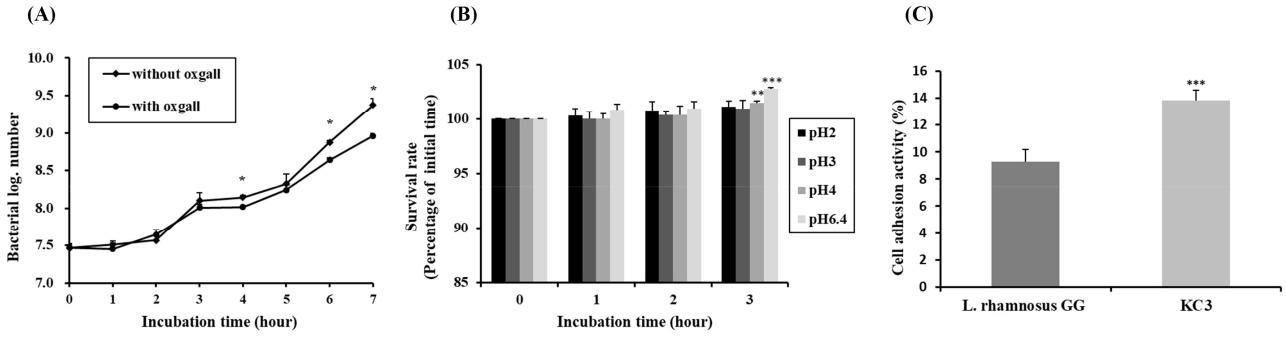

Fig. 2. Bile acid tolerance, acid tolerance, and adhesive property of *Lactobacillus plantarum* KC3. (A) Growth in MRS broth containing 0.05% L-cysteine with/without 0.3% ox gall. (B) Survival rates after three hours in HCl solution (pH 2.0, 3.0, 4.0, and 6.4). (C) Adhesion ability to HT-29 cell line, compared with that of *Lactobacillus rhamnosus* GG. All values are within the mean±SD of the three replicates. * p<0.05, ** p<0.01, and *** p<0.001 compared with the control (t-test). MRS, man rogosa sharpe.

(Erkkilä and Petäjä, 2000). High acid tolerance was detected in strain KC3. The results of pH tolerance of *L. plantarum* KC3 and other strains are shown in Fig. 2B, indicating that, in comparison to 6.4, the growth of the strain was not significantly influenced by the pH values 2, 3, and 4. Based on its survival rates, *L. plantarum* KC3 showed the highest bile and acid tolerance among the tested strains. Because a comparatively high percentage of the strain survived under bile acid and acidic conditions, *L. plantarum* KC3 has probiotic potential under *in vivo* conditions.

Antimicrobial activity of *Lactiplantibacillus plantarum* KC3

Table 3 shows the antimicrobial activity of *L. plantarum* KC3 against various pathogenic strains. *L. plantarum* KC3 showed resistance to *E. coli*, *S.* Typhimurium, *L. monocytogenes* and *S. aureus* at rates of 53.78%, 76.80%, 26.27%, and 34.61%, respectively. After incubation for 6 h, the pH value of the pathogens was around 5.98–6.10, while that of the mixed culture of *L. plantarum* KC3 and pathogens was around pH 4.98–5.54, which indicates that even though the lactic acid produced during incubation had an effect on antimicrobial activity it was not large.

The antimicrobial effects of LAB in the GIT are related to inhibition during pH reduction, the competition for consumption between nutrients and pathogens, a reduction of redox potential, the production of hydrogen peroxide under aerobic conditions, and the secretion of antimicrobial active substances such as bacteriocin (Havenaar et al., 1992). Some strains of LAB produce different antimicrobial compounds which can prevent the growth of pathogenic and spoilage bacteria (Ahmadova

Table 3. Inhibition of pathogens by *Lactobacillus plantarum* KC3 in MRS broth

Pathogens	Viable numbers (CFU/mL) of pathogens[1)]				Inhibition (%)
	Without KC3		With KC3[2)]		
	CFU/mL	pH	CFU/mL	pH	
Escherichia coli	$3.23\pm0.25\times10^6$	5.98	$1.51\pm0.15\times10^6$	4.98	53.78
Salmonella Typhimurium	$6.46\pm0.35\times10^6$	6.10	$1.50\pm0.26\times10^6$	5.54	76.80
Listeria monocytogenes	$1.57\pm0.20\times10^5$	6.06	$1.16\pm0.12\times10^5$	5.07	26.27
Staphyloccous aureus	$3.46\pm0.87\times10^6$	6.08	$2.26\pm0.11\times10^6$	5.05	34.61

All values are within the mean±SD of the three replicates.
[1)] As determined after 6 h of incubation at 37°C.
[2)] Initial count of *Lactobacillus plantarum* KC3: $2.10\pm0.17\times10^6$ CFU/mL.
MRS, man rogosa sharpe.

et al., 2013). The antimicrobial activity of a strain varies depending on the pathogen, even if it is a strain of the same species (Jacobsen et al., 1999).

Adhesive property of *Lactiplantibacillus plantarum* KC3

The ability to adhere to the intestinal epithelium is one of the key criteria when selecting probiotic strains (Sanders et al., 2010). In a recent study, HT-29 cells were used as an *in vitro* model of epithelial cell adherence (Lee et al., 2011). The ability of *L. plantarum* KC3 to adhere to the human intestinal cell line HT-29 is shown in Fig. 2C. *L. plantarum* KC3 (13.85%) adhered to the HT-20 cells, thus showing greater adhesiveness than *L. rhamnosus* GG (with only 9.26%), the positive control. Although *L. rhamnosus GG's* strong ability to adhere to HT-29 cells has been reported in several previous studies, our data were similar to those of Verdenelli et al. (2009).

Biogenic amine (BA) formation of *Lactiplantibacillus plantarum* KC3

Some LAB strains may form BA by amino acid decarboxylation. BA is an alkaline organic substance with biological activity that is commonly found in fermented foods or fermented beans, and is formed mainly by the enzymes of food or by decarboxylation of the amino acids in microorganisms (Silla Santos, 1996). BAs are found in various foods such as non-fermented foods (fish, fruits, vegetables, and meat), dairy products, fermented fish/meat products, soybean fermented products, and alcoholic drinks such as wine and beer (Silla Santos, 1996). BAs have diverse biological activities, including negative effects such as toxicity and causing allergenic responses. Especially histamine and tyramine can cause migraines, flushing, nervous disorders, headaches, vomiting, nausea, heart palpitations, respiratory distress, hypertension, and blood pressure instability (BIOHAZ, 2011). *L. plantarum* KC3 did not show any BA formation from the precursor amino acids used in this study (data not shown), namely tyrosine, histidine, ornithine, and lysine.

Anti-obesity effect of *Lactiplantibacillus plantarum* KC3 on diet-induced-obesity mice

To evaluate the anti-obesity effect of *L. plantarum* KC3 on the aberrant host conditions, lyophilized probiotic strains were incorporated with the IF diet at a level of 5.0×10^9 CFU/mouse/day. LGG and 299V were used in the studies as reference probiotic strains, and Xenical (Xen) was used as the positive chemical control for anti-obesity. Each animal group that received the LGG strain, Xen and KC3 showed a significantly lower bodyweight and total weight gain compared to the high-fat diet group (Table 4). Moreover, the weight of the liver and other adipose tissues of the LGG and KC3 groups, but not that of the 299v group, showed a significant reduction. The concentrations of other parameters such as the glucose/lipid metabolism-related biomarkers, total cholesterol, triacylglycerol (TG) and low-density lipoprotein cholesterol, were alleviated in the LGG and KC3 groups, indicating an amelioration of the biomarkers of metabolic disease induced by the IF diet (Table 4).

Bile acids play an essential role in maintaining TG and cholesterol homeostasis (Li et al., 2013). According to Kwon et al. (2020), it was reported that the expression of genes involved in bile acid synthesis was significantly increased in the liver of *L. plantarum* treated mice. These assessments have shown that *L. plantarum* KC3 is adequate for use in anti-obesity investigations in an *in vivo* study. Freeze-dried *L. plantarum* KC3 was incorporated into the IF diet and administered during an abnormal host status of diet- induced obesity. As a result, *L. plantarum* KC3 showed an ability to reduce fat accumulation. Based on these results, it is necessary to confirm the change in the intestinal microbiota during administration of *L. plantarum* KC3. The regulation of gene expression related to lipid metabolism in the adipose tissue has probably been altered. As such,

Table 4. Baseline characteristics of the animal experiments on weight and other obesity-related indicators in the blood of diet-induced obesity mice receiving a high-fat diet

Parameters	Groups					
	ND	HFD	Xen	LGG	299v	KC3
Bodyweight (g)						
Final	29.3±2.51***	41.4±2.32	30.5±1.56***	36.0±2.11***	39.0±3.12	34.4±2.81***
Weight gain	11.5±1.87***	22.5±1.98	11.7±1.05***	18.9±1.60***	21.4±2.46	16.7±2.36***
Organ weight (g)						
Liver	1.04±0.29***	1.528±0.13	1.26±0.13***	1.22±0.05***	1.44±0.27	1.20±0.09***
EAT	0.81±0.14***	2.026±0.17	0.75±0.260***	1.73±0.39	1.81±0.28	1.64±0.37*
MAT	0.34±0.07***	0.893±0.27	0.246±0.10***	0.57±0.18*	0.76±0.29	0.52±0.14***
SAT	0.38±0.08***	1.247±0.39	0.363±0.16***	0.97±0.33	1.12±0.20	0.91±0.28*
Serum (mg/dL)						
Total cholesterol	102.9±8.55***	164.4±25.3	92.0±20.1***	126.7±26.0**	133.3±49.0	90.7±18.0***
TG	106.3±24.3	109.3±12.0	74.0±9.32***	97.3±29.2	100.0±14.1	76.0±13.2***
LDLC	9.14±3.02***	24.9±7.42	10.0±3.70***	16.0±5.06*	18.7±10.9	10.7±4.13***

The whole bodyweight, liver and adipose tissues were measured after 12 weeks from the initial point (n=6–9). The indicators for the serum analysis were measured by the blood analyzer. The data are represented as the mean±SD and analyzed in a comparison with the HFD group. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ by Fisher's LSD test.
ND, control group with a normal chow diet; HFD, positive control group with a high-fat diet; Xen, negative control group treated with Xenical; LGG, receiving *Lactobacillus rhamnosus* strain GG mixed with a high-fat diet; 299v, receiving *Lactiplantibacillus plantarum* strain 299v mixed with a high-fat diet; KC3, receiving *L. plantarum* strain KC3 mixed with a high-fat diet; EAT, epididymal adipose tissue; MAT, mesenteric adipose tissue; SAT, subcutaneous adipose tissue; TG, triacyl-glyceride; LDLC, low density lipoprotein cholesterol.

it would be necessary to adopt a mechanistic approach in order to determine the exact way in which *L. plantarum* KC3 modulated this interactive cascade.

Conflicts of Interest

The authors declare no potential conflicts of interest.

Acknowledgements

This work was supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, and Forestry (IPET) through the Agricultural Microbiome R&D Program (No. 916002-02-1-CG000) funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA), and by a grant from the Korea Food Research Institute (project no. E0201100-03).

Author Contributions

Conceptualization: Holzapfel WH, Lim SD. Data curation: Kim S, Huang E. Formal analysis: Kim S, Ji Y. Methodology: Kim S, Huang E. Software: Kim S, Huang E. Investigation: Kim S, Ji Y. Writing - original draft: Kim S, Huang E, Lim SD. Writing - review & editing: Kim S, Huang E, Ji Y, Holzapfel WH, Lim SD.

Ethics Approval

The Committee on the Ethics of Animal Experiments of Handong Global University approved the animal experiments (20160615-002).

References

Ahmadova A, Todorov SD, Choiset Y, Rabesona H, Zadi TM, Kuliyev A, Franco BDGM, Chobert JM, Haertlé T. 2013. Evaluation of antimicrobial activity, probiotic properties and safety of wild strain *Enterococcus faecium* AQ71 isolated from Azerbaijani Motal cheese. Food Control 30:631-641.

Chang M, Chang HC. 2012. Development of a screening method for biogenic amine producing *Bacillus* spp. Int J Food Microbiol 153:269-274.

Clark PA, Cotton LN, Martin JH. 1993. Selection of bifidobacteria for use as dietary adjuncts in cultured dairy foods. II. Tolerance to simulated pH of human stomachs. Cult Dairy Prod J 28:11-14.

de Vrese M, Stegelmann A, Richter B, Fenselau S, Laue C, Schrezenmeir J. 2001. Probiotics: Compensation for lactase insufficiency. Am J Clin Nutr 73:421S-429S.

EFSA Panel on Additives and Products or Substances used in Animal Feed [FEEDAP]. 2012. Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. EFSA J 10:2740.

EFSA Panel on Biological Hazards [BIOHAZ]. 2011. Scientific opinion on the risk based control of biogenic amine formation in fermented foods. EFSA J 9:2393.

Erkkilä S, Petäjä E. 2000. Screening of commercial meat starter cultures at low pH and in the presence of bile salts for potential probiotic use. Meat Sci 55:297-300.

European Commission. 2001. Opinion of the scientific committee on animal nutrition on the criteria for assessing the safety of microorganisms resistant to antibiotics of human clinical and veterinary importance. Available from: https://food.ec.europa.eu/system/files/2020-12/sci-com_scan-old_report_out108.pdf. Accessed at Apr 27, 2022.

European Food Safety Authority [EFSA]. 2008. Technical guidance prepared by the panel on additives and products or substances used in animal feed (FEEDAP) on the update of the criteria used in the assessment of bacterial resistance to antibiotics of human or veterinary importance. EFSA J 732:1-15.

Food and Agriculture Organization of the United Nations [FAO], World Health Organization [WHO]. 2002. Guidelines for the evaluation of probiotics in food. Available from: http://4cau4jsaler1zglkq3wnmje1-wpengine.netdna-ssl.com/wp-content/uploads/2019/04/probiotic_guidelines.pdf. Accessed at Apr 20, 2022.

Gilliland SE, Speck ML. 1977. Deconjugation of bile acids by intestinal lactobacilli. Appl Environ Microbiol 33:15-18.

Gilliland SE, Walker DK. 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.

Havenaar R, Brink BT, Huis In't Veld JHJ. 1992. Selection of strains for probiotic use. In Probiotics: The scientific basis. Fuller R (ed). Chapman & Hall, New York, NY, USA. pp 209-224.

Heller KJ. 2001. Probiotic bacteria in fermented foods: Product characteristics and starter organisms. Am J Clin Nutr 73:374S-379S.

Hirose M, Ando T, Shofiqur R, Umeda K, Kodama Y, Nguyen SV, Goto T, Shimada M, Nagaoka S. 2013. Anti-obesity activity of hen egg anti-lipase immunoglobulin yolk, a novel pancreatic lipase inhibitor. Nutr Metab 10:70.

Huang E, Kim S, Park H, Park S, Ji Y, Todorov SD, Lim SD, Holzapfel WH. 2021. Modulation of the gut microbiome and obesity biomarkers by *Lactobacillus plantarum* KC28 in a diet-induced obesity murine model. Probiotics Antimicrob Proteins 13:677-697.

Jacobsen CN, Nielsen VR, Hayford AE, Møller PL, Michaelsen KF, Paerregaard A, Sandström B, Tvede M, Jakobsen M. 1999. Screening of probiotic activities of forty-seven strains of *Lactobacillus* spp. by *in vitro* techniques and evaluation of the colonization ability of five selected strains in humans. Appl Environ Microbiol 65:4949-4956.

Jang YS, Jeong JM. 2010. Antioxidative effect and digestive enzyme inhibition of grape seed extract (GSE). J Korean Soc Food Sci Nutr 39:783-788.

Jeon HK. 1998. Enzymology. Pusan National University Press, Busan, Korea. p 486.

Kim S, Huang E, Park S, Holzapfel W, Lim SD. 2018. Physiological characteristics and anti-obesity effect of *Lactobacillus plantarum* K10. Korean J Food Sci Anim Resour 38:554-569.

Klare I, Konstabel C, Werner G, Huys G, Vankerckhoven V, Kahlmeter G, Hildebrandt B, Müller-Bertling S, Witte W, Goossens H. 2007. Antimicrobial susceptibilities of *Lactobacillus*, *Pediococcus* and *Lactococcus* human isolates and cultures intended for probiotic or nutritional use. J Antimicrob Chemother 59:900-912.

Klarin B, Larsson A, Molin G, Jeppsson B. 2019. Susceptibility to antibiotics in isolates of *Lactobacillus plantarum* RAPD-type Lp299v, harvested from antibiotic treated, critically ill patients after administration of probiotics. Microbiologyopen 8:e00642.

Kwon J, Kim B, Lee C, Joung H, Kim BK, Choi IS, Hyun CK. 2020. Comprehensive amelioration of high-fat diet-induced metabolic dysfunctions through activation of the PGC-1α pathway by probiotics treatment in mice. PLOS ONE 15:e0228932.

Lee J, Yun HS, Cho KW, Oh S, Kim SH, Chun T, Kim B, Whang KY. 2011. Evaluation of probiotic characteristics of newly isolated *Lactobacillus* spp.: Immune modulation and longevity. Int J Food Microbiol 148:80-86.

Li T, Francl JM, Boehme S, Chiang JYL. 2013. Regulation of cholesterol and bile acid homeostasis by the cholesterol 7α-hydroxylase/steroid response element-binding protein 2/microRNA-33a axis in mice. Hepatology 58:1111-1121.

Lim SD, Kim KS, Do JR. 2011. Physiological characteristics and production of vitamin K_2 by *Lactobacillus fermentum* LC272 isolated from raw milk. Korean J Food Sci Anim Resour 31:513-520.

Marco ML, Heeney D, Binda S, Cifelli CJ, Cotter PD, Foligné B, Gänzle M, Kort R, Pasin G, Pihlanto A, Smid EJ, Hutkins R. 2017. Health benefits of fermented foods: Microbiota and beyond. Curr Opin Biotechnol 44:94-102.

Phillips I, Andrews JM, Bridson E, Cooke EM, Holt HA, Spencer RC, Wise R, Bint AJ, Brown DFJ, Greenwood D, King A, Williams RJ. 1991. A guide to sensitivity testing. J Antimicrob Chemother 27:1-50.

Rhee SJ, Lee JE, Lee CH. 2011. Importance of lactic acid bacteria in Asian fermented foods. Microb Cell Fact 10:S5.

Rhee YK, Kim DH, Han MJ. 1998. Inhibitory effect of *Zizyphi fructus* on β-glucuronidase and tryptophanase of human intestinal bacteria. Korean J Food Sci Technol 30:199-205.

Saarela M, Mogensen G, Fondén R, Mättö J, Mattila-Sandholm T. 2000. Probiotic bacteria: Safety, functional and technological properties. J Biotechnol 84:197-215.

Sanders ME, Akkermans LMA, Haller D, Hammerman C, Heimbach J, Hörmannsperger G, Huys G, Levy DD, Lutgendorff F, Mack D, Phothirath P, Solano-Aguilar G, Vaughan E. 2010. Safety assessment of probiotics for human use. Gut Microbes 1:164-185.

Silla Santos MH. 1996. Biogenic amines: Their importance in foods. Int J Food Microbiol 29:213-231.

Verdenelli MC, Ghelfi F, Silvi S, Orpianesi C, Cecchini C, Cresci A. 2009. Probiotic properties of *Lactobacillus rhamnosus* and *Lactobacillus paracasei* isolated from human faeces. Eur J Nutr 48:355-363.

Walsh GA, Power RF, Headon DR. 1993. Enzymes in the animal-feed industry. Trends Biotechnol 11:424-430.

Wang YW, Jones PJH. 2004. Conjugated linoleic acid and obesity control: Efficacy and mechanisms. Int J Obes 28:941-955.