Probiotic property and anti-obesity effect of *Lactiplantibacillus plantarum* KC3

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Abstract

Lactic acid bacteria (LAB) are representative probiotics that have beneficial effects on humans. Nineteen strains among the 167 single strains from kimchi was selected and investigated their physiological features. 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; *L. 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/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 (EFSA, 2012), bile salt and low pH tolerance, biogenic amine formation (EFSA, 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.

Material 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

Lactic acid bacteria were isolated from 40 kinds of homemade kimchi by using a modified

MRS medium (Lim et al., 2011). The strain was cultured for 18 h at 37℃ in *Lactobacillus* MRS broth (Difco, USA) and stored at -80℃. Before use, the stock cultures were grown twice at 37℃ 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 Corp., 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, 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: 3.5mg/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

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 InstaGeneTM Matrix (Bio-Rad, 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 0.3g/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) with or without 0.3% ox gall (Sigma). 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). Biogenic amine formation was tested with LB agar (pH 5.0; 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 (2008), 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 x 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 2000 x *g* for 20 minutes. 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 Inc., USA).

Results and discussion

Isolation and screening of lactic acid bacteria

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 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

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 *Lactiplantibacillus plantarum* type strain. Based on the nucleotide sequence of the 16S rDNA gene, it was confirmed that it was identical to *Lactiplantibacillus plantarum*, and it was named *Lactiplantibacillus plantarum* KC3.

Antibiotic susceptibility of *L. plantarum* KC3

193 The tolerance of the *L. plantarum* KC3 to 16 types of antibiotics is shown in Table 2.
194 According to Klarin et al. (2019), the ampicillin MIC values of *L. plantarum* 299v and *L.*
195 *plantarum* 299 were both 0.094 μg/mL. The MIC of *L. plantarum* KC3 showed a high
196 resistance to ampicillin (MIC>256 μg/mL); while its resistance to penicillin, vancomycin,
197 gentamicin, streptomycin, erythromycin, clindamycin, and chloramphenicol was found to be
198 similar to the 46 *L. plantarum* strains reported by Klare et al. (2007). KC3 was more sensitive
199 to clindamycin and erythromycin than to other antibiotics, but showed the highest resistance to
200 ampicillin and vancomycin. The resistance of KC3 to kanamycin, streptomycin, clindamycin,
201 rifampicin, and chloramphenicol was within the range accepted by the European Food Safety
202 Authority (EFSA, 2008) and the Scientific Committee for Animal Nutrition (European
203 Commission, 2001). However, KC3 was resistant to gentamycin, ampicillin, ciprofloxacin,
204 tetracycline, and vancomycin, and had an equal or higher MICs according to the European
205 Food Safety Authority (EFSA, 2008) and the Scientific Committee for Animal Nutrition
206 (European Commission, 2001).

207 **Enzyme activity of *L. plantarum* KC3**

208 Unlike *Bacillus* spp. and fungi, *Lactobacillus* is known for producing intracellular enzymes
209 (Jeon, 1998). The results of the enzyme activity of *L. plantarum* KC3 are shown in Table 2. *L.*
210 *plantarum* KC3 produced enzymes such as esterase, lipase, leucine arylamidase, valine
211 arylamidase, cystimearylamidase, acid phosphatase, naphtol-AS-BI-phosphohydrolase, β-
212 galactosidase, α-glucosidase, β-lucosidase, and N-acetyl-β-glucosaminidase. In particular, it
213 produced large amounts of such enzymes as leucine arylamidase, valine arylamidase, β-
214 galactosidase, and β-glucosidase. However, no activity associated with β-glucuronidase, a pro-
215 carcinogenic enzyme that converts benzopyrene to a carcinogenic substance (Rhee et al., 1998)
216 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 can alleviate the symptoms of lactose intolerance by converting lactose into galactose and glucose in milk (De Verse et al., 2003). The β-galactosidase activity in *L. plantarum* KC3 was determined at 5 degree.

Bile acid and acid tolerance of *L. 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. 1(A). 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 (Erkkila and Petaja, 2000). High acid tolerance was detected in strain KC3. The results of pH tolerance of *L. plantarum* KC3 and

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

246 **Antimicrobial activity of *L. plantarum* KC3**

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

254 The antimicrobial effects of LAB in the GIT are related to inhibition during pH reduction, the
255 competition for consumption between nutrients and pathogens, a reduction of redox potential,
256 the production of hydrogen peroxide under aerobic conditions, and the secretion of
257 antimicrobial active substances such as bacteriocin (Havenaar et al., 1992). Some strains of
258 LAB produce different antimicrobial compounds which can prevent the growth of pathogenic
259 and spoilage bacteria (Ahmadova et al., 2013). The antimicrobial activity of a strain varies
260 depending on the pathogen, even if it is a strain of the same species (Jacobsen et al., 1999).

261 **Adhesive property of *L. plantarum* KC3**

262 The ability to adhere to the intestinal epithelium is one of the key criteria when selecting
263 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. 2(C). *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 formation of *L. plantarum* KC3

Some LAB strains may form biogenic amine (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 (EFSA, 2011). *L. plantarum* KC3 did not show any biogenic amine formation from the precursor amino acids used in this study (Data not shown), namely tyrosine, histidine, ornithine, and lysine,.

Anti-obesity effect of *L. 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 was used as the positive chemical control for anti-obesity. Each animal group that received the LGG strain, Xenical (Xen) and KC3 showed a significantly lower bodyweight and total weight gain compared to the high-fat diet group (HFD) (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 (LDLC) - 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, 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.

Conflict of Interest

The authors declare no potential conflicts of interest.

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Auther Contributions

Conceptualization: Lim SD, Holzapfel WH. 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: Lim SD, Holzapfel WH.

Ethics Approval

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

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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

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*
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	2048	-	α-glucosidase	3
Choloramphenicol	4	8	β-glucosidase	4
			N-acetyl-β-glucosaminidase	3
			α-mannosidase	0
			α-fucosidase	0

*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.

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

Pathogens	Viable numbers (CFU/mL) of pathogens[a]				Inhibition (%)
	Without KC3[a]		With KC3*[a]		
	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%

*Initial count of *Lactobacillus plantarum* KC3: $2.10\pm0.17\times10^6$ CFU/mL[a] as determined after 6 h of incubation at 37 ℃. All values are within the mean ± standard deviation of the three replicates.

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 *L. rhamnosus* strain GG mixed with a high-fat diet, *299v* = receiving *L. 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.

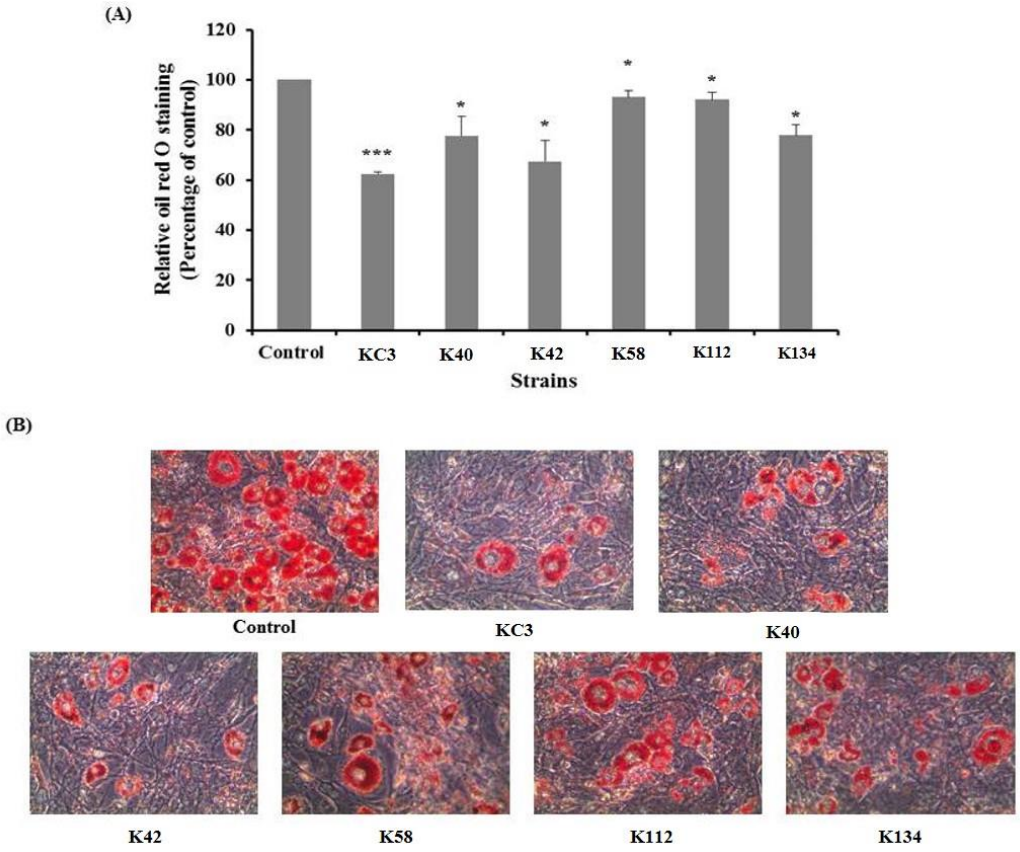

Fig. 1. The effect of the 6 selected strains on adipocytes revealed by oil red O staining in 3T3-L1. (A) quantification of oil red O staining. (B) photograph of oil red O staining. The cells were stained with oil red O and observed with a microscope (original magnification×200). *$p<0.05$ and ***$p<0.001$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

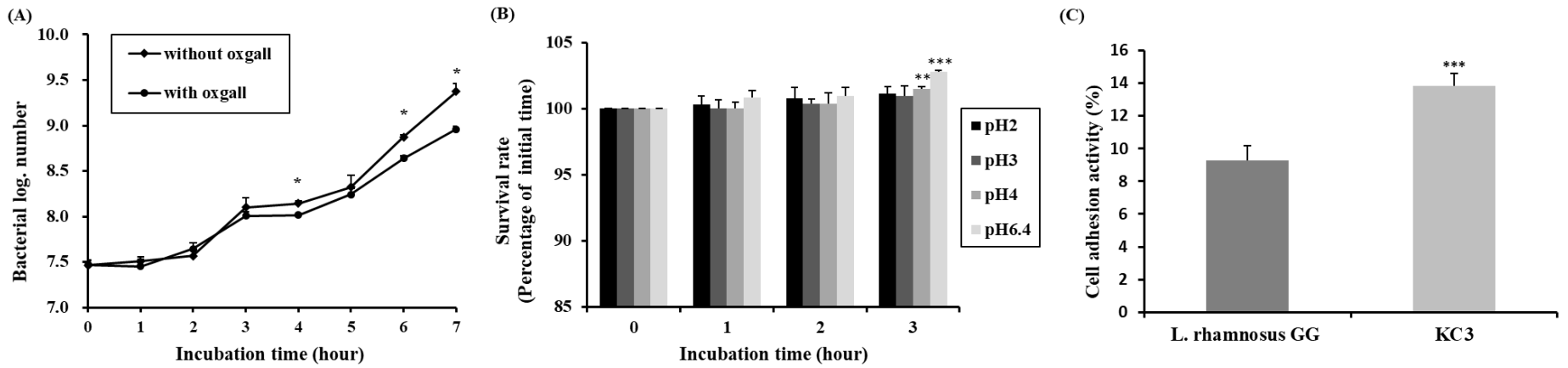

Fig. 2. Bile acid tolerance, acid tolerance, and adhesive property of *Lactobacillus plantarum* KC3. (A) Growth in MRS broth containing 0.05% L-cysteine with/without 0.3% ox gall. (B) Survival rates after three hours in HCl solution (pH 2.0, 3.0, 4.0 and 6.4). (C) Adhesion ability to HT-29 cell line,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