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Anti-adipogenic Effects of the Probiotic *Lactiplantibacillus plantarum* KU15117 on 3T3-L1 Adipocytes

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Abstract

In this study, we investigated the probiotic properties and anti-obesity effects of bacterial strains isolated from homemade kimchi. *Lactiplantibacillus plantarum* KU15117 was isolated using lactobacilli selective medium. *L. plantarum* KU15117 did not produce β-glucuronidase and showed high tolerance to artificial gastric juice and bile salt, acceptable resistance to antibiotics, and high adhesion ability to HT-29 cells. The anti-adipogenic activity of *L. plantarum* KU15117 at 10^9 CFU/well was confirmed by the reduction of oil red O staining and intracellular triglyceride level. Additionally, the expression levels of fatty acid synthase, CCAAT/enhance-binding protein-α, and peroxisome proliferator-activated receptor-γ, which are associated with the early stage of adipocyte differentiation, were significantly lower in the probiotic-treated group than in the control group. These results suggest that *L. plantarum* KU15117 has probiotic properties and anti-obesity effects and could be used as a prophylactic probiotics.

Keywords Probiotics · Kimchi · *Lactiplantibacillus plantarum* · Anti-adipogenic effect · Obesity

Abbreviations

FAS	Fatty acid synthase
C/EBP-α	CCAAT/enhance-binding protein-α
PPAR-γ	Peroxisome proliferator-activated receptor-γ

Introduction

Changes in lifestyles, such as reduction in physical activities and convenient lifestyles, may induce obesity, which is not only a cosmetic problem but also a major health issue. Obesity is associated with abnormal or excessive fat accumulation leading to various diseases, including non-alcoholic fatty liver disease, cardiovascular diseases, type 2 diabetes, cancer, and hypertension [1, 2]. Particularly, obesity is caused by an imbalance between lipogenesis and lipolysis, which are complex processes regulated by various signaling molecules. Adipogenesis is characterized by changes in cell morphology, accumulation of triglycerides, and expression of related gene [3, 4]. Peroxisome activated receptor-γ (PPAR-γ) and CCAAT/enhancer-binding protein-α (C/EBP-α) are involved in the early stage of adipocyte differentiation, and some enzymes, including adipose-specific fatty acid-binding protein (aP2), fatty acid synthase (FAS), sterol regulatory element-binding protein-1c (SREBP-1c), and carnitine palmitoyltransferase-1 (CPT-1), are involved in the formation of mature adipocytes [5, 6].

Probiotics are live bacteria, mainly lactic acid bacteria, which are beneficial to humans and animals by improving intestinal microbial balance [7]. Common probiotics include representatives of *Lactobacillus acidophilus*, *Lacticaseibacillus casei*, *Lactiplantibacillus plantarum*, *Lacticaseibacillus rhamnosus*, *Bifidobacterium bifidum*, and *Bifidobacterium longum*. Some of these probiotics can be components of functional foods and may be sold as dietary supplements. Few studies have reported the pleiotropic effects of probiotics, including antimicrobial, anticancer, anti-inflammatory, antioxidant, anti-biofilm, anti-obesity, antidiabetic, and cholesterol-lowering activities [8, 9].

The gut microbiome influences human health and consists of more than 100 trillion bacterial species [10]. They include the commensal bacteria, which are involved in digestion related to metabolic disorders [11]. The cell-free extract (CFE) of *Lactobacillus fermentum* MG4231 and

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MG4244 strains showed anti-obesity effects through the inhibition of adipogenesis and lipid accumulation in 3T3-L1 preadipocytes. The anti-obesity effects of CFE also involved the downregulation of FAS, aP2, PPAR-γ, and C/EBP-α expression, as well as upregulating of AMP-activated protein kinase (AMPK) and hormone-sensitive lipase (HSL) phosphorylation [4, 12]. The adjustment of the gut microbiome has been suggested as a therapeutic approach against obesity and metabolic disorders. Therefore, in this study, we determined the probiotic properties and anti-adipogenic effects of *Lactobacillus* strains isolated from homemade kimchi.

Materials and Methods

Bacterial Strains and Culture Conditions

Lactiplantibacillus plantarum KU15117 (KCCM 12212P) and *Latilactobacillus curvatus* KU15031 were isolated using Lactobacillus Selective Medium (BD BBL, Franklin Lakes, NJ, USA) from Korean homemade diced-radish kimchi and cabbage kimchi. The commercial probiotic strain *Lacticaseibacillus rhamnosus* GG (KCTC 5033) was used as the reference strain. *Lactobacillus* strains were cultured in MRS broth at 37 °C for 24 h.

Tolerance of Bacterial Strains to Artificial Acid and Bile Salt

The tolerance of bacterial strains to artificial acid and bile salt conditions was determined as previously described by Lee et al. [9] and Son et al. [13]. To determine the tolerance of the strains to artificial acid, overnight cultures of bacterial strains were resuspended in artificial gastric acid (pH 2.5) (MRS medium containing 0.3% (w/v) of pepsin (Sigma-Aldrich, St. Louis, MO, USA)), followed by incubation at 37 °C for 3 h. To determine tolerance to bile acid, overnight cultures were resuspended in MRS medium containing 0.3% (w/v) of oxgall (BD BBL), followed by incubation at 37 °C for 24 h. Viable cells were counted after plating and incubated on MRS agar at 48 °C for 24 h. The survival rate was calculated as follows:

$$\text{Survival rate (\%)} = \frac{\text{Cell no. of after reaction (CFU)}}{\text{Initial cell no. (CFU)}} \times 100$$

Enzyme Production

Enzyme production was measured using the API ZYM kit (BioMerieux, Lyon, France). Bacterial strains were centrifuged (12,000 × *g*, 4 °C, 10 min), and the harvested cells were resuspended in PBS at 10^5 CFU/mL. The resuspended cultures were inoculated in each well and incubated at 37 °C for 4 h. Next, ZYM reagents A and B were added to the cupules. The enzyme activity was determined as 0 to ≥ 40 nM based on the color change.

Adhesion of Bacterial Strains to HT-29 Cells

HT-29 (human colon adenocarcinoma, KCLB 30038) cell line was cultivated in RPMI 1640 (HyClone Laboratories, Inc., Logan, UT, USA) with 10% fetal bovine serum (FBS; HyClone Laboratories, Inc.) and 1% streptomycin/penicillin solution at 37 °C in 5% CO_2 atmosphere.

The adherence of bacterial strains to HT-29 cells was performed according to the method of Son et al. [13]. HT-29 cells were seeded by 1×10^5 cells/well in a 24-well plate and cultured at 37 °C for 24 h. Bacterial strains were inoculated into each well at approximately 10^7 CFU and cultured at 37 °C for 2 h. Non-adhered bacteria were removed by washing thrice with PBS, followed by the addition of 1 mL Triton X-100 (1% (v/v); Sigma-Aldrich) into each well and incubation at 37 °C for 10 min. Incubated cells were harvested from each well, and adherent bacterial cells were plated on MRS plates. Adhesion activity was calculated as follows:

$$\text{Adhesion activity (\%)} = \frac{\text{Adhered cell no. (CFU)}}{\text{Initial cell no. (CFU)}} \times 100$$

Antibiotic Sensitivity of Bacterial Strains

The sensitivity of the bacterial strains was measured according to the guidelines of the Clinical and Laboratory Standards Institute [15]. Each bacterial strain, at a concentration of 10^7 CFU/mL, was dispersed on MRS agar, and paper discs containing the antibiotics were placed on the plate after a few minutes. The antibiotics used were ampicillin (10 μg/disc), chloramphenicol (30 μg/disc), ciprofloxacin (5 μg/disc), doxycycline (30 μg/disc), gentamicin (10 μg/disc), kanamycin (30 μg/disc), streptomycin (10 μg/disc), and tetracycline (30 μg/disc). The plates were cultured at 37 °C for 24 h, and the inhibition zones were measured.

Anti-adipogenic Effect of Bacterial Strains

Preparation of Heat-Killed Bacteria

Bacterial strains were grown in MRS broth and washed twice with PBS by centrifugation at 12,000 × *g* at 4 °C for 10 min. The washed bacteria were resuspended in PBS at a final concentration of 10^8 and 10^9 CFU/mL, respectively, and the cells were plated to confirm the number of viable

cells. Each bacterial sample was heated in a water bath at 80 °C for 30 min.

Cell Culture and Differentiation of 3T3-L1 cells

3T3-L1 preadipocytes (ATCC CL-173) were cultured in Dulbecco's modified Eagle's medium (DMEM; HyClone Laboratories, Inc.) supplemented with 10% bovine calf serum (HyClone Laboratories, Inc.) and 1% streptomycin/penicillin solution at 37 °C at 5% CO_2. For adipocyte differentiation, the cells were seeded in 6-cm cell culture dishes at a density of 1.5×10^4 cells/dish and cultured until confluence (approximately 3 days). After confluence, the growth medium was replaced with the differentiation medium (MDI), consisting of DMEM, 10% FBS, 0.5 mM 3-isobutyl-1-methylxanthine (IBMX; Sigma-Aldrich), 10 µM dexamethasone (Sigma-Aldrich), and 5 µg/mL insulin (Sigma-Aldrich), and the cells were cultured for 2 days. Next, the medium was changed to insulin media containing DMEM, 10% FBS, and 5 µg/mL insulin, which was replaced at 2, 4, and 6 days.

Cell Viability of 3T3-L1

The effect of bacterial strains on the viability of 3T3-L1 cells was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich) assay. 3T3-L1 cells were plated at 1×10^4 cells/well in 24-well plates until confluence. Next, the *Lactobacillus* strains were added at 10^8 and 10^9 CFU/well and incubated at 37 °C for 48 h in an incubator (5% CO_2). The supernatant was aspirated, and the cells were incubated with MTT solution (2.5 mg/mL) for 1 h. After discarding the supernatant, DMSO (Sigma-Aldrich) was added to each well to dissolve the generated formazan. The dissolved solution was measured at 570 nm using a microplate reader, and cell viability was calculated.

Oil Red O Staining and Intracellular Triglyceride Contents

The effects of bacterial strains on oil red O-stained differentiated 3T3-L1 were determined as described by Park et al. [16]. Differentiated 3T3-L1 cells were fixed with 10% formaldehyde solution for 20 min, followed by the addition of 0.5% oil red O solution (Sigma-Aldrich) to each dish and incubation at room temperature for 20 min. After staining, the cells were washed twice with PBS and isopropanol was added to each dish, and the absorbance was measured at 520 nm.

To determine the intracellular triglyceride level, a triglyceride quantification kit (BioVision, Milpitas, CA, USA) was used. Differentiated 3T3-L1 cells were harvested and centrifuged at $14{,}000 \times g$ for 25 min at 4 °C. Triglyceride levels were determined according to the manufacturer's protocol.

Semi-Quantitative RT-PCR Analysis

The 3T3-L1 cells were seeded in 6-cm cell culture dishes (1.5×10^4 cells/dish) and differentiated into mature adipocytes. This was followed by the addition of bacterial strains (10^8 and 10^9 CFU/well). RNA was isolated from the treated 3T3-L1 cells using the RNeasy Mini Kit (Qiagen, Germany), and cDNA was synthesized using the Revert Aid First Strand cDNA Synthesis Kit (ThermoFisher Scientific, MA, USA). The expression of adipogenesis-related genes was measured by RT-PCR using synthesized cDNA, primers (shown in Table 1), and SYBR Green PCR Master Mix (PikoReal 96, ThermoFisher Scientific). The RT-PCR conditions were 95 °C for 2 min, 40 cycles of 95 °C for 5 s, and 60 °C for 15 s. Gene expression was determined by relative quantification with β-actin as the house-keeping gene.

Western Blot Analysis

The expression of obesity-related proteins was investigated by western blotting. Differentiated 3T3-L1 adipocytes treated with bacterial strains (10^8 and 10^9 CFU/well) were harvested by using RIPA lysis and extraction buffer with Halt™ Protease and Phosphatase Inhibitor Cocktail (ThermoFisher Scientific), and the cell lysates were sonicated (5 AMP; pulse on, 3 s; pulse off 3 s) for a total period of 9 s and placed on ice. The sonicated cell lysate was harvested, and the supernatant was obtained by centrifugation at $14{,}000 \times g$ at 4 °C for 25 min. The protein concentration of the supernatant was measured using a DC™ protein assay kit (Bio-Rad Laboratories Inc., Hercules, CA, USA). Each protein was separated using sodium dodecylsulfate–polyacrylamide gel electrophoresis gel. The separated proteins were transferred onto a polyvinylidene fluoride membrane (Millipore, Bedford, MA, USA). The membrane was blocked with 5% skim milk for 30 min and reacted with a specific primary antibody at 4 °C for 20 h.

Table 1 Primer sequences for semiquantitative reverse-transcription polymerase chain reaction analysis

Gene	Primer sequence (5′→3′)	
β-Actin	Sense	5′-TGT CCA CCT TCC AGC AGA TGT-3′
	Antisense	5′-AGC TCA GTA ACA GTC CGC CTA GA-3′
FAS	Sense	5′-AGG GGT CGA CCT GGT CCT CA-3′
	Antisense	5′-GCC ATG CCC AGA GGG TGG TT-3′
C/EBPα	Sense	5′-GGA ACT TGA AGC ACA ATC GAT C-3′
	Antisense	5′-TGG TTT AGC ATA GAC GTG CAC A-3′
PPARγ	Sense	5′-TTG ATT TCT CCA GCA TTT CT-3′
	Antisense	5′-RTG TTG TAG AGC TGG GTC TTT-3′

FAS fatty acid synthase, *C/EBP-α* CCAAT/enhancer-binding protein-α, *PPAR-γ* peroxisome proliferator-activated receptor-γ

The membrane was then incubated with a horseradish peroxidase–conjugated secondary antibody for 2 h. The protein bands were visualized using a chemiluminescence detection kit (Thermo FisherScientific), and the thickness was analyzed using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Statistical Analysis

All experiments were performed in triplicate and presented as the mean ± standard deviation using one-way analysis of variance (ANOVA) and Duncan's multiple range test. Values were considered significant at $P < 0.05$. All analyses were conducted using the Statistical Package for the Social Sciences (SPSS), version 24 (IBM, Chicago, IL, USA).

Results

Gastric Acid and Bile Tolerance, Enzyme-Production Ability, Antibiotic Susceptibility, and Adhesion Ability of Bacterial Strains

The tolerance of *L. rhamnosus* GG, *L. curvatus* KU15031, and *L. plantarum* KU15117 to gastric acids and bile salts is shown in Table 2. All bacterial strains exhibited high resistance with over 96% of survival rate in gastric conditions (0.3% pepsin, pH 2.5). *L. rhamnosus* GG (102.88%) exhibited higher resistance than *L. curvatus* KU15031 (92.33%) and *L. plantarum* KU15117 (84.70%) in bile conditions (0.3% oxgall).

The enzymes produced by the different bacterial strains are shown in Table 3. The tested bacterial strains did not produce α-galactosidase (diabetes-related enzyme) or β-glucuronidase. However, *L. rhamnosus* GG and *L. plantarum* KU15117 produced β-galactosidase.

The antibiotic sensitivity of bacterial strains is presented in Table 4. *L. curvatus* KU15031 had a similar sensitivity to *L. rhamnosus* GG, except for ampicillin (10 μg). *L. plantarum* KU15117 was resistant to ampicillin (10 μg) and doxycycline (30 μg).

Table 3 Enzymatic activities of bacterial strains

Enzyme (nM)	*L. rhamnosus* GG	*L. curvatus* KU15031	*L. plantarum* KU15117
Control	0	0	0
Alkaline phosphatase	0	5	0
Esterase	10	0	0
Esterase lipase	5	0	0
Lipase	0	0	0
Leucine arylamidase	20	20	30
Valine arylamidase	20	10	20
Cystine-arylamidase	0	0	0
Trypsin	0	0	0
α-Chymotrypsin	0	0	0
Acid phosphatase	5	5	5
Naphthol-AS-BI-phosphohydrolase	10	5	5
α-Galactosidase	0	0	0
β-Galactosidase	5	0	10
β-Glucuronidase	0	0	0
α-Glucosidase	0	0	5
β-Glucosidase	5	0	30
N-Acetyl-β-glucosaminidase	0	0	10
α-Mannosidase	0	0	0
α-Fucosidase	0	0	0

The bacterial strains showed a high adhesion rate to HT-29 cells (Fig. 1). *L. rhamnosus* GG exhibited a higher adhesion (6.37%) than *L. curvatus* KU15031 (1.33%) and *L. plantarum* KU15117 (2.34%).

Effects of Bacterial Strains on Lipid Accumulation during Adipogenesis

The viability of 3T3-L1 cells treated with *L. plantarum* KU15117 and *L. rhamnosus* GG at 10^8 and 10^9 CFU/well

Table 2 Tolerance of bacterial strains to gastric acid and bile salt conditions

Lactobacillus strains	Viable cell no. (Log CFU/mL)		
	L. rhamnosus GG	*L. curvatus* KU15031	*L. plantarum* KU15117
Initial cell number	8.31 ± 0.09	7.73 ± 0.03	8.50 ± 0.04
Gastric acid condition (0.3% Pepsin, pH 2.5, 3 h)	8.25 ± 0.10	7.72 ± 0.08	8.24 ± 0.11
Survival rate (%)	99.27 ± 00.73[a]	99.15 ± 1.33[a]	96.93 ± 0.12[b]
Bile salt condition (0.3% Oxgall, 24 h)	8.55 ± 0.02	7.31 ± 0.44	7.20 ± 0.01
Survival rate (%)	102.88 ± 0.17[a]	92.33 ± 4.88[b]	84.70 ± 0.05[c]

All values are expressed as mean ± standard deviation. Values with different letters in the same row are significant different for each characteristic ($P < 0.05$)

Table 4 Antibiotic susceptibility of bacterial strains

Antibiotics	*L. rhamnosus* GG	*L. curvatus* KU15031	*L. plantarum* KU15117
Ampicillin (10 μg)	S	R	S
Gentamycin (10 μg)	R	R	R
Kanamycin (30 μg)	R	R	R
Streptomycin (10 μg)	R	R	R
Tetracycline (30 μg)	S	S	R
Ciprofloxacin (30 μg)	R	R	R
Chloramphenicol (30 μg)	S	S	R
Doxycycline (30 μg)	S	S	S

Resistant according to the CLSI breakpoints [15]

S susceptible, *I* intermediate, *R* resistant

was over 96% (Fig. 2). However, the viability of the cells treated with *L. curvatus* KU15031 was 34.58% and 26.79% at 10^8 and 10^9 CFU/well, respectively. Therefore, *L. plantarum* KU15117 and *L. rhamnosus* GG were used to determine lipid accumulation.

L. plantarum KU15117 and *L. rhamnosus* GG inhibited lipid accumulation, as shown by oil red O staining (Fig. 3A). In comparison with that of the positive control (100.81%), the oil red stain contents was as follows: *L. plantarum* KU15117 (31.88%) at 10^9 CFU/well < *L. rhamnosus* GG (45.02%) at 10^9 CFU/well < *L. plantarum* KU15117 (86.95%) at 10^8 CFU/well < *L. rhamnosus* GG (96.78%) at 10^8 CFU/well (Fig. 3B). In addition, triglyceride contents of positive control were 5.661 mM, while *L. plantarum* KU15117 and *L. rhamnosus* GG showed 0.399 mM and 1.684 mM at 10^9 CFU/well, respectively (Fig. 3C).

Effects of Bacterial Strains on the mRNA and Protein Levels of Adipogenesis-Related Genes in Differentiated 3T3-L1 Adipocytes

Figure 4 shows the regulation of *FAS*, *C/EBP-α*, and *PPAR-γ* mRNA levels in differentiated 3T3-L1 adipocytes. *L. rhamnosus* GG and *L. plantarum* KU15117 at 10^9 CFU/well significantly decreased the mRNA levels of the genes in 3T3-L1 cells. Particularly, *L. plantarum* KU15117 at 10^9 CFU/well decreased the expression of *FAS* (92.96%), *C/EBP-α* (99.41%), and *PPAR-γ* (95.26%) (Fig. 4A–C). *L. rhamnosus* GG at 10^8 CFU increased the expression of *FAS*, *C/EBP-α*, and *PPAR-γ* mRNA.

The protein expression of adipogenic transcription factors and enzymes, including FAS, C/EBP-α, and PPAR-γ (Fig. 4D) was confirmed by western blot analysis. In the positive control, the expression levels of FAS, C/EBP-α, and PPAR-γ proteins were 3.28, 6.29, and 3.67, respectively. *L. rhamnosus* GG at 10^9 CFU/well decreased the protein expression by 0.96, 1.33, and 0.24, respectively. *L. plantarum* KU15117 at 10^8 CFU/well and *L. plantarum* KU15117 at 10^9 CFU/well significantly decreased the expression levels of FAS (3.08 and 0.91, respectively), C/EBP-α (2.74 and 1.15, respectively), and PPAR-γ (1.97 and 0, respectively). The expression of FAS, C/EBP-α, and PPAR-γ proteins following treatment with LGG-8 was 3.32, 6.36, and 2.64, respectively. These results display a similar trend to those of mRNA expression levels.

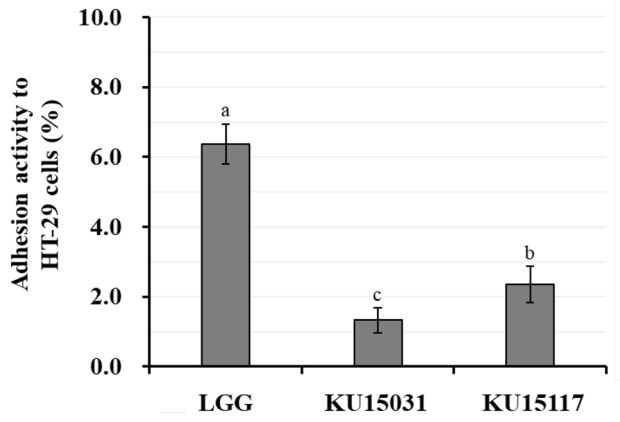

Fig. 1 Adhesion activity of bacterial strains to HT-29 cells. LGG, *L. rhamnosus* GG; KU15031, *L. curvartus* KU15031; KU15117, *L. plantarum* KU15117. Error bars indicate standard deviation of three independent experiments. All values are expressed as mean ± standard deviation. Letters denote statistical significance ($P < 0.05$) as determined by Duncan's multiple range test

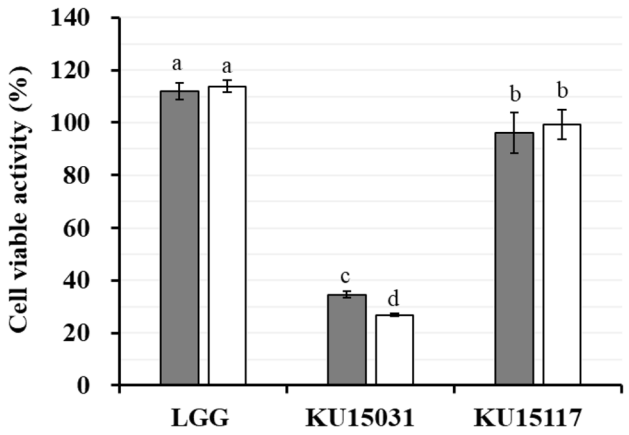

Fig. 2 Effects of bacterial strains on the viability of 3T3-L1 adipocytes. LGG, *L. rhamnosus* GG; KU15031, *L. curvartus* KU15031; KU15117, *L. plantarum* KU15117. Filled square, 10^8 CFU/well; empty square, 10^9 CFU/well. All values are expressed as mean ± standard deviation. Letters denote significance ($P < 0.05$) as determined by Duncan's multiple range test

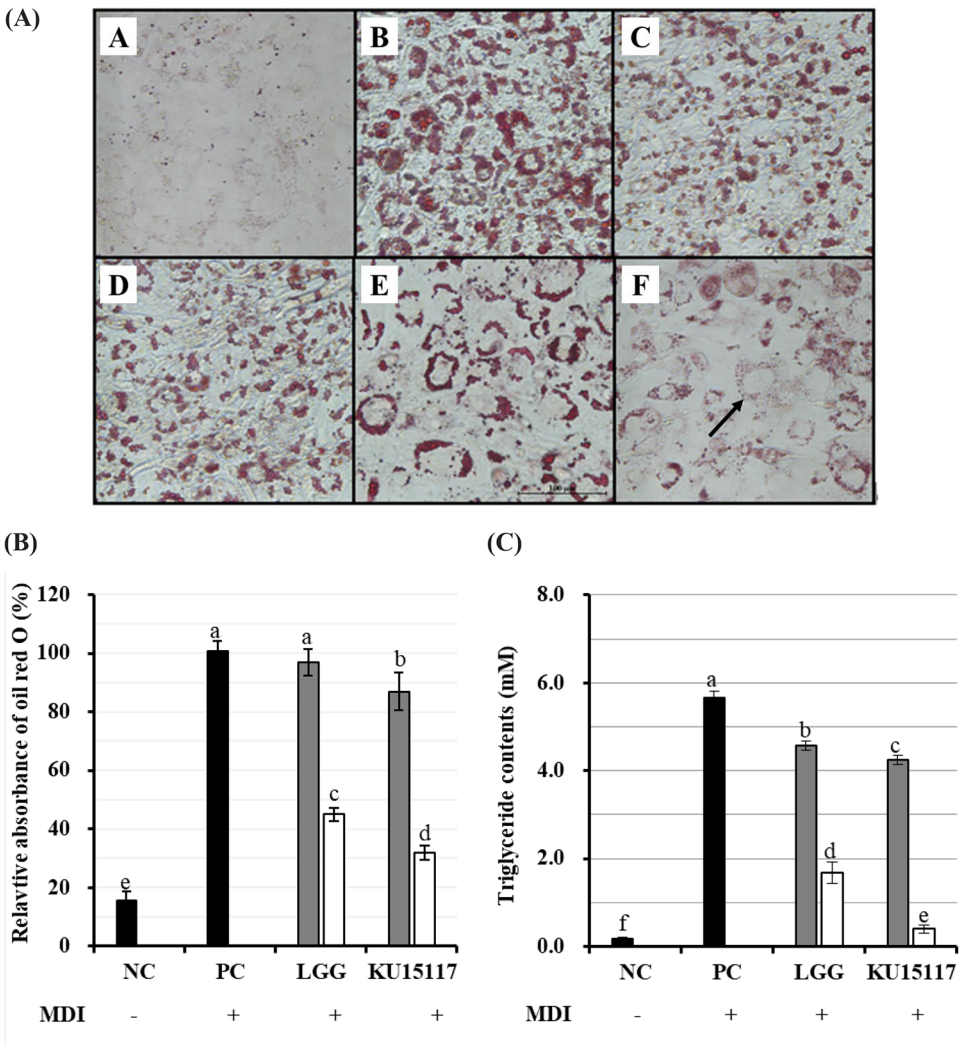

Fig. 3 Anti-obesity effects of bacterial strains on 3T3-L1 adipocytes. **A** Photograph of oil red O staining, **B** related absorbance of oil red O staining, and **C** triglyceride content. A, NC (negative control, non-treated with MDI in adipocytes); B, PC (positive control, treated with MDI in adipocytes); C, *L. rhamnosus* GG (10^8 CFU/well); D, *L. rhamnosus* GG (10^9 CFU/well); E, *L. plantarum* KU15117 (10^8 CFU/well); F, *L. plantarum* KU15117 (10^9 CFU/well); gray square, 10^8 CFU/well; white square, 10^9 CFU/well, LGG, *L. rhamnosus* GG; KU15117, *L. plantarum* KU15117. All values are expressed as mean ± standard deviation. Letters denote significance ($P < 0.05$) as determined by Duncan's multiple range test

Discussion

Probiotics are used as food formulations for prophylactic therapy against metabolic syndrome. In this study, the anti-obesity effects of lactic acid bacteria isolated for probiotic use were investigated. Tolerance to gastric conditions is an essential characteristic, which influences the probiotic properties of bacterial strains in the intestine [14]. Under strongly acidic and bile salt conditions, the survival rate of *Levilactobacillus brevis* KU15153 reduced by 70.79% and increased by > 104.47%, respectively [17]. *L. curvatus* KU15031 and *L. plantarum* possessed probiotic properties, as indicated by the resistance to gastric conditions.

Some probiotic bacteria produce useful β-galactosidase, which decreases lactose intolerance [18]. However, some probiotic bacteria also produce deleterious enzymes, such as β-glucuronidase, which have been associated with the induction of carcinogenesis, mutagenesis, and toxicity [19]. Son et al. [13] indicated that probiotic *L. plantarum* FI10604 and *L. brevis* FI10700 do not produce β-glucuronidase. *Lactococcus lactis* KC24 produces various enzymes, including acid phosphatase, β-galactosidase, and naphthol-AS-BI-phosphohydrolase, but not β-glucuronidase [14]. Similarly, we showed that *L. curvatus* KU15031 and *L. plantarum* KU15117 do not produce α-galactosidase and β-glucuronidase.

The sensitivity of probiotic bacteria to antibiotics is a fundamental factor because antibiotic-resistant strains may not be easily eliminated if required, and the antibiotic resistance may be transmitted to pathogenic or potentially pathogenic bacteria [20]. *L. plantarum* Ln4 is sensitive to commercial antibiotics, such as chloramphenicol, doxycycline, ampicillin, and tetracycline [13]. *Lactobacillus* spp. have intrinsic resistance to aminoglycosides (kanamycin and streptomycin) or quinolones (ciprofloxacin) [21]. Therefore, these results confirm that *L. curvatus* KU15031 and *L. plantarum* KU15117 are safe in accordance with the CLSI guidelines [15].

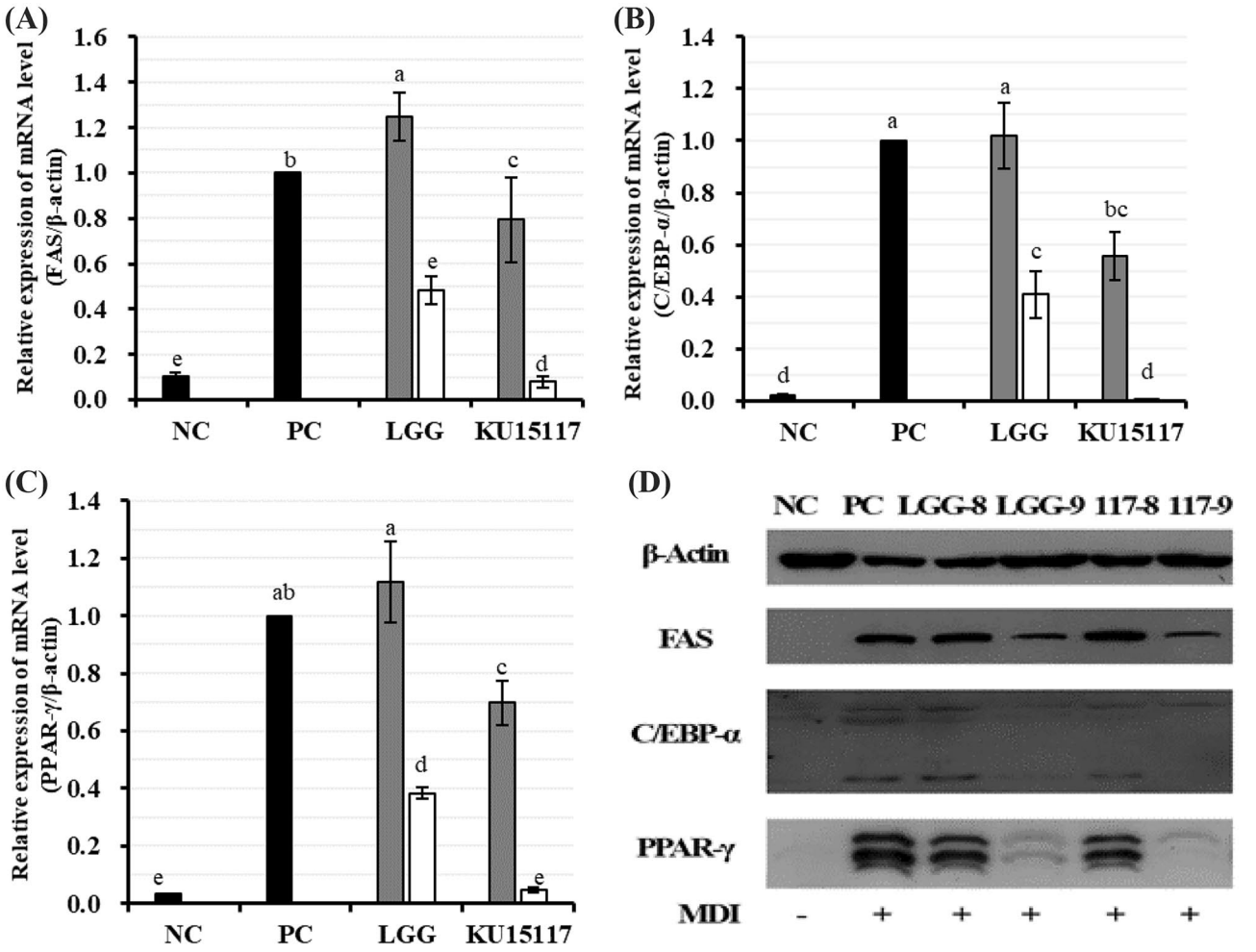

Fig. 4 Anti-adipogenic effects of bacterial strains in MDI-induced differentiation of 3T3-L1 preadipocytes. **A** FAS, **B** C/EBPα, **C** PPAR-γ gene expression of lipid metabolism-related genes, and **D** adipogenic protein expression. NC (Negative control), not treated with MDI in adipocytes; PC (Positive control), treated with MDI in adipocytes; LGG, *L. rhamnosus* GG; KU15117, *L. plantarum* KU15117. Gray square, 10^8 CFU/well; white square, 10^9 CFU/well; β-actin, loading control; LGG-8, 10^8 CFU/well of *L. rhamnosus* GG; LGG-9, 10^9 CFU/well of *L. rhamnosus* GG; 117–8, 10^8 CFU/well of *L. plantarum* KU15117; 117–9, 10^9 CFU/well of *L. plantarum* KU15117. All values are expressed as the mean ± standard deviation and standardized against the β-actin housekeeping gene. Values with different letters in the same row indicate significant differences for each characteristic ($P < 0.05$)

The adhesion of bacterial strains to intestinal cells is the most important factor associated with their probiotic properties [22]. Song et al. [23] showed that *L. brevis* KCCM 12203P (6.84%) and *L. rhamnosus* GG (6.21%) have similar adhesion rates. Zhang et al. [24] and Jeon et al. [25] showed that *L. plantarum* strains (< 2%) and *B. subtilis* P223 (1.33%) have diminished adhesion to intestinal epithelial cells, respectively. Therefore, *L. curvatus* KU15031 (1.33%) and *L. plantarum* KU15117 (2.34%) have acceptable adhesion rates to HT-29 cells.

Obesity is related to the differentiation, expansion, and lipid accumulation of adipocytes [4]. *L. brevis* B151, *L. fermentum* KCCM 200060, and *L. plantarum* Ln4 exhibited reduced lipid accumulation in both heat-killed cells and freeze-dried broth [11]. During the differentiation period, *L. plantarum* Q180 dose-dependently inhibited 3T3-L1 adipogenesis in terms of lipid accumulation by 14.63% compared with that by control cells [26]. Park et al. [27] reported that the addition of *L. brevis* OPK-3 (40 μg/mL) showed 40% reduction in triglyceride accumulation. In our data, *L. plantarum* KU15117 (92.95%) and *L. rhamnosus* GG (68.93%) inhibited lipid accumulation by triglyceride accumulation.

In the early stage of adipocyte differentiation, adipocyte-specific FAS, C/EBP-α, and PPAR-γ are regulated [28]. *Weissella koreensis* OK1-6 significantly reduced the mRNA expression levels of *SREBP1*, *aP2*, *FAS*, and *C/EBP-α* [29]. *L. brevis* OPK-3 significantly downregulated the mRNA expression of *C/EBP-α* and *PPAR-γ* in differentiating 3T3-L1 adipocytes [27]. Similarly, *L. plantarum* KY1032 decreased

the expression of PPAR-γ, C/EBP-α, FAS, and A-FABP proteins [30]. *L. acidophilus* and cocktail of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* were reduced adipogenesis genes (*PPAR-γ*, *CD36*, and *aP2*) expression [31]. These results showed that the mRNA and protein expression levels of heat-killed *L. plantarum* KU15117 at 10^8 CFU/well and 10^9 CFU/well might have been downregulated during adipocyte differentiation. Therefore, *L. plantarum* KU15117 could be influenced in the early stage of adipocyte differentiation in animal models.

In conclusion, we demonstrated the probiotic properties and anti-obesity effects of *L. plantarum* KU15117. *L. plantarum* KU15117 showed high tolerance to gastric conditions, safe enzyme activity, high adhesion rate to intestinal cells, and safe antibiotic sensitivity. Additionally, the anti-adipogenic activity of *L. plantarum* KU15117 was demonstrated by reduced lipid accumulation, low level of intercellular triglyceride, and suppressed expression of adipocyte-specific genes and proteins that are associated with the early stage of adipocyte differentiation. Therefore, *L. plantarum* KU15117 is a probiotic strain with anti-obesity effects. In addition, this study should be confirmed in animal model for further study.

Author Contribution Kyoung Jun Han: investigation, methodology, writing—original draft, validation. Na-Kyoung Lee: conceptualization, investigation, methodology, validation, writing—review and editing. Hyung-Seok Yu: investigation, methodology. Hoon Park: writing—review and editing, validation. Hyun-Dong Paik: conceptualization, supervision, writing—review and editing, validation.

Data Availability Statement All data generated or analyzed during this study are included in this article.

Declarations

Conflict of Interest The authors declare no competing interests.

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