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Effect of postbiotic *Lactiplantibacillus plantarum* LRCC5282 on obesity improvement by modulation of the gut microbiome

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ABSTRACT

This study aimed to evaluate the ameliorating effects of *Lactiplantibacillus plantarum* LRCC5282 (5282-P) postbiotic on obesity. Treatment with 5282-P decreased the ratio of *Firmicutes/Bacteroidetes*, increased the abundance of obesity regulation-associated genera, including *Bacteroides*, and elevated the production of short-chain fatty acids. These microbiome alterations correlated with reductions in adipose tissue weight, improvements in serum lipid profiles, and alleviation of obesity symptoms, including body weight and BMI. Additionally, the mRNA expression levels of adipogenesis-related genes, including leptin, PPARγ, SREBPα1, and CEBPα, were significantly downregulated. These findings suggest that 5282-P enhances metabolic health and improves obesity by promoting a diverse and balanced gut microbiome, making it a promising therapeutic candidate for obesity treatment.

1. Introduction

Obesity is a global health issue associated with numerous comorbidities, including diabetes, cardiovascular diseases, hypertension, and metabolic syndrome. In 1997, the World Health Organization defined obesity as a chronic disease. The prevalence of obesity has increased dramatically over the past few decades, and the market for obesity prevention and treatment is the highest in the fields of pharmaceuticals and healthcare. Despite extensive research and the development of several drugs for obesity treatment, long-term administration of these drugs has often resulted in decreased efficacy and side effects. Consequently, the discovery of safe and functional therapeutic agents to treat obesity is urgently needed.

Recent studies have highlighted the important role of the gut microbiota in regulating host metabolism, energy balance, and inflammation, making it a promising therapeutic agent. The gut microbiome, which is composed of trillions of microorganisms, plays a crucial role in maintaining metabolic homeostasis. Hence, it has potential advantages in preventing and managing various diseases, including diabetes, depression, atopic dermatitis, and obesity (Bielka, Przezak, & Pawlik, 2022; Maurya, Mogra, & Bajpai, 2014; Rios et al., 2017; Vallianou, Stratigou, Christodoulatos, & Dalamaga, 2019). Notably, the *Firmicutes/Bacteroidetes* ratio is often elevated in individuals with obesity, suggesting that specific microbial profiles contribute to the pathophysiology of obesity (Le Chatelier et al., 2013).

Probiotics and postbiotics have emerged as potential interventions to modulate the gut microbiota and improve metabolic health. Probiotics, which restore the composition of the gut microbiome, are microorganisms that influence host health. Postbiotics are new functional food ingredients derived from probiotics, and they include cellular components and metabolites produced during fermentation (Jackson et al., 2019).

In contrast to probiotics, postbiotics are not affected by gastric or bile acids and can be delivered to the intestines to exert their effects. In addition, their high stability even in high-temperature or high-pressure

Abbreviations: LAB, lactic acid bacteria; LRCC, LOTTE R&D Culture Collection; PCR, polymerase chain reaction; NCBI, National Center for Biotechnology Information; ATCC, American Type Culture Collection; DMEM, Dulbecco's modified Eagle's medium; ELISA, enzyme-linked immunosorbent assay; SCFAs, short-chain fatty acids; AL, alleviation; PR, prophylactic; RI, refractive index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglycerides; BW, body weight; BMI, body mass index; RNA, ribonucleic acid; DNA, deoxyribonucleic acid; cDNA, complementary DNA; DMI, dexamethasone, 3-isobutyl-1-methylxanthine, and insulin; CNT, control.

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environments make them applicable to various fields. Considering these advantages, numerous studies have reported the potential use of postbiotics in treating or preventing obesity (Kim et al., 2021; Lee et al., 2021; Yoon et al., 2022). However, whether postbiotics improve obesity remains to be verified.

Thus, this study aimed to investigate the effects of *Lactiplantibacillus plantarum* LRCC5282 postbiotic (5282-P) on obesity. Specifically, the effects of 5282-P treatment on metabolic health, as well as the underlying mechanisms, were explored by analyzing changes in microbial diversity, microbiota composition, and short-chain fatty acid (SCFA) production. This study provides insights into the relationship between gut microbiota changes and obesity and into the potential use of postbiotics in obesity treatment.

2. Material and methods

2.1. Isolation of lactic acid bacteria and postbiotic preparation

Traditionally fermented kimchi samples were macerated and blended with sterile saline (0.85 % NaCl). The obtained dilutes were spread onto de Man–Rogosa–Sharpe agar (Difco, USA) with 0.02 % bromocresol purple and then incubated for 24 h at 37 °C. The cultured colonies were identified as lactic acid bacteria (LAB) by 16S rRNA sequencing as previously described by Lane (1991), followed by the Basic Local Alignment Search Tool (BLAST) analysis. A total of 263 LAB strains were identified, and *Lactiplantibacillus plantarum* LOTTE R&D Culture Collection 5282 (LRCC5282) was selected for further experiments based on preliminary screening tests, which revealed genes associated with carbohydrate and lipid metabolism through whole genome sequencing (WGS). Additionally, in vitro studies demonstrated that LRCC5282 produces high levels of SCFAs when various sugars are provided as carbon sources (data not presented). Commercial L. *gasseri* BNRThin S30 was used as a positive control (P-CNT). This strain has been individually recognized by the Ministry of Food and Drug Safety (MFDS) of Korea for its approved functionality in body fat reduction. A commercial product containing this strain was purchased and utilized for the experiments.

The postbiotics of LRCC5282 were manufactured by Lactomason Co., Ltd. (Jinju-si, Gyeongsangnam-do, Korea). Briefly, LRCC5282 was inoculated into a 100 L fermenter containing sterilized media (4 % glucose, 0.3 % yeast extract, 0.05 % KH_2PO_4, 0.02 % $MgSO_4$, and 0.02 % $MnSO_4$) and then fermented under controlled conditions (37 °C, 48 h, pH 6.8 ± 0.2). The cultured broth was heat-treated at 90 °C for 30 min. The cell lysates were collected via centrifugation (15,000 ×*g*, 30 min, 4 °C), freeze-dried at −80 °C for 48 h, and then harvested. The lyophilized powder was considered as 5282-P, and these processes were repeated three times to standardize the manufacturing process.

2.2. Animals and experiments

Specific pathogen-free C57BL/6 J mice aged 6 weeks were obtained from the central laboratory (Seoul, Korea) and used in the experiments after a week of adaptation. During adaptation, the mice were provided with normal diet and water ad libitum, and five animals per group were assigned to cages. Subsequently, the animals were grouped randomly to ensure a balanced distribution of baseline characteristics, including initial body weight and age, across the experimental groups. The animals were housed under the following conditions: temperature of 23 ± 1 °C, humidity of 50 % ± 5 %, noise of 60 phones or less, light/dark cycle of 12 h/12 h, illumination of 150–300 Lux, and ventilation of 10–12 times per hour.

Seven animal groups were designed, including two types of obesity-inducing models, to investigate the alleviating and prophylactic effects of 5282-P treatment. To investigate the alleviating effect, we administered 5282-P (1×10^8 cells/mouse) and *Lactobacillus gasseri* BNR17 (1×10^8 cells/mouse dissolved in 200 μL of PBS to the mice for 4 weeks via oral gavage and then induced obesity by feeding them a high-fat diet (HFD) consisting of 60 % total calories (DIO Rodent Purified Diet, 58 V8, Test Diet, UK) for 6 weeks (AL-P-CNT, AL-5282-P, respectively). *L. gasseri* BNR17 (P-CNT), a commercial product named BNRThin (S30 (AceBiome, Seoul 06164, Korea), approved functionality in body fat reduction by the Ministry of Food and Drug Safety (MFDS) of Korea (Kim, Yun, Kim, Kwon, & Cho, 2018) was used as a positive control. The negative control group in the alleviation model (AL-N-CNT) was administered with only phosphate buffer saline (PBS; Sigma-Aldrich, USA), followed by HFD feeding. For prophylactic tests, commercial L. *gasseri* product B and 5282-P were administered simultaneously with HFD feeding for 10 weeks (PR-P-CNT, PR-5282, respectively). The negative control group in the prophylactic model (PR-N-CNT) was administered simultaneously with PBS and HFD feeding for 10 weeks. The normal control group (Normal) was fed a normal diet consisting of 18 % protein (Teklad Protein Rodent diet, 2918C, Envigo, UK). Thereafter, the mice were euthanized, and their visceral fat, liver, and blood were harvested for further analyses. All animal experiments and procedures were conducted in accordance with the regulations of the Animal Experimental Ethics Committee of Chung-Ang University (Approval No. 2018–00022).

2.3. Gut microbiome analysis

Microbiome analysis was performed as described previously (Kim, Kim, & Kim, 2019). A FastDNA Spin Kit for Soil (MP Biomedical, California, USA) was used to extract whole genomic DNA from the microbiome in the fecal samples. Polymerase chain reaction (PCR) amplification of the 16S rRNA gene (V3–V4 region) was performed using an Illumina MiSeq system (Macrogen Inc., Seoul, Korea) with a bacterial primer set (encoded by 347F / 803R). Raw reads with quality trimming were run using the Quantitative Insights into Microbial Ecology (Qiime2, version 2021.2) software. Demultiplexing resulted in artifacts, and noise removal was performed using DADA2 (Callahan et al., 2016). In this step, the chimeric sequences were filtered to maintain high-quality reads. Amplicon sequence variation analysis was performed for taxonomic and diversity analyses. Taxonomic assignments derived from individual datasets were determined using the Green Genes database (gg-13_8, 99 %). (McDonald et al., 2012). All data visualizations were analyzed using R Studio software (version 3.6.1). The α and β diversities were analyzed statistically using the phyloseq R package, and the permutational multivariate analysis of variance test was performed with 999 permutations using the adonis function of the vegan R package. Taxonomy and heatmap were analyzed using the phyloseq R and DESeq2 R packages (Kim et al., 2019).

2.4. Determination of SCFAs

SCFAs were determined as described previously (Kim et al., 2021). For SCFA sample preparation, fecal samples were collected from the mice and then centrifuged at 12,000 *g* for 20 min at 4 °C). The samples were filtered through a membrane filter (C18 Sep-Pak cartridges; Millipore, Waters, USA). The contents of SCFAs, including acetic acid, butyric acid, and propionic acid, were determined through high-performance liquid chromatography (Ultimate 3000, Thermo Dionex, USA) on an Aminex 87H column (300 × 10 mm, Bio-Rad, USA). They were measured at a wavelength of 210 nm using an RI detector (ERC, RefractoMAX520, Japan).

2.5. Measurement of adipocyte size and lipid profile in serum

Visceral fat tissues were collected from the sacrificed mice and fixed in 10 % (*v*/v) neutral-buffered formalin for 24 h at room temperature. The samples were fixed in paraffin, sectioned with a thickness of 4–5 μm, and then stained with hematoxylin and eosin. The size of the adipocytes was estimated under a light microscope (Leica, Wetzlar, Germany), and

their area was measured using ImageJ software (http://imagej.nih.gov/ij/). Blood samples were collected from the mice and centrifuged at 6000 *g* for 10 min at 4 °C to obtain the serum. Serum lipids, including high-density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol (TC), and triglyceride (TG), were analyzed at GC Labs (Green Cross Biopharmaceutical, Yongin).

2.6. Evaluation of obesity symptoms

The body weight (BW) and body length were measured weekly during the study period. BMI was calculated using the following formula: [BW (kg)/height^2 (m^2)]. In addition, food intake was measured once a week at a fixed time the next day after feeding.

2.7. mRNA expression of genes related to obesity and inflammation in vitro and in vivo

Total RNA was extracted from 3 T3-L1 cells and mouse fat tissue using the RNeasy mini kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's protocol and then dissolved in nuclease-free water. After the concentration of the extracted total RNA was measured with a Nano-Quant Spectrophotometer (Infinite 200; Tecan, Switzerland), the same concentration of RNA was prepared in a new 1.5 mL tube. The total RNA extract was reverse transcribed into cDNAs using the PrimeScript first-strand cDNA synthesis kit (Takara Bio, Shiga, Japan).

The mRNA expression levels of adipogenesis-related genes, including leptin, FAS, PPARγ, SREBPα1, and CEBP/α, were measured using ELISA kits (Abcam, Cambridge, UK; MyBioSource, San Diego, CA, USA) in accordance with the manufacturer's instructions. The mRNA expression levels of inflammation-related genes, including IL-6, IL-10, and TNF-α, were also analyzed using the above process. RT-qPCR was performed using the SYBR green PCR kit (Qiagen) and the 7500 Fast Real-time PCR system. The fold change of gene expression was calculated using the ΔΔCt method.

2.8. Inhibitory effect of LRCC5282-P on 3 T3-L1 adipocytes

3 T3-L1 cells were obtained from the American Type Culture Collection (ATCC, USA) and cultured at 37 °C in a 5 % CO_2 atmosphere. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, NY, USA) containing 10 % bovine calf serum (Sigma-Aldrich) and 1 % penicillin–streptomycin (Gibco, NY, USA). The cells were seeded at a density of 5×10^4 cells/well in 12-well plates and then cultured for 48 h at 37 °C. The cultured cells were treated with 10 % FBS-DMEM-DMI (dexamethasone, 3-isobutyl-1-methylxanthine, and insulin) for 2 days to induce adipocyte differentiation. Following DMI treatment, FBS-DMEM with insulin was administered for the next 4 days. Finally, adipogenesis was stopped by using FBS-DMEM only.

The 3 T3-L1 cells differentiated with DMI were treated with 5282-P at a concentration of 10^8 cells/well. Oil Red O staining was performed to assess the effect of 5282-P treatment on adipogenic differentiation. Briefly, the cells were fixed with 10 % formalin for 1 h at 37 °C. Formalin was removed, and then the cells were stained with 0.5 % Oil Red O solution for 30 min at 37 °C. The cells were washed with isopropyl alcohol for 10 min at room temperature, and absorbance was measured using a microplate reader (Infinite M200 Nano-Quant) at OD_{450}. Lipid droplets were observed under a microscope (CKX41, Olympus, Tokyo, Japan). The triglyceride (TG) concentration was measured using a TG assay kit (Abcam, Cambridge, MA, USA) in accordance with the manufacturer's instructions. The TGs were eluted with 5 % Triton X-100. Adipocyte differentiation experiments using 3 T3-L1 cells were conducted in triplicate ($n = 3$).

2.9. Statistical analysis

Statistical analysis was performed using GraphPad Prism (v.9.0), and data are presented as mean ± standard error of the mean. To evaluate relative differences, we determined statistically significant differences between groups by using the non-parametric Kruskal–Wallis test for microbial phyla and genera. One-way ANOVA was used for multiple group comparisons. Differences between groups were considered significant at $P < 0.05$.

3. Results

3.1. Effects of 5195-P on the gut microbiota

The effects of 5282-P treatment on the gut microbiome were examined (Figs. 1 and 2). The α-diversity (evenness and richness) of the bacterial community was statistically assessed using sequencing data based on the observed number of species and Shannon, Chao1, and Simpson indices. Significant differences in the observed number of species and Shannon and Chao1 indices were observed in the AL-5282 and PR-5282 groups compared with each N-CNT group ($P < 0.05$, Fig. 1A), with the Chao1 index showing significant differences only in the prophylactic model. The Simpson index did not significantly differ compared with the control groups. β-diversity analysis revealed that the gut microbiota of the 5282-P-treated groups differed from that of their respective control groups in the principal coordinate analysis (PCoA) plot of the Adonis analysis (Fig. 1B). Furthermore, significant differences in microbiota composition among all groups were confirmed by ANOSIM using the Bray–Curtis distance rank. The relative abundances of bacterial communities at the phylum, family, and genus levels were analyzed (Fig. 2A). *Bacteroidetes, Firmicutes*, and *Proteobacteria* were the dominant bacteria across all groups at the phylum level. *Firmicutes* was predominant in the obesity-induced group, whereas *Bacteroidetes* was dominant in the normal group. In the prophylactic model, the relative abundance of *Verrucomicrobia* significantly decreased, whereas that of *Deferribacteres* slightly increased. At the family level, the obesity-induced groups exhibited a relative predominance of *Ruminococcaceae* and *Lachnospiraceae*. Additionally, the prophylactic model demonstrated a slightly higher abundance of *Bacteroidales* than the alleviation model. The relative abundance at the genus level highlights notable differences in *Proteobacteria, Bacteroides*, and *Papillibacter*. Twenty-nine OTUs derived from the experimental groups were visualized as a heatmap (Fig. 2B).

Various bacterial phyla and genera were identified across the experimental groups. Notably, significant differences in the abundance of nine families and eight genera were found among the groups (Fig. 3). At the phylum level (Fig. 3A), *Bacteroidetes*, *Bacteroidaceae*, *Lactobacillaceae*, and *Desulfovibrionaceae* were significantly more abundant in the 5282-P-treated groups than in the N-CNT group in both the alleviation and prophylactic models. In addition, *Oscillospiraceae, Erysipelotrichaceae,* and *Lachnospiraceae* were significantly more abundant in the PR-5282 group than in the PR-N-CNT group ($P < 0.001$). The *Firmicutes/Bacteroidetes* ratio was significantly lower in the 5282-P-treated groups than in their respective control groups, with the PR-5282 group showing the lowest ratio. Conversely, no significant differences in *Ruminococcaceae* abundance were observed among the groups, except for the Normal group. In the P-CNT group, *Bacteroidaceae* and *Desulfovibrionaceae* showed significantly higher abundance in the alleviation model, whereas *Oscillospiraceae* and *Lachnospiraceae* exhibited significant differences compared with the PR-N-CNT group in the prophylactic model.

At the genus level (Fig. 3B), *Bacteroides*, *Desulfovibrio, Dorea, Lactobacillus*, and *Macellibacteroides* were more abundant in the 5282-P-treated groups than in their respective control groups. In addition, *Alistipes* was significantly more abundant in the PR-5282 group than in the PR-N-CNT group. No significant differences in *Clostridium* and *Ruminococcus* were observed between the groups. In the P-CNT group,

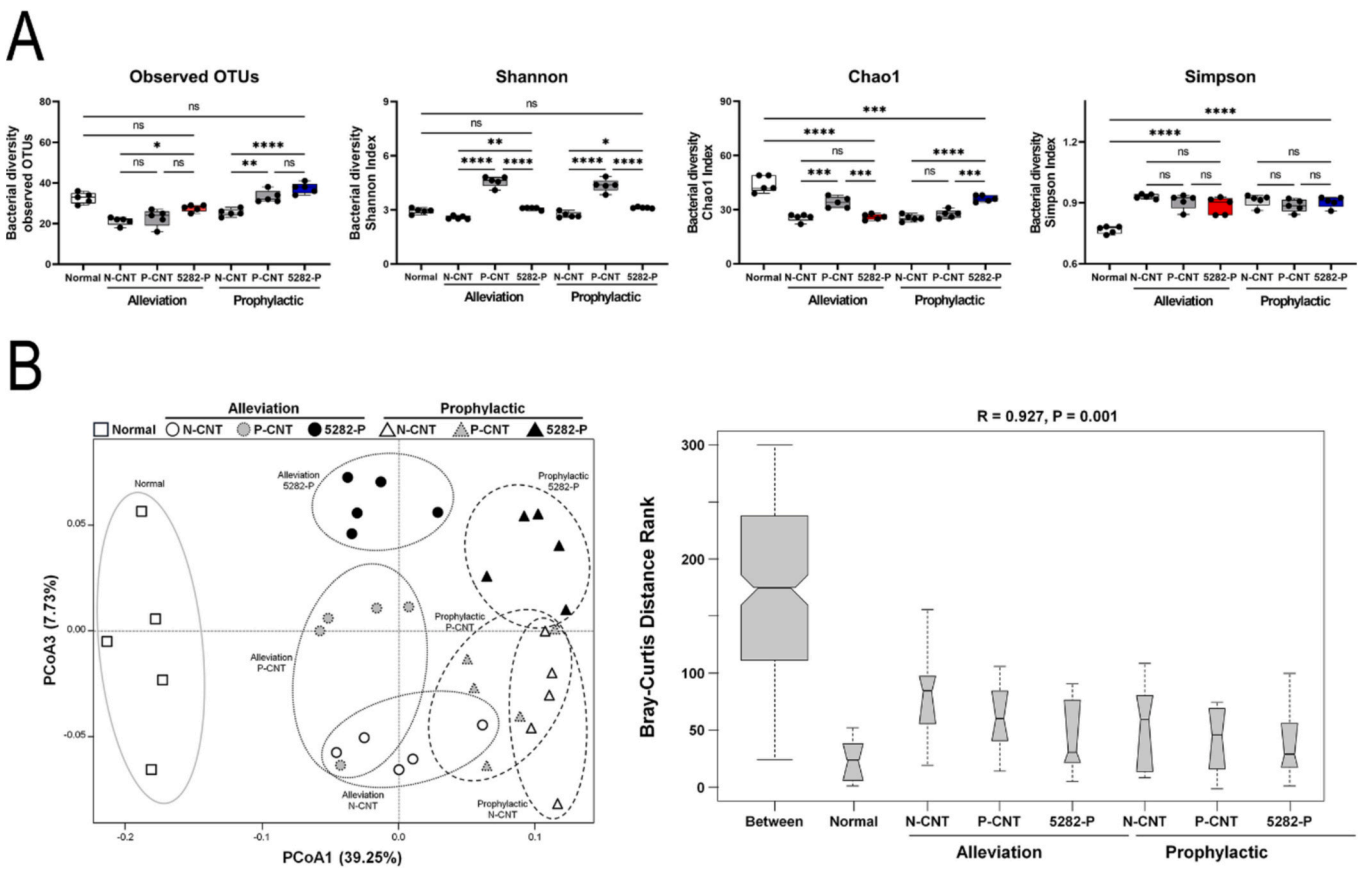

Fig. 1. Effect of *Lactiplantibacillus plantarum* LRCC5282 postbiotic on bacterial diversity.
(A) α-diversity indices including observed number of species, Shannon, Chao1, and Simpson. (B) β-diversity and principal coordinate analysis plot.
Normal: animal groups without any treatment and fed a normal diet; N-CNT: animal groups without any treatment and fed a high-fat diet; P-CNT: animal groups treated with commercial L. *gasseri* product B and fed a high-fat diet; 5282-P: animal groups treated with L. *plantarum* 5282 postbiotic and fed a high-fat diet.
Significant differences between the groups, denoted by underscores, are indicated as follows: *($P < 0.05$); **($P < 0.001$); ***($P < 0.0005$); and ****($P < 0.0001$). ^{ns}Means no significant differences between the groups, as shown by the underscores.

Desulfovibrio and *Bacteroides* showed significant differences compared with the N-CNT group in both models, whereas *Dorea* and *Lactobacillus* exhibited significant differences only in the alleviation and prophylactic models, respectively.

3.2. Effects of 5195-P on SCFA levels

The SCFAs in the fecal samples from the experimental groups were analyzed (Fig. 4A). The obesity-induced control groups, AL-N-CNT and PR-N-CNT, had lower levels of acetate, butyrate, and propionic acid than the Normal group. However, the levels of all the SCFAs were higher in the 5282-P-treated groups than in their respective N-CNT groups. No significant differences in the levels of acetic acid were found between the 5282-P-treated groups and the Normal group. Conversely, the butyric acid levels were significantly higher in the PR-5282 group than in the Normal group. By contrast, propionic acid levels were lower in the PR-5282 group than in the Normal group. Fig. 4B shows the bacterial genera correlated with the levels of various SCFAs in the obesity-induced groups, excluding the normal group. Seven genera showed significant correlations. Specifically, *Bacteroides* correlated with acetic acid, butyric acid, and propionic acid, with correlation coefficients (R) ranging from 0.74 to 0.89. By contrast, *Alistipes* was correlated with butyric acid only in the alleviation model, with an *R*-value of 0.76.

3.3. Adipose tissues and serum profile

The weight of adipose tissue was measured and is shown in Fig. 5A. The white fat weights in the neck, subcutaneous, and epididymal tissues were lower in the 5282-P-treated groups than in their respective control groups. Notably, neck fat weight was slightly but significantly higher in the PR-5282 group than in the Normal group, whereas subcutaneous and epididymal fat weights reduced to levels comparable to those in the Normal group. Brown fat weight was lowest in the AL-N-CNT group and highest in the PR-5282 group, although no significant difference was observed compared with the Normal group. Additionally, the ratio of white/brown fat was highest in the PR-5282 group, although no statistical significance was observed. Histological analysis of the neck, subcutaneous, and epididymal adipose tissues revealed the largest adipocyte areas in both the N-CNT groups, with reduced sizes in the 5282-P-treated groups, particularly in the PR-5282 group (Fig. 5A–5B). Compared with the N-CNT group, the P-CNT group showed significantly lower organ weight, including neck, subcutaneous, epididymal, brown, and brown/white fat ratios. Histological analysis also revealed significantly smaller adipose tissues in the neck, subcutaneous, and epididymal areas in the P-CNT group. However, the results were similar to or slightly lower than those observed in the 5282-P-treated group.

Serum profiles, including biomarkers of hepatic toxicity and cardiovascular health, are shown in Fig. 5C. The AST and ALT levels were lower in both 5282-P-treated groups than in their respective N-CNT groups. Triglyceride levels were significantly lower in all 5282-P-treated groups than in their respective control groups, with no significant difference compared with the Normal group. No significant differences in total cholesterol levels were found between the PR-5282 and PR-N-CNT groups. However, LDL cholesterol levels were lower in the AL-5282 and

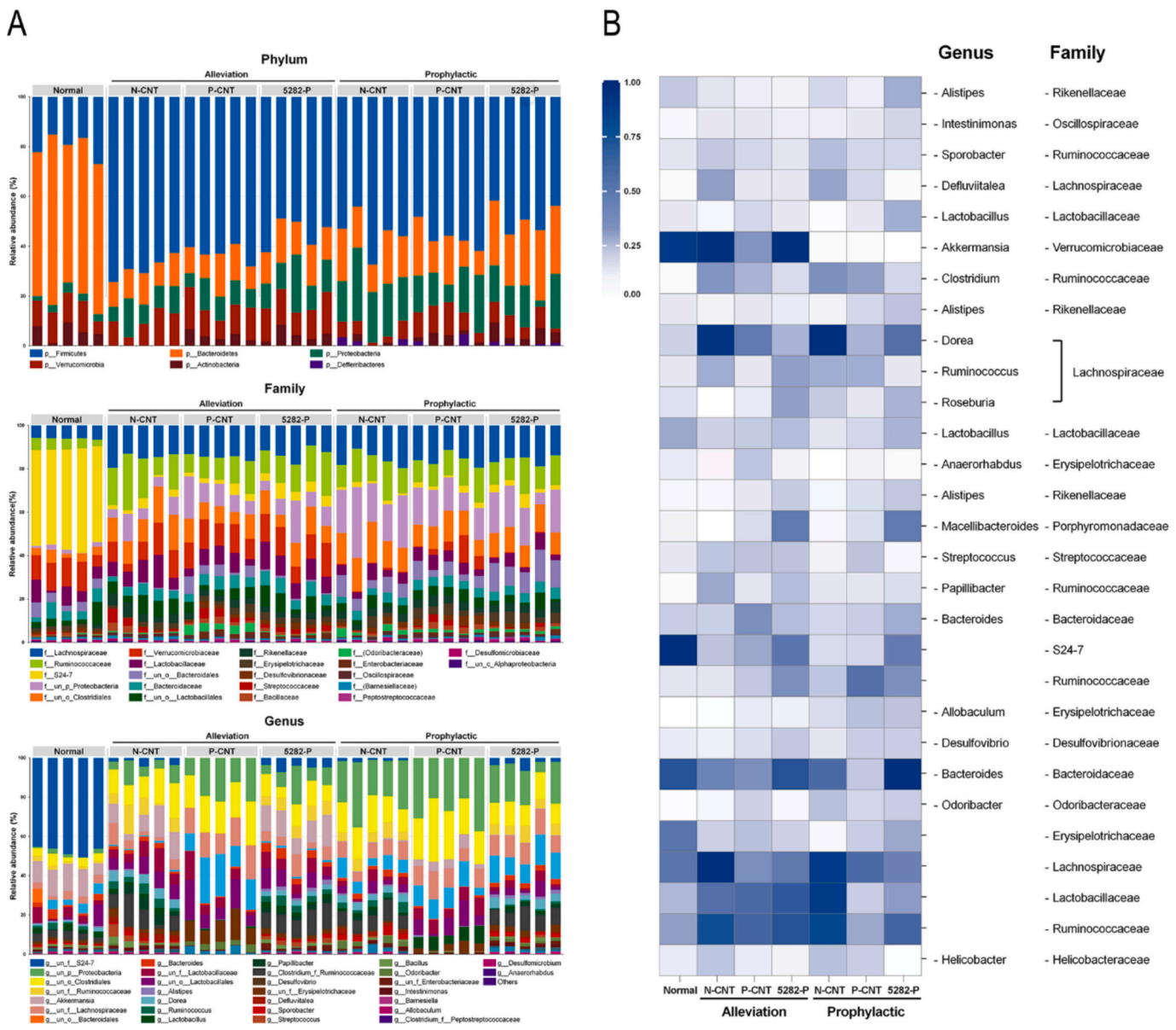

Fig. 2. Effect of *Lactiplantibacillus plantarum* LRCC5282 postbiotic on bacterial abundance.
(A) Relative abundance at the phylum, family, and genus levels. (B) Heatmap hierarchical clustering of the discriminant key OTUs.
Normal: animal groups without any treatment and fed a normal diet; N-CNT: animal groups without any treatment and fed a high-fat diet; P-CNT: animal groups treated with commercial *L. gasseri* product B and fed a high-fat diet; 5282-P: animal groups treated with *L. plantarum* 5282 postbiotic and fed a high-fat diet.

PR-5282 groups than in their respective N-CNT groups. Conversely, HDL cholesterol levels showed a significant difference only in the AL-5282 group. Significant differences in ALT, AST, and triglyceride levels were observed between the AL-P-CNT and PR-P-CNT groups and their respective N-CNT groups. HDL-cholesterol levels significantly increased in both models.

3.4. mRNA expression of genes related to obesity and inflammation

The effects of 5282-P treatment on the mRNA expression levels of genes related to adipogenesis are shown in Fig. 6A. The mRNA expression levels of PPAR/γ, FAS, SREBPα1, and CEBP/α were lower in the 5282-P-treated groups than in their respective N-CNT groups. Leptin levels were lower in the AL-5282 group than in the AL-N-CNT group, whereas adiponectin levels were significantly higher in the PR-5282 group than in the PR-N-CNT group. Furthermore, the levels of leptin, PPAR/γ, FAS, SREBPα1, and CEBP/α in the 5282-treated groups were regulated to the levels comparable to those in the Normal group. The mRNA expression levels of the inflammatory cytokines are demonstrated in Fig. 6B. The mRNA expression levels of pro-inflammatory cytokines TNF-α and IL-6 were lower in the 5282-P-treated groups than in their respective N-CNT groups. By contrast, the levels of the anti-inflammatory cytokine IL-10 were higher in the 5282-P-treated groups than in their respective N-CNT groups. Additionally, the levels of CCL2 were lower in the 5282-P-treated groups than in their respective N-CNT. In the P-CNT group, significant differences were observed in PPAR/γ in the alleviation model and in FAS in the prophylactic model compared with the N-CNT group. The levels of inflammatory cytokines, including TNF-α, IL-6, and CCL2, were significantly lower in both the alleviation and prophylactic models, whereas the level of IL-10 was significantly higher in both models.

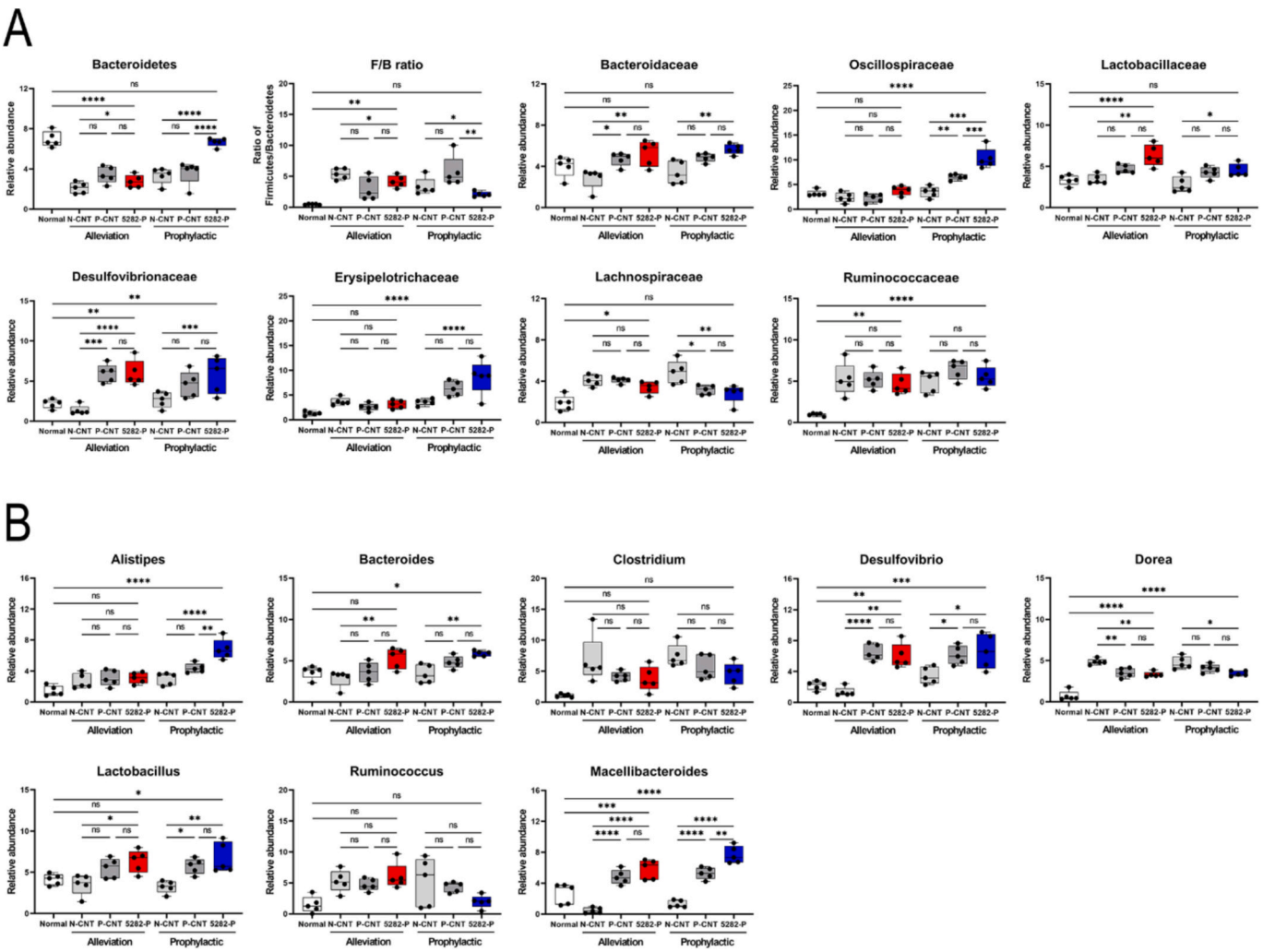

Fig. 3. Effect of *Lactiplantibacillus plantarum* LRCC5282 postbiotic on gut microbial communities and relative abundance of genera.
(A) Relative abundance of genera at the family level; (B) Relative abundance of genera at the genus level.
Normal: animal groups without any treatment and fed a normal diet; N-CNT: animal groups without any treatment and fed a high-fat diet; P-CNT: animal groups treated with commercial *L. gasseri* product B and fed a high-fat diet; 5282-P: animal groups treated with *L. plantarum* 5282 postbiotic and fed a high-fat diet.
Significant differences between the groups, denoted by underscores, are indicated as follows: *($P < 0.05$); **($P < 0.001$); ***($P < 0.0005$); and ****($P < 0.0001$). ^{ns}Means no significant differences between the groups, as shown by the underscores.

3.5. Weight gain and body mass index

The changes in body weight and BMI during the experiments are shown in Table 1. The initial body weights of the mice belonging to the Normal, AL-N-CNT, AL-P-CNT, AL-5282, PR-N-CNT, PR-P-CNT, and PR-5282 groups were 23.6 ± 0.4, 22.7 ± 0.6, 21.4 ± 0.7, 23.4 ± 0.4, 20.5 ± 0.5, 21.4 ± 0.3, and 21.2 ± 0.4 g, respectively. The weight gains after the experiments were 7.6 ± 2.0, 22.6 ± 0.5, 21.2 ± 0.8, 20.6 ± 2.1, 24.5 ± 0.4, 17.8 ± 0.9, and 15.2 ± 0.6 g, respectively. The PR-N-CNT and PR-N-5282 groups showed the highest and lowest weight gains, respectively, relative to their initial weights. The average BMI of the mice at the beginning of the experiment was 2.6 ± 0.3, and their BMIs after 19 weeks were 2.9 ± 0.1, 4.8 ± 0.2, 4.6 ± 0.4, 4.3 ± 0.2, 5.2 ± 0.0, 4.5 ± 0.4, and 3.9 ± 0.1 g, respectively. The PR-N-CNT and PR-5282 groups showed the highest and lowest changes in BMI, respectively, relative to their initial BMIs. Compared with the respective N-CNT group, the PR-5282 group showed significant differences in weight gain and BMI. In the P-CNT group, significant differences compared with the N-CNT group were observed only in weight change in the prophylactic model.

3.6. Effect of 5282-P on adipogenesis in vitro

Fig. 7 shows the results of lipid accumulation, TG levels, and Oil Red O staining. The lipid accumulation rate and TG levels in the 5282-P-treated 3 T3-L1 cells were 22.4 % ± 1.1 % and 73.4 % ± 1.2 %, respectively, compared with the cells without treatment (i.e., DMI-induced, PBS-treated 3 T3-L1 cells). Following treatment with P-CNT, the lipid accumulation rate was 31.4 % ± 7.7 %, and TG levels were 78.6 % ± 7.4 %, respectively. The mRNA expression levels of adiponectin, PPAR/γ, CEBP/α, and FAS in the 5282-P-treated 3 T3-L1 cells were 0.3 % ± 0.2 %, 15.4 % ± 1.2 %, 2.1 % ± 0.4 %, and 7.3 % ± 0.3 %, respectively, compared with the DMI-induced cells without treatment. All data on the lipid accumulation, TG levels, and mRNA expression levels of the 5282-P-treated 3 T3-L1 cells were significantly different from those of the DMI-induced cells without treatment. Treatment with P-CNT significantly altered the mRNA expression levels of adiponectin, PPAR/γ, CEBP/α, and FAS, following a pattern similar to that observed in the 5282-P-treated cells. The results of Oil Red O staining of the differentiated 3 T3-L1, P-CNT-treated, and 5282-P-treated cells are shown in Fig. 7B. Compared with the differentiated 3 T3-L1 cells, the 5282-P-treated cells exhibited a markedly lower number of lipid

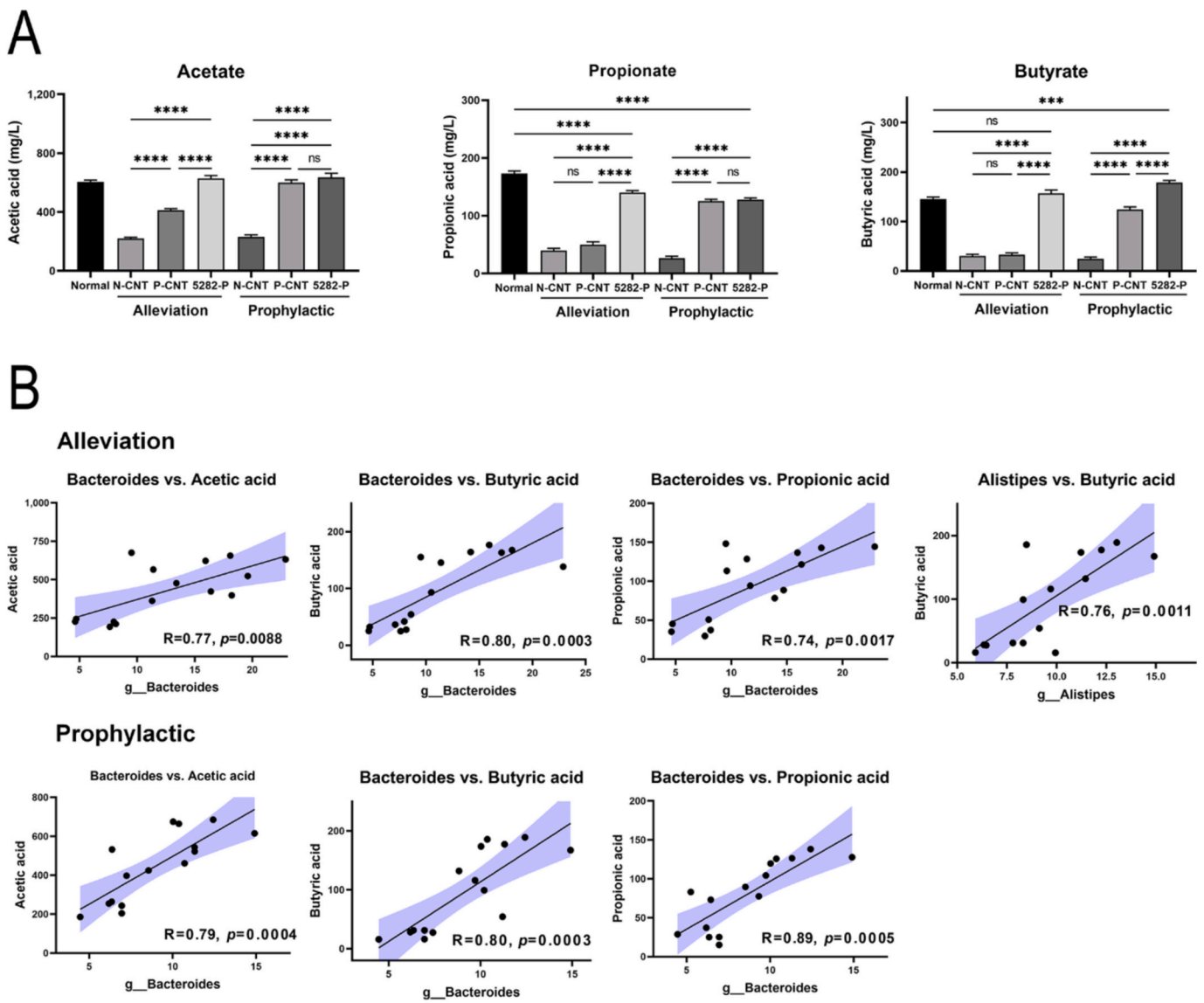

Fig. 4. Effect of *Lactiplantibacillus plantarum* LRCC5282 postbiotic on short-chain fatty acids (SCFAs).
(A) Acetic, butyric, and propionic acid. (B) Correlations with bacterial genera and SCFA levels. Normal: animal groups without any treatment and fed a normal diet; N-CNT: animal groups without any treatment and fed a high-fat diet; P-CNT: animal groups treated with commercial *L. gasseri* product B and fed a high-fat diet; 5282-P: animal groups treated with *L. plantarum* 5282 postbiotic and fed a high-fat diet.
Significant differences between the groups, denoted by underscores, are indicated as follows: *($P < 0.05$); **($P < 0.001$); ***($P < 0.0005$); and ****($P < 0.0001$). ^{ns}Means no significant differences between the groups, as shown by the underscores.

droplets (marked as red dots).

4. Discussion

Obesity is a metabolic disorder characterized by the accumulation of excess energy in the adipose tissue, which leads to an imbalance in energy metabolism. This imbalance is a known risk factor for various pathological conditions, including cardiovascular disease, hyperlipidemia, diabetes, nonalcoholic hepatitis, cancer, dementia, and arthritis (Spiegelman & Flier, 2001). Recently, many studies have reported the role of microbiome regulation in improving obesity, and LAB, including *Lactobacillus* spp. and *Bifidobacterium* spp., are known to play key roles in this process. Consequently, developing LAB into therapeutic agents with no side effects and excellent efficacy for obesity improvement will have a great impact not only scientifically and technologically but also socially. Therefore, the results of the present study highlight that *L. plantarum* LRCC5282 postbiotic can significantly improve obesity by modulating the gut microbiome.

Gut microbial diversity is an index that measures the variety of microorganisms associated with health. Loss of diversity has been observed in many disease states, including inflammatory bowel disease and diabetes (Gilbert et al., 2016). A previous meta-analysis of microbiota studies on obesity revealed a significant reduction in microbial diversity in individuals with obesity, as measured by indices, including the Shannon index (Sze & Schloss, 2017). Le Chatelier et al. (2013) reported that obesity is not only associated with low taxonomic diversity levels but also with low bacterial gene richness, which is involved in the regulation of inflammation and metabolic disorders (Le Chatelier et al., 2013). Cotillard et al. (2013) proposed a high-protein diet as a way to improve obesity, and their results suggested that the increased compositional diversity observed in high-protein diets may correspond to greater microbial gene abundance (Cotillard et al., 2013). In the present study, the α-diversity indices, including Shannon and Chao1, indicated significant increases in bacterial evenness and richness, in the AL-5282

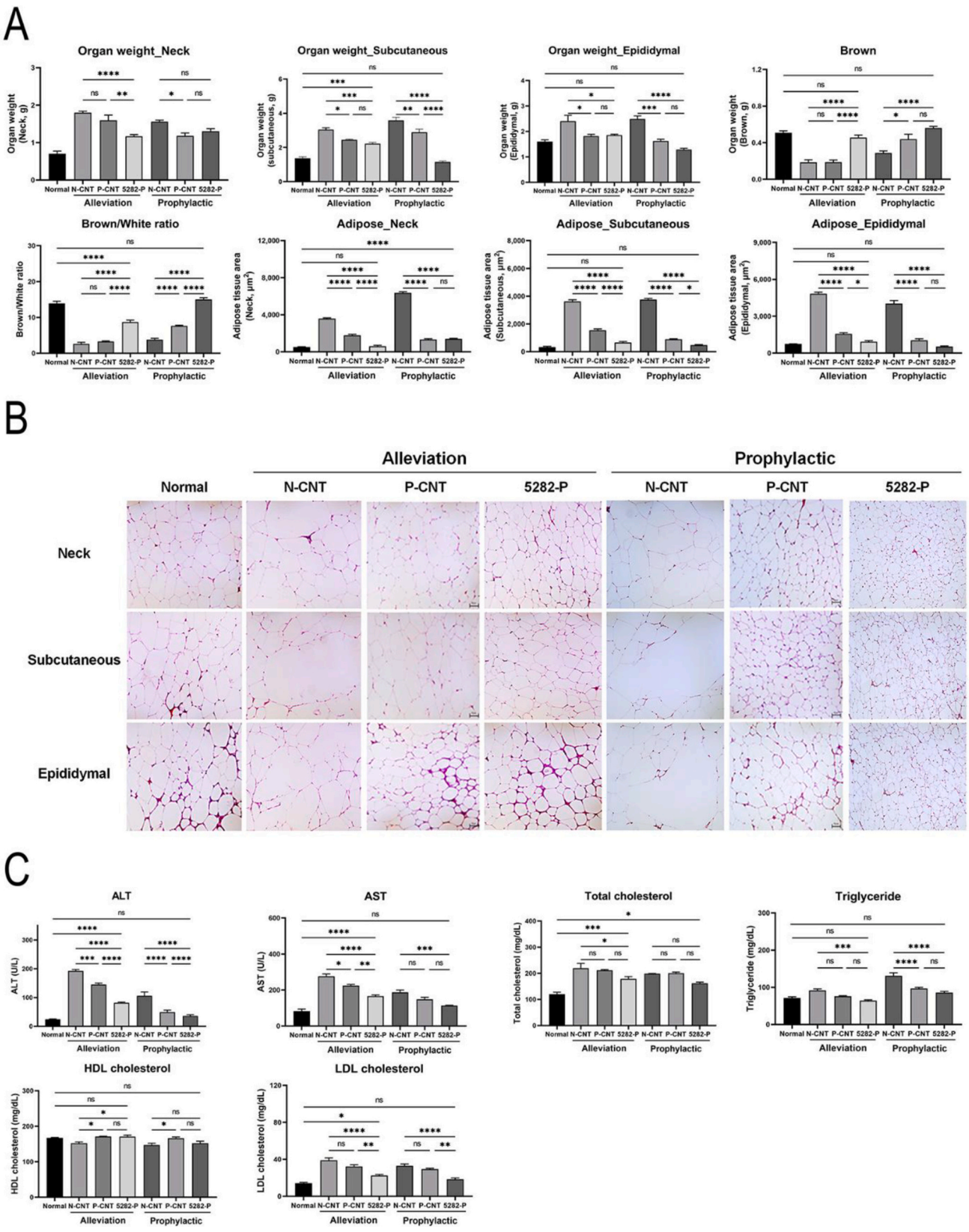

Fig. 5. Effect of *Lactiplantibacillus plantarum* LRCC5282 postbiotic on the organ lipid weights and adipose size in high-fat diet (HFD)-fed mouse.
(A) Weight of white/brown fat in each organ, and area of adipose tissue. (B) Observation of adipose tissue. (C) Serum profile.
Normal: Animal groups without any treatment and fed a normal diet; N-CNT: animal groups without any treatment and fed a high-fat diet; P-CNT: animal groups treated with commercial *L. gasseri* product B and fed a high-fat diet; 5282-P: animal groups treated with *L. plantarum* 5282 postbiotic and fed a high-fat diet.
Significant differences between the groups, denoted by underscores, are indicated as follows: *($P < 0.05$); **($P < 0.001$); ***($P < 0.0005$); and ****($P < 0.0001$). ^{ns}Means no significant differences between the groups, as shown by the underscores. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

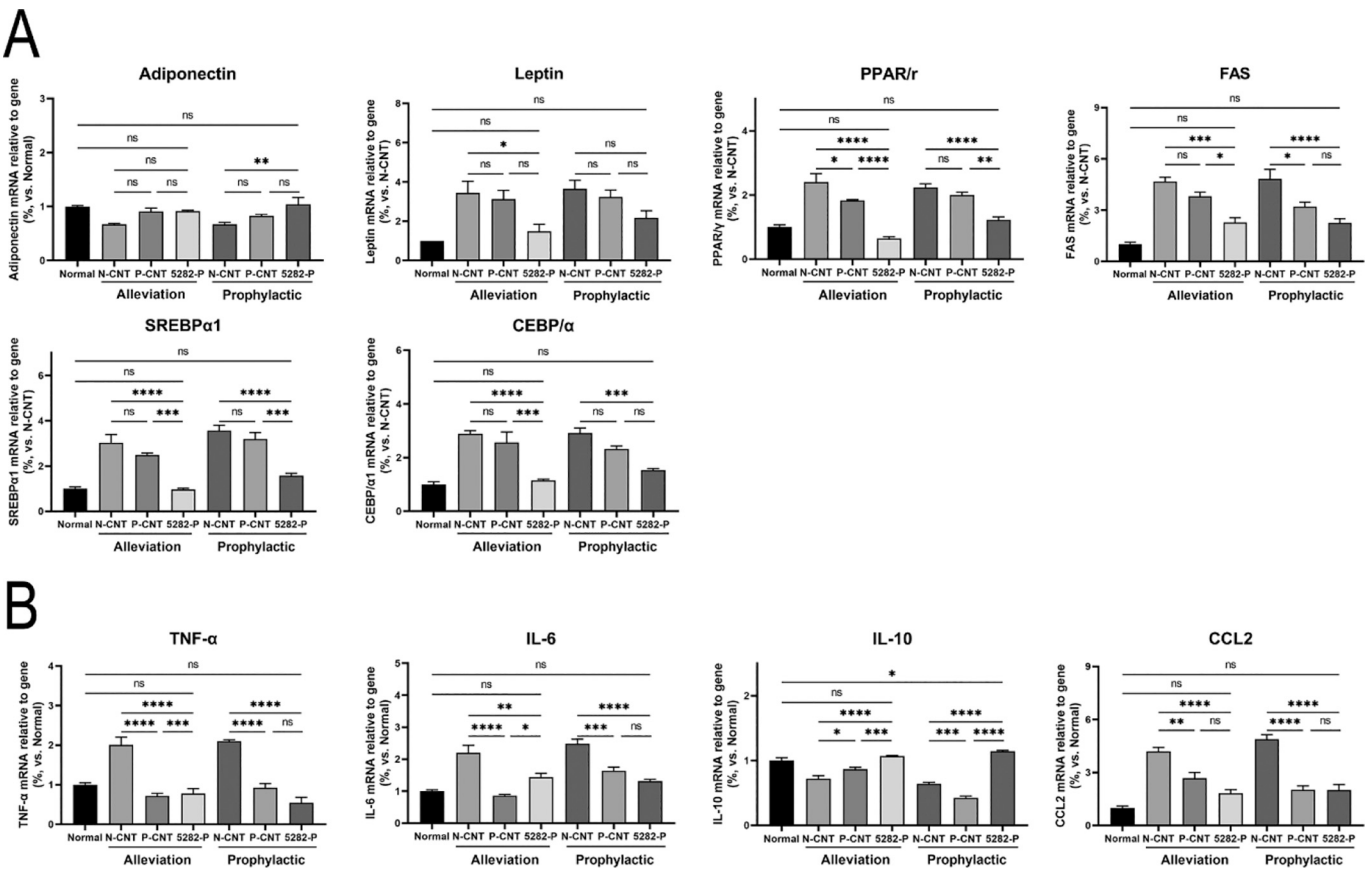

Fig. 6. Effect of *Lactiplantibacillus plantarum* LRCC5282 postbiotic on mRNA expressions levels.
(A) mRNA expression of genes related to adipogenesis. (B) mRNA expression of genes related to inflammatory cytokines.
Normal: animal groups without any treatment and fed a normal diet; N-CNT: animal groups without any treatment and fed a high-fat diet; P-CNT: animal groups treated with commercial *L. gasseri* product B and fed a high-fat diet; 5282-P: animal groups treated with *L. plantarum* 5282 postbiotic and fed a high-fat diet.
Significant differences between the groups, denoted by underscores, are indicated as follows: *($P < 0.05$); **($P < 0.001$); ***($P < 0.0005$); and ****($P < 0.0001$).
[ns]Means no significant differences between the groups, as shown by the underscores.

Table 1
Effect of *Lactiplantibacillus plantarum* LRCC5282 postbiotics on the body weight and body mass index.

Group		Body weight(g)			Body Mass Index		
		Initial	12-weeks	Δweight (12wks-0wks)	Initial	12-weeks	ΔBMI (12wks-0wks)
Normal-control		23.6 ± 0.4	31.2 ± 1.9	7.6 ± 2.0	2.6 ± 0.0	2.9 ± 0.1	0.3 ± 0.1
	N-CNT	22.7 ± 0.6	45.3 ± 0.3	22.6 ± 0.5	2.6 ± 0.1	4.8 ± 0.2	2.2 ± 0.2
Alleviation	P-CNT	21.4 ± 0.7	41.7 ± 1.1	21.2 ± 0.8	2.5 ± 0.3	4.6 ± 0.4	2.1 ± 0.2
	5282-P	23.4 ± 0.4	44.0 ± 2.0	20.6 ± 2.1	2.5 ± 0.1	4.3 ± 0.2	1.7 ± 0.2
	N-CNT	20.5 ± 0.5	45.3 ± 0.4	24.5 ± 0.4	2.5 ± 0.0	5.2 ± 0.0	2.6 ± 0.1
Prophylactic	P-CNT	21.4 ± 0.3	39.2 ± 1.4*	17.8 ± 0.9*	2.6 ± 0.2	4.5 ± 0.4	1.9 ± 0.4
	5282-P	21.2 ± 0.4	36.5 ± 0.6*	15.2 ± 0.6*	2.6 ± 0.1	3.9 ± 0.1*	1.3 ± 0.1*

Normal: animal groups without any treatment and fed a normal diet; N-CNT: animal groups without any treatment and fed a high-fat diet;
P-CNT: animal groups treated with commercial L. *gasseri* product B and fed a high-fat diet;
5282-P: animal groups treated with L. *plantarum* 5282 postbiotic and fed a high-fat diet.
* Significant difference between each column (vs. respective control group, *$P < 0.05$).

and PR-5282 groups compared with their respective control groups. Moreover, the β-diversity analysis further confirmed that the gut microbiota composition in the 5282-P-treated groups was distinctly different from each control group, as evidenced by the PCoA plot of the Adonis analysis. These findings suggest that 5282-P promotes a diverse and balanced gut microbiome that is important for metabolic health and has high potential for obesity improvement.

Relative abundance is also an important indicator of gut microbiome regulation. At the phylum level, the *Firmicutes/Bacteroidetes* ratio reportedly increases in diet-induced obese mice, ob/ob mice, and humans with obesity and conversely decreases with weight loss (Ley, Turnbaugh, Klein, & Gordon, 2006). A significant increase in the *Firmicutes/Bacteroidetes* ratio is thought to cause increased obesity sensitivity and imbalanced energy harvesting. The increased energy is stored in the liver and body fat tissue, which can lead to body weight gain. Therefore, significantly lowering the *Firmicutes/Bacteroidetes* ratio may be an effective strategy for improving obesity. In addition, *Oscillospiraceae*, *Lactobacillaceae* (Lee et al., 2021), *Lachnospiraceae, Erysipelotrichaceae*, and *Desulfovibrionaceae* (Huang et al., 2021) are important families associated with obesity symptoms in animals and humans.

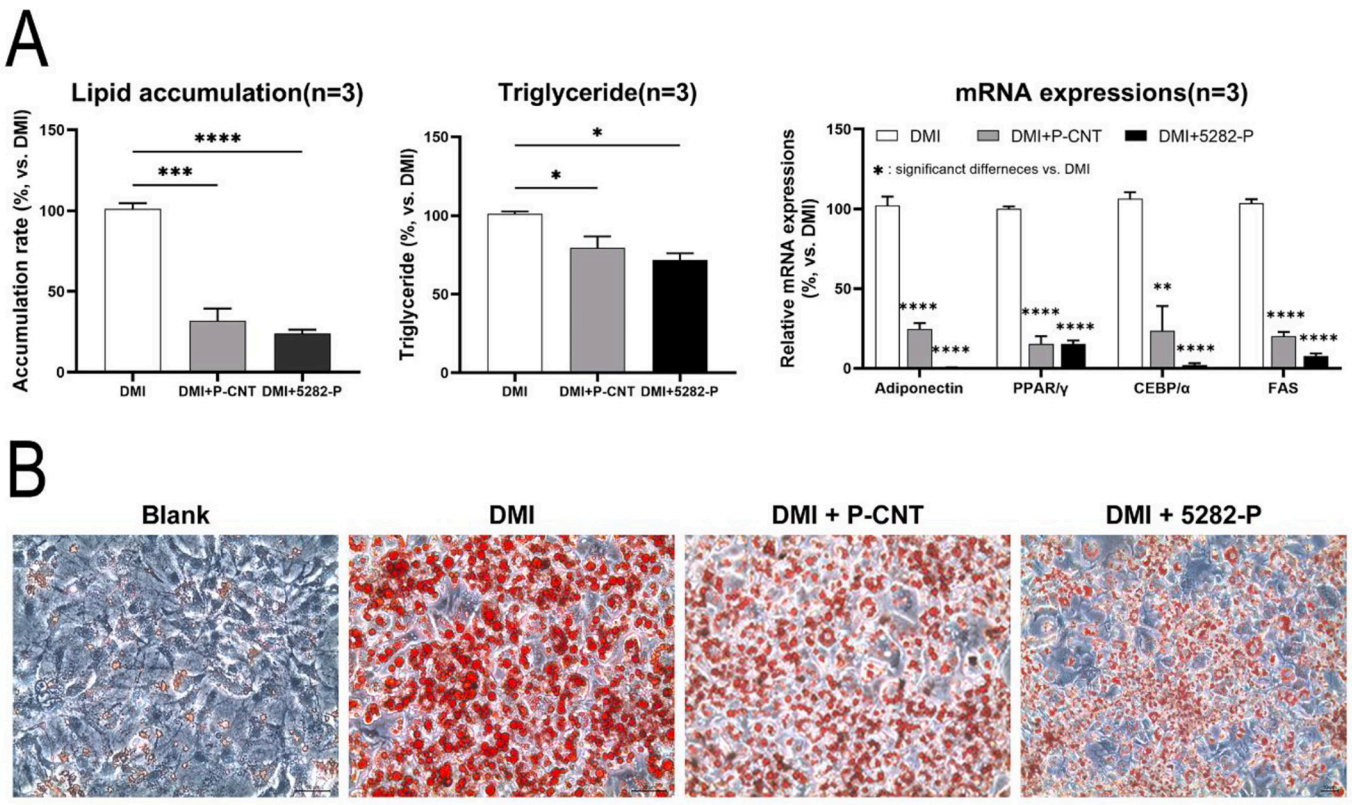

Fig. 7. Effect of *Lactiplantibacillus plantarum* LRCC5282 postbiotic on the lipid accumulation and triglycerides in 3 T3-L1 cells.
(A) Lipid accumulation and triglycerides. (B) Observation of lipid droplets.
Normal: animal groups without any treatment and fed a normal diet; N-CNT: animal groups without any treatment and fed a high-fat diet; P-CNT: animal groups treated with commercial *L. gasseri* product B and fed a high-fat diet; 5282-P: animal groups treated with *L. plantarum* 5282 postbiotic and fed with high-fat diet.
The results are expressed as mean ± SEM (standard error of the mean) (n = 3).
Significant differences between the groups, denoted by underscores, are indicated as follows: *($P < 0.05$); **($P < 0.001$); ***($P < 0.0005$); and ****($P < 0.0001$).
^{ns}Means no significant differences between the groups, as shown by the underscores.

Previous studies have reported that treatment with *Lactobacillus* sp. increases the abundance of *Bacteroidaceae, Oscillospiraceae, Lactobacillaceae, Desulfovibrionaceae*, and *Erysipelotrichaceae*. This result agrees with the effects of 5282-P treatment in the present study. Moreover, Kang et al. reported that treatment with *Lactobacillus acidophilus* decreases *Lachnospiraceae* abundance, which was also consistent with the results of this study.

Numerous studies have reported that treatment with *Lactobacillus* species increases the abundance of *Alistipes*, *Bacteroides*, *Desulfovibrio*, *Lactobacillus*, and *Macellibacteroides*. Notably, *Alistipes* species are indole-positive bacteria involved in the metabolism of the serotonin precursor tryptophan and maintenance of gut immune homeostasis (Verdu, Hayes, & O'Mahony, 2016). Additionally, an increase in *Lactobacillus* abundance inhibits the activity of intestinal pathogens and exhibits anti-obesity effects (Liang et al., 2021). Various studies have also reported that probiotic treatment reduces the abundance of *Clostridium, Dorea,* and *Ruminococcus* in the guts of humans or animals with obesity. In particular, *Ruminococcus* has been consistently identified in many studies as a characteristic microbe of inflammatory bowel disease. It reduces gut bacterial diversity and is categorized as a harmful bacterium that induces inflammatory responses detrimental to human health (Gilbert et al., 2016). Conversely, Gulnaz et al. reported that treatment with *Lactobacillus* species significantly decreases *Desulfovibrio* levels. Specifically, *Latilactobacillus sakei* Probio65 treatment reduces *Desulfovibrio* abundance (Gulnaz et al., 2021). The results of the present study are consistent with previous findings demonstrating the beneficial effects of 5282-P on HFD-induced obesity. The treatment groups showed lower *Firmicutes/Bacteroidetes* ratios than the respective control groups, which is consistent with the results of prior studies associating a reduced *Firmicutes/Bacteroidetes* ratio with improved metabolic health (Ley et al., 2006). In addition to the lower *Firmicutes/Bacteroidetes* ratio, we observed an increase in the abundance of phyla and genera known to be favorable for obesity improvement, including *Bacteroidetes* and *Lactobacillus*. Conversely, the abundance of phyla and genera often associated with obesity, such as *Firmicutes* and *Ruminococcus*, decreased. These findings suggest that 5282-P treatment promotes gut microbiome health, which is crucial for overall metabolic health and obesity reduction.

Many studies have suggested that these changes in microbiota are caused by metabolites. Among these, SCFAs have received much attention because of their important roles in disease control (Kim et al., 2019; Tan et al., 2014). Generally, SCFAs are used as energy sources or signaling molecules and are closely related to the metabolism of carbohydrates, lipids, and proteins (Kim et al., 2019; McNabney & Henagan, 2017). Butyrate is an important SCFA that stimulates anti-inflammatory activity and energy homeostasis. It can be produced by members of the families *Lachnospiraceae*, including the genus *Dorea*, or *Ruminococcaceae.* In the present study, the secretion of acetate, propionate, and lactate increased markedly in the 5282-P-treated groups compared with their respective control groups. Additionally, certain bacterial genera, particularly *Bacteroides*, were highly correlated with acetic, butyric, and propionic acids. These results suggest that 5282-P enhances the production of beneficial metabolites through intestinal microflora regulation by stimulating SCFA production. Changes in gut microbiota composition significantly influence host immune responses by strengthening gut barrier integrity and regulating inflammatory

cytokine production. An intact gut barrier is essential to prevent pathogenic bacteria and their metabolites from entering the bloodstream, thereby maintaining immune homeostasis (Chelakkot, Ghim, & Ryu, 2018). In the present study, 5282-P improved the gut environment by promoting beneficial microbial populations; specifically, the increased abundance of *Lactobacillus* and *Bacteroides* has been linked to enhanced mucosal defense mechanisms and gut integrity. These microorganisms stimulate the expression of tight junction proteins in intestinal epithelial cells, which reinforces the barrier function (Chelakkot et al., 2018). Moreover, the gut microbiota plays a crucial role in modulating inflammatory cytokine expression. This shift in cytokine profiles may be attributed to the production of SCFAs by gut bacteria. SCFAs, particularly acetate and butyrate, foster an anti-inflammatory gut environment by regulating immune cell function and inhibiting inflammatory pathways (Kim et al., 2019; Tan et al., 2014). Additionally, the increase in *Bacteroides* abundance may contribute to reducing endotoxemia and systemic inflammation, consistent with the anti-inflammatory effects observed following 5282-P treatment in the present study (Ríos-Covián, González, Valdés, & Gueimonde, 2016).

Evidence of obesity can appear in various organs in animals, including humans, and should be considered based on various corresponding parameters rather than analyzing only specific indicators. In terms of adipose tissue and serum profiles, the present study demonstrated that the 5282-P-treated groups had lower white fat and higher brown fat ratios, which correlated with reduced adipocyte size and enhanced metabolic activity. This result was particularly evident in the PR-5282 group, which showed the lowest adipocyte area and highest white/brown fat ratio. The reduction in triglycerides, LDL cholesterol, and inflammatory cytokines (TNF-α and IL-6), coupled with an increase in anti-inflammatory IL-10, highlights the systemic benefits of 5282-P in managing obesity and associated metabolic disorders. The significant decreases in the mRNA expression levels of adipogenesis-related genes PPAR/γ, SREBPα1, and CEBP/α further support the potential of 5282-P in reducing adipogenesis and inflammation. The gut microbiota plays a critical role in the metabolism of bile acids and production of other bioactive metabolites that can indirectly affect adipogenesis-related genes. In particular, genera such as *Lactobacillus* and *Bacteroides* facilitate bile acid transformation in the intestine, which positively impacts metabolic health. Bile acids function as metabolites and act as signaling molecules that regulate host energy homeostasis and lipid metabolism. For instance, secondary bile acids produced by specific gut bacteria modulate the expression of genes such as PPAR/γ in adipocytes, thereby inhibiting adipogenesis (Ridlon, Harris, Bhowmik, Kang, & Hylemon, 2016; Wahlström, Sayin, Marschall, & Bäckhed, 2016). These metabolites also impact immune cells and metabolic pathways, which in turn indirectly modulate the expression of adipogenesis-related genes (Canfora, Meex, Venema, & Blaak, 2019; Tan et al., 2014). Therefore, the downregulated expression of adipogenesis-related genes following 5282-P treatment in the present study is possibly closely related to changes in bile acid and SCFA metabolism. In vitro experiments corroborated these findings, showing decreased accumulation of lipids and mRNA expression of adipogenesis markers in 3 T3-L1 cells treated with 5282-P.

5. Conclusions

Treatment with 5282-P demonstrated a significant potential for improving obesity by modulating the gut microbiome in mice fed a HFD. It increased gut microbiota diversity, obesity-associated genera, and beneficial SCFA production while decreasing the *Firmicutes/Bacteroidetes* ratio. These changes contributed to reduced adipogenesis, enhanced metabolic activity, and overall improvement in obesity-related parameters. The primary objective of this study was to investigate the mechanisms underlying the anti-obesity effects of 5282-P, with an additional aim of assessing its potential applicability in future clinical studies. The findings suggest that 5282-P could serve as a promising agent for obesity prevention and management. Further studies are necessary to explore the underlying mechanisms and validate these effects in larger clinical trials.

Ethical statement

Animal experiments were performed in accordance with the principles and guidelines of the Chung-Ang University Institutional Animal Care and Use Committee of the Laboratory Animal Research Center (IACUC no. 2018–00022).

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CRediT authorship contribution statement

Ahyoung Lim: Writing – original draft, Methodology, Formal analysis. **Jihye Baek:** Software, Methodology, Investigation, Formal analysis. **YoHan Nam:** Methodology, Investigation, Formal analysis. **Seokmin Yoon:** Visualization, Validation, Data curation. **Jong-Hwa Kim:** Validation, Data curation. **Jungki Kwak:** Supervision, Project administration. **Gyeong-hwon Lee:** Supervision, Project administration, Funding acquisition, Conceptualization. **Wonyong Kim:** Writing – review & editing, Project administration, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no competing financial or non-financial interests.

Data availability

No data was used for the research described in the article.

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