pISSN 1225-4339 eISSN 2287-4992
Korean J. Food Nutr. Vol. 32. No. 6, 738~744 (2019)
https://doi.org/10.9799/ksfan.2019.32.6.738

THE KOREAN JOURNAL OF
한국식품영양학회지
FOOD AND NUTRITION

Inhibitory Effects of *Lactobacillus plantarum* Q180 on Lipid Accumulation in HepG2 Cells

Jaeryang Chu, Hyunchae Joung, Byung-Kook Kim*, In-Suk Choi** and †Tae-Sik Park***

Researcher, Probiotics Research Laboratory, CKDBiO Research Institute, Ansan 15064, Korea
**Principal Research Scientist, Probiotics Research Laboratory, CKDBiO Research Institute, Ansan 15064, Korea*
***Research Institute, CKDBiO Research Institute, Ansan 15064, Korea*
****Professor, Dept. of Life Science, Gachon University, Sungnam 13120, Korea*

Abstract

Recently, the prevalence of hyperlipidemia has been increasing, and consequently, the need to identify safe and effective treatments to control this chronic disease has also increased. The beneficial effects of probiotics have been revealed by several studies over the past few years, including their effects on hypertriglyceridemia. However, the mechanisms of action of probiotics are still unclear. The anti-obesity effects of *Lactobacillus plantarum* Q180 on lipid accumulation have already been demonstrated using an *in vitro* HepG2 cell model, and therefore, we investigated its efficacy and mechanism of action. Lipid accumulation was induced in HepG2 cells by palmitic acid treatment and then the cells were incubated with *L. plantarum* Q180 lysate or supernatant to investigate changes in lipid accumulation and expression of lipid metabolism-related genes. The results showed that the *L. plantarum* Q180-treated group exhibited significantly lower levels of lipid accumulation and mRNA expression of lipid synthesis- and adipogenesis-related genes than the palmitic acid-treated group did. These results indicate that *L. plantarum* Q180 may contribute to alleviating hypertriglyceridemia by inhibiting lipid synthesis.

Key words: probiotics, *Lactobacillus plantarum* Q180, hypertriglyceridemia, dyslipidemia

Introduction

Metabolic syndrome includes obesity, hypertension, diabetes, and dyslipidemia, which are major risk factors for cardiovascular disease. In addition, dyslipidemia indicates that the blood lipid concentration is above normal range with abnormalities of cholesterol (total, low-density lipoprotein [LDL], and high-density lipoprotein [HDL] cholesterol) and triglyceride levels (Franssen et al. 2011). In the US, approximately 53% of adults have increased LDL cholesterol concentrations; however, >50% of all patients received proper care and only 35% were managed at the normal levels (Karr S 2017). The prevalence of dyslipidemia in Korea has been increasing gradually in the last few years, accompanied by increased hypertriglyceridemia, which indicates abnormally high blood triglyceride concentrations. Especially, the proportion of men with hypertriglyceridemia is two times that of women. Accordingly, medical expenses for hypertriglyceridemia care have been constantly increasing, but 60% of patients have not received proper therapy (Korean Society of Lipidology and Atherosclerosis 2018).

Probiotics are live microorganisms that contribute to improving the host's gut health and have been sold as supplements for this purpose, including bowel movement improvement (Ann YG 2011; Yoon et al. 2018). *Bifidobacterium* and *Lactobacillus* species are widely used as the main ingredients of probiotics. Recently, the various studies have demonstrated that probiotics might contribute to the amelioration of several symptoms of metabolic syndrome including hypertriglyceridemia (Yousaf et al. 2016; Ueda et al. 2018; Jang et al. 2019; Wang et al. 2019).A study showed that administration of a probiotic mixture consisting of *Bifidobacterium* and *Lactobacillus* species ameliorated hypertriglyceridemia and other metabolic syndrome symptoms in a hypertriglyceridemia-induced mouse model (Al-Muzafar et al. 2017). Another study showed that administration of a *Lactobacillus*

† Corresponding author: Tae-Sik Park, Professor, Dept. of Life Science, Gachon University, Sungnam 13120, Korea. Tel: +82-31-750-8824, Fax: +82-32-899-6039, E-mail: tspark@gachon.ac.kr

plantarum and *Lactobacillus curvatus* mixture ameliorated the blood lipid profile and lipoprotein levels in a mouse model (Choi et al. 2016). Although probiotics have shown a wide range of efficacies in various studies, their mechanism of action remains unclear. The anti-obesity activity of *L. plantarum* Q180 has already been examined in *in vivo* studies (Park et al. 2014a; Park et al. 2014b; Park et al. 2016; Park et al. 2018) and, therefore, in this study, we investigated its beneficial effects on lipid accumulation in HepG2 cell and the underlying mechanisms.

Materials and Methods

1. Preparation of *L. plantarum* Q180

The *L. plantarum* Q180 used in this study was isolated from healthy human feces (Park et al. 2014a). Its physiological characteristics were assessed using the API 50 CHL test and the identification was confirmed using 16s rRNA sequencing. *L. plantarum* Q180 was cultured in Man-Rogosa-Sharpe (MRS) medium at 37°C for 18 hours. The cell suspensions were then centrifuged, washed twice with phosphate-buffered saline (PBS), and the collected cells were suspended in cold PBS and fragmentized using sonication to prepare the lysate. The lysate was centrifuged, the debris was discarded, and then the pellet was resuspended in PBS. The supernatant was extracted from the bacterial culture medium by centrifugation and the lysate and supernatant were stored at −20℃ until use. In addition, *Lactobacillus rhamnosus* GG (LGG) lysate and supernatant were similarly prepared for comparison.

2. Cell line culture

The HepG2 human liver adherent cell line used in this study was purchased from Korean Cell Line Bank (KCLB, Seoul, Korea). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), and 1% penicillin/streptomycin (all from Gibco, Waltham, MA, USA) until achieved confluency. Cells were then subcultured at 70% confluency and maintained at 37°Cin an atmosphere of 5% CO_2.

3. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

To assess cell viability, the Vybrant™ 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay kit (Invitrogen, Carlsbad, MA, USA) was used following the manufacturer's instruction. Briefly, HepG2 cells were seeded in a 96-well plate at a density of 5×10^4 cells per well for 2 days and then the medium was changed to an FBS and antibiotic-free medium, followed by incubation overnight. Then, the cells were incubated with the vehicle, lysates, or supernatants for 24 hours, and then the medium was changed to serum and antibiotic-free DMEM without phenol red. We used PBS as the vehicle. MTT solution was added to the cells, followed by incubation in the dark for 4 hours at 37℃ in an atmosphere of 5% CO_2. Then, the cells were treated with sodium dodecyl sulfate (SDS) dissolved 0.01 N hydrochloride (HCl) for 4 hours and the absorbance of the resultant suspension in the culture plate was read at 540nm using Epoch2 Microplate Spectrophotometer (BioTek, Winooski, VT, USA). The cell viability of the test sample was calculated as the percentage absorbance relative to that of the untreated control sample.

4. Lipid accumulation in HepG2 cell

To assess the effect of *L. plantarum* Q180 on lipid accumulation, we experimentally induced lipid accumulation in HepG2 cells using a previously reported method (Liu et al. 2011; Kwon et al. 2016) with modifications. Briefly, palmitic acid was dissolved in 0.1 M sodium hydroxide (NaOH) to a concentration of 100 mM, which was then slowly mixed with 5% bovine serum albumin (BSA) in a 60℃ water bath. Then, the BSA-conjugated palmitic acid was filtered, dissolved in serum-free medium, and added to HepG2 cells seeded in a six-well plate at a density of 5×10^5 cells per well. After a 2-day incubation, the cells were serum-starved overnight and then the medium was changed to serum-free DMEM containing BSA-conjugated palmitic acid. The lysate and supernatants were incubated with BSA-conjugated palmitic acid medium. After 1-day incubation, the medium was changed to serum-free DMEM. To investigate lipid accumulation, HepG2 cells were analyzed using Oil Red O staining. To analyze the relative mRNA expression following lipid accumulation, HepG2 cells were harvested and we performed RNA extraction and cDNA synthesis. The relative mRNA expression was determined using real-time quantitative polymerase chain reaction (qPCR).

5. Oil Red O staining

We assessed lipid accumulation in HepG2 cell using a commercially available Oil Red O staining kit (Biovision, Milpitas, CA, USA), following the manufacturer's instructions. Briefly, cells were fixed with 10% formalin, washed twice, stained,

examined using a microscope, and then the lipid accumulated in the cells was extracted with 100% isopropanol and quantified by reading the absorbance at 492nm.

6. RNA extraction and real-time qPCR analysis

Total RNA was extracted from HepG2 cells after lipid accumulation and treatment using a kit (Promega, Madison, WI, USA) following the manufacturer's instructions. The extracted RNA was quantified, and then 2μg was reverse-transcribed using a cDNA synthesis kit (Bioneer, Daejeon, Korea). Real-time qPCR was performed using a 7500 Fast Real-Time PCR system using TB Green® Premix Ex Taq™ II (Takara Bio Inc., Shiga, Japan) followed the manufacturer's instructions and conditions.

7. Statistical analysis

Statistical analysis was performed using the R program (R Core Team, R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org). All data from the experiment are expressed as means±standard error of the mean. Data were verified for homogeneity of variance and normality before the statistical analysis, which was performed using a one way-analysis of variance (ANOVA), followed by Duncan variance test. All p-values were two-sided and differences were considered statistically significant at $p<0.05$.

Results and Discussion

1. Results

As shown in Fig. 1, there was no significant difference in cell viability 24 hours after treatment with the *L. plantarum* Q180 lysate or supernatant compared with untreated cells. Treatment with the supernatant decreased the cell viability more than the lysate treatment did, although the difference was not statistically significant. This result implies that treatment with the *L. plantarum* Q180 lysate or supernatant was not harmful to the cells.

The Oil Red O staining to investigate the effects of the *L. plantarum* Q180 lysate and supernatant on lipid accumulation showed that they both significantly decreased lipid accumulation more than palmitic acid did (Fig. 2). Although the *L. plantarum* Q180 supernatant-treated group showed a statistical difference in lipid accumulation compared to the *L. plantarum* Q180 lysate-treated and *L. rhamnosus* GG (LGG) groups, the accumulation was significantly lower than that of the palmitic acid- treated group. The LGG treatment group showed a similar decrease in lipid accumulation to that of the *L. plantarum* Q180 lysate-treated group. These results suggest that the lysates and supernatant of *L. plantarum* Q180 contributes to reducing lipid accumulation.

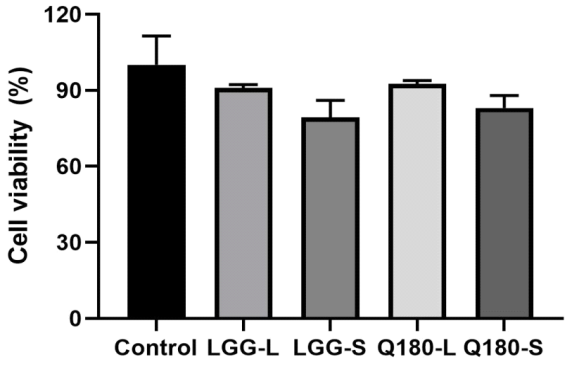

Fig. 1. Cell viability after treatment *Lactobacillus plantarum* Q180 to HepG2 cell. Bar charts show the mean ±SEM. * $p<0.05$. Significantly different from the values between groups, using the one-way ANOVA test followed by Duncan's multiple range test. LGG-L: *Lactobacillus rhamnosus* GG lysate solution treated group; LGG-S: *Lactobacillus rhamnosus* GG supernatant treated group; Q180-L: *Lactobacillus plantarum* Q180 lysate solution treated group; Q180-S: *Lactobacillus plantarum* Q180 supernatants treated group.

We investigated differences in mRNA expression of genes related to lipid metabolism induced by *L. plantarum* Q180 treatment. The relative mRNA expression of the lipid synthesis-related genes such as acetyl-CoA carboxylase (*ACC*) and sterol regulatory element-binding protein-1c (*SREBP1c*) were significantly down regulated in the *L. plantarum* Q180 supernatant-treated group than those of the palmitic acid-treated group. *L. plantarum* Q180 lysate-treated group, but only the *ACC* mRNA expression showed a significant difference. In the fatty acid synthase (*FAS*) mRNA analysis, the *L. plantarum* Q180-treated groups showed a tendency to decrease more than the other groups but there was no significant difference among the groups.

In addition, the relative mRNA expression of CCAAT-enhancer-binding protein-α (*C/EBPα*), which is an adipogenesis-related gene, decreased significantly in the *L. plantarum* Q180-treated group compared to palmitic acid-treated group. The relative mRNA expression of peroxisome proliferator-activated receptor-γ (*PPARγ*) in the *L. plantarum* Q180-treated group showed a

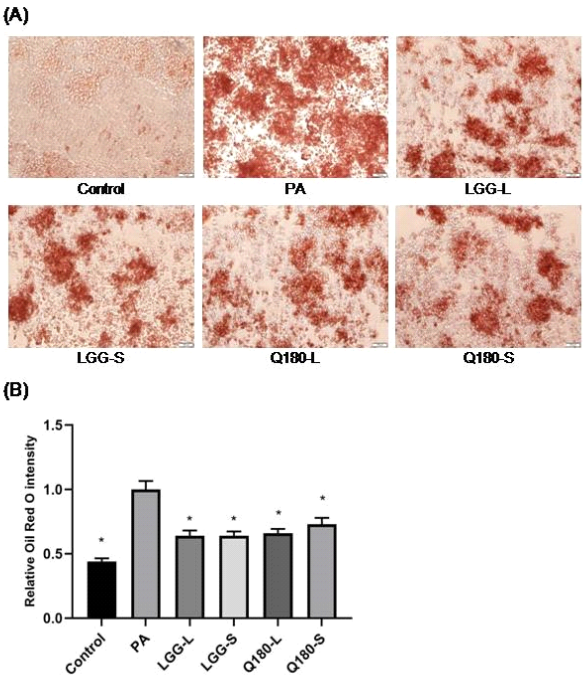

Fig. 2. Lipid accumulation after treatment *Lactobacillus plantarum* Q180 in HepG2 cells (A) Microscopic observation determined by Oil Red O staining. (B) Quantitative analysis of lipid accumulation measured by absorbance of stained cells. Bar charts show the mean±SEM. $^{*}p<0.05$. Significantly different from the values between groups, using the one-way ANOVA test followed by Duncan's multiple range test. PA, palmitic acid treated group; LGG-L, *Lactobacillus rhamnosus* GG lysate solution treated group; LGG-S, *Lactobacillus rhamnosus* GG supernatant treated group; Q180-L, *Lactobacillus plantarum* Q180 lysate solution treated group; Q180-S, *Lactobacillus plantarum* Q180 supernatants treated group.

slight decreasing trend, although it was not significantly different compared to that of the palmitic acid-treated group (Fig. 3B). As shown in Fig. 3C, analysis of the mRNA expression levels of the lipid oxidation-related *PPARα* and *CPT* genes of the *L. plantarum* Q180-treated group revealed they were not different from those of the palmitic acid-treated group. These results suggest that *L. plantarum* Q180 contributed to inhibiting lipid synthesis and adipogenesis.

2. Discussion

In the present *in vitro* experimental study, we investigated the effect of *L. plantarum* Q180 on lipid accumulation and changes in lipid metabolism-related gene expression. This *in vitro* study showed that *L. plantarum* Q180 decreased the lipid accumulation in a palmitic acid-induced model of HepG2 cells. In addition, an increment in the mRNA expression of lipid oxidation-related genes and decrease in that of lipid synthesis-related genes was observed.

The *SREBP1c* gene is a representative regulatory factor in *de novo* lipogenesis (Ferre et al. 2007) and its overexpression was shown to induce accumulation of fatty acids and lower lipid oxidation in bovine hepatocytes (Li et al. 2014). Furthermore, another study showed increased mRNA expression in a high-fat diet-induced obese mouse model (Ponugoti et al. 2010). Transcription of *SREBP1c* regulates both *ACC* and *FAS* genes, which are related to lipogenic metabolism (Sekiya et al. 2008). In this study, the expression levels of SREBP1c and ACC in the *L. plantarum* Q180-treated group were significantly decreased compared to that in palmitic acid-treated group while *FAS* expression showed a decreasing trend, but it was not significant. In addition, our findings showed decreased expression levels of *PPARɣ* and *C/EBPα*, which are important activators of lipogenesis (Madsen et al. 2014). However, the change in mRNA expression levels of *PPARɣ* was not significant, whereas that of C/EBPα was. Taken together, these results indicated that treatment with *L. plantarum* Q180 might control the expression of *SREBP1c* and its downstream genes, leading to the inhibition of lipid synthesis.

The *PPARα* gene is one of the essential factors for ß-oxidation, which stimulates mitochondrial fatty acid oxidation (Chinetti et al. 2000) and regulates other genes that control fatty acid import and lipid oxidation pathway such as carnitine palmitoyl transferase 1 (*CPT1*) (Rakhshandehroo et al. 2010). This signaling pathway mediates the catabolism of fatty acids and lowers triglyceride concentrations in circulation. However, *L. plantarum* Q180 did not stimulate ß-oxidation-related gene expression in this study. The increased expression of these genes in the palmitic acid-treated group might have been affected by excess lipid supplementation to maintain homeostasis (Esposito et al. 2009; Alves et al. 2017). The expression of *CPT1* is influenced by that of *PPARα* and, therefore, insufficient, *PPARα* expression might also affect CPT1. Therefore, we did not identify clear differences in ß-oxidation-related gene expression levels between the palmitic acid-treated and *L. plantarum* Q180 groups.

The mechanisms underlying the action of probiotics in gene expression modification have not been previously elucidated. However, research studies have proposed diverse hypothesis to

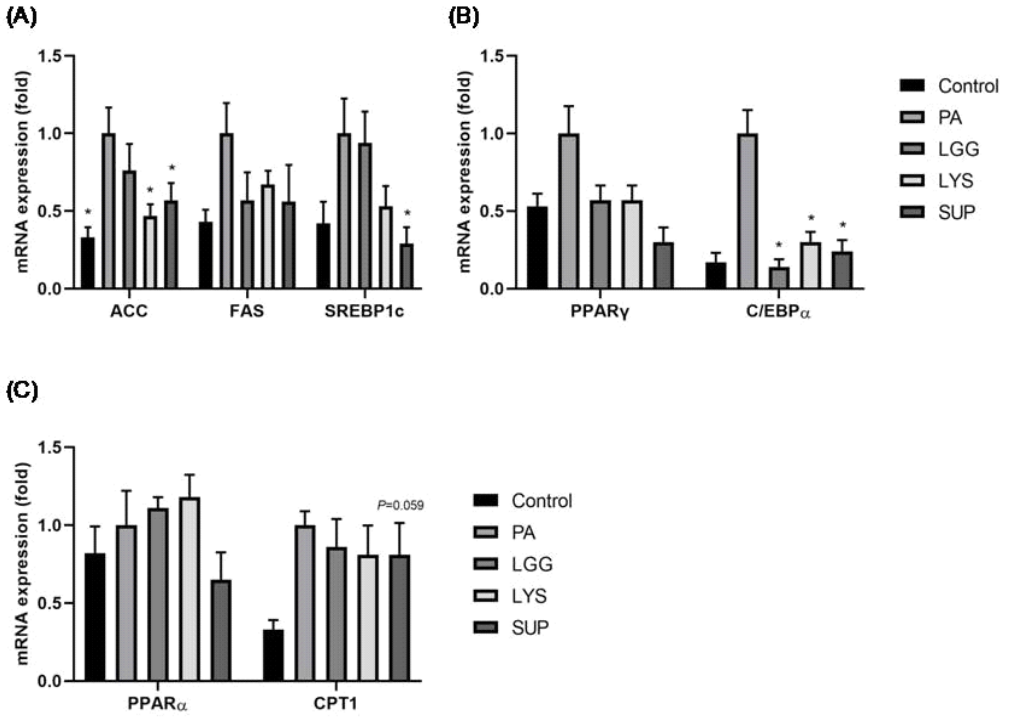

Fig. 3. Lipid synthesis, adipogenesis and ß-oxidation related genes expression in HepG2 cells treated with *Lactobacillus plantarum* Q180. (A) ACC, FAS and SREBP1c gene expression, (B) PPARɣ and C/EBPαgene expression, (C) PPARα and CPT1 gene expression were measured by real-time quantitative PCR. Bar charts show the mean±SEM. $^{*}p<0.05$. Significantly different from the values between groups, using the one-way ANOVA test followed by Duncan's multiple range test. PA, palmitic acid treated group; LGG: *Lactobacillus rhamnosus* GG treated group; LYS, *Lactobacillus plantarum* Q180 lysate solution treated group; SUP, *Lactobacillus plantarum* Q180 supernatants treated group.

explain the mechanisms, including the involvement of metabolites produced by bacterial fermentation, which have been suggested as the most likely factors (Plaza-Diaz et al. 2019). We considered that probiotics may not act directly to improve blood hypertriglyceridemia, and so we used lysates and culture media of *L. plantarum* Q180 to investigate its probiotic activity. We observed significant changes in some analyses including lipid accumulation and the relative mRNA analysis, however, we did not identify the specific metabolite that induced those changes. Therefore, further, more comprehensive studies are needed to determine the active ingredients that directly mediate the changes in gene expression and establish the specific mechanism of action of *L. plantarum* Q180.

Conclusion

This study investigated the anti-obesity effects of *Lactobacillus plantarum* Q180 on lipid accumulation using an *in vitro* HepG2 cell model. HepG2 cells were induced lipid accumulation and the cells were incubated with *L. plantarum* Q180 lysate or supernatant to investigate changes in lipid accumulation and expression of lipid metabolism-related genes. Our results showed that the *L. plantarum* Q180-treated group significantly decreased lipid accumulation and mRNA expression of lipid synthesis- and adipogenesis-related genes compared to the palmitic acid-treated group. These results indicate that *L. plantarum* Q180 may contribute to ameliorating hypertriglyceridemia by inhibiting lipid synthesis and adipogenesis.

Acknowledgements

This research was supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through the Technology Commercialization Support Program, funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA) (Grant Number: 818028-2).

References

Al-Muzafar HM, Amin KA. 2017. Probiotic mixture improves fatty liver disease by virtue of its action on lipid profiles, leptin, and inflammatory biomarkers. *BMC Complement Altern Med* 17:43

Alves CC, Waitzberg DL, de Andrade LS, Dos Santos Aguiar L, Reis MB, Guanabara CC, Junior OA, Ribeiro DA, Sala P. 2017. Prebiotic and synbiotic modifications of beta oxidation and lipogenic gene expression after experimental hypercholesterolemia in rat liver. *Front Microbiol* 8:2010

Ann YG. 2011. [Lactic acid bacteria] probiotic lactic acid bacteria. *Korean J Food Nutr* 24:817-832

Chinetti G, Fruchart JC, Staels B. 2000. Peroxisome proliferator-activated receptors (PPARs): nuclear receptors at the crossroads between lipid metabolism and inflammation. *Inflamm Res* 49:497-505

Choi ID, Kim SH, Jeong JW, Lee DE, Huh CS, Hong SS, Sim JH, Ahn YT. 2016. Triglyceride-lowering effects of two probiotics, *Lactobacillus plantarum* KY1032 and *Lactobacillus curvatus* HY7601, in a rat model of high-fat diet-induced hypertriglyceridemia. *J Microbiol Biotechnol* 26:483-487

Esposito E, Iacono A, Bianco G, Autore G, Cuzzocrea S, Vajro P, Canani RB, Calignano A, Raso GM, Meli R. 2009. Probiotics reduce the inflammatory response induced by a high-fat diet in the liver of young rats. *J Nutr* 139:905-911

Ferre P, Foufelle F. 2007. SREBP-1c transcription factor and lipid homeostasis: clinical perspective. *Horm Res* 68:72-82

Franssen R, Monajemi H, Stroes ES, Kastelein JJ. 2011. Obesity and dyslipidemia. *Med Clin North Am* 95:893-902

Jang HM, Han SK, Kim JK, Oh SJ, Jang HB, Kim DH. 2019. *Lactobacillus sakei* alleviates high-fat-diet-induced obesity and anxiety in mice by inducing AMPK activation and SIRT1 expression and inhibiting gut microbiota-mediated NF-kappaB activation. *Mol Nutr Food Res* 63:e1800978

Karr S. 2017. Epidemiology and management of hyperlipidemia. *Am J Manag Care* 23:S139-S148

Korean Society of Lipidology and Atherosclerosis. 2018. Dyslipidemia fact sheets in Korea. Available from: http://www.lipid.or.kr/file/Dyslipidemia%20Fact%20Sheets%20in%20Korea%202018.pdf [cited 2019 Jun 20]

Kwon YS, Koh WJ. 2016. Diagnosis and treatment of nontuberculous mycobacterial lung disease. *J Korean Med Sci* 31:649-659

Li X, Li Y, Yang W, Xiao C, Fu S, Deng Q, Ding H, Wang Z, Liu G, Li X. 2014. SREBP-1c overexpression induces triglycerides accumulation through increasing lipid synthesis and decreasing lipid oxidation and VLDL assembly in bovine hepatocytes. *J Steroid Biochem Mol Biol* 143:174-182

Liu JF, Ma Y, Wang Y, Du ZY, Shen JK, Peng HL. 2011. Reduction of lipid accumulation in HepG2 cells by luteolin is associated with activation of AMPK and mitigation of oxidative stress. *Phytother Res* 25:588-596

Madsen MS, Siersbaek R, Boergesen M, Nielsen R, Mandrup S. 2014. Peroxisome proliferator-activated receptor gamma and C/EBP alpha synergistically activate key metabolic adipocyte genes by assisted loading. *Mol Cell Biol* 34:939-954

Park SY, Cho SA, Kim SH, Lim SD. 2014a. Physiological characteristics and anti-obesity effect of *Lactobacillus plantarum* Q180 isolated from feces. *Korean J Food Sci Anim Resour* 34:647-655

Park SY, Cho SA, Lim SD. 2014b. Application of response surface methodology (RSM) for optimization of anti-obesity effect in fermented milk by *Lactobacillus plantarum* Q180. *Korean J Food Sci Anim Resour* 34:836-843

Park SY, Kim S, Lim SD. 2018. The inhibitory effect of *L. plantarum* Q180 on adipocyte differentiation in 3T3-L1 and reduction of adipocyte size in mice fed high-fat diet. *Korean J Food Sci Anim Resour* 38:99-109

Park SY, Seong KS, Lim SD. 2016. Anti-obesity effect of yogurt fermented by *Lactobacillus plantarum* Q180 in diet-induced obese rats. *Korean J Food Sci Anim Resour* 36:77-83

Plaza-Diaz J, Ruiz-Ojeda FJ, Gil-Campos M, Gil A. 2019. Mechanisms of action of probiotics. *Adv Nutr* 10:S49-S66

Ponugoti B, Kim DH, Xiao Z, Smith Z, Miao J, Zang M, Wu SY, Chiang CM, Veenstra TD, Kemper JK. 2010. SIRT1 deacetylates and inhibits SREBP-1C activity in regulation of hepatic lipid metabolism. *J Biol Chem* 285:33959-33970

Rakhshandehroo M, Knoch B, Muller M, Kersten S. 2010. Peroxisome proliferator-activated receptor alpha target genes. *PPAR Res* 2010:1-20

Sekiya M, Hiraishi A, Touyama M, Sakamoto K. 2008. Oxidative stress induced lipid accumulation via SREBP1c activation in HepG2 cells. *Biochem Biophys Res Commun* 375:602-607

Ueda T, Tategaki A, Hamada K, Kishida H, Hosoe K, Morikawa H, Nakagawa K. 2018. Effects of *Pediococcus acidilactici* R037 on serum triglyceride levels in mice and rats after oral

administration. *J Nutr Sci Vitaminol* 64:41-47

Wang G, Huang W, Xia Y, Xiong Z, Ai L. 2019. Cholesterol-lowering potentials of *Lactobacillus* strain overexpression of bile salt hydrolase on high cholesterol diet-induced hypercholesterolemic mice. *Food Funct* 10:1684-1695

Yoon JA, Shin KO. 2018. Studies on the biological activity of synbiotics: A review. *Korean J Food Nutr* 31:319-327

Yousaf S, Hussain A, Rehman S, Aslam MS, Abbas Z. 2016. Hypoglycemic and hypolipidemic effects of *Lactobacillus fermentum*, fruit extracts of *Syzygium cumini* and *Momordica charantia* on diabetes induced mice. *Pak J Pharm Sci* 29:1535-1540

Received 09 December, 2019
Revised 12 December, 2019
Accepted 16 December, 2019