Basic nutritional investigation

Hypocholesterolemic effects of *Lactobacillus plantarum* KCTC3928 by increased bile acid excretion in C57BL/6 mice

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Abstract

**Objective:** We doubly coated *Lactobacillus plantarum* KCTC3928 with proteins and polysaccharide compounds to enhance its acid and bile resistance. The present study investigated the hypocholesterolemic effects of double-coated *L. plantarum* KCTC3928 in C57BL/6 mice fed a high-fat diet. The effects of live and dead bacteria were compared.

**Methods:** Six-week-old C57BL/6 male mice were divided into three groups: the control group was fed no *L. plantarum* KCTC3928, and the two treatment groups were orally fed live or dead *L. plantarum* KCTC3928 daily. Plasma and liver cholesterol and lipid levels, fecal bile acid, and gene and protein expressions were measured.

**Results:** Low-density lipoprotein cholesterol and plasma triacylglycerol levels were significantly lower in the group fed live bacteria, by 42% and 32%, respectively (*P* < 0.05), and fecal bile acid excretion was accelerated (+45%). Expression of the low-density lipoprotein receptor and 3-hydroxy-3-methylglutaryl coenzyme A reductase were marginally affected by the feeding of coated cells; however, the gene expression and protein levels of CYP7A1 were significantly upregulated after live *L. plantarum* KCTC3928 feeding (+80% for mRNA and +60% for protein expression).

**Conclusion:** Double-coated live *L. plantarum* KCTC3928 may have hypocholesterolemic effects in mice primarily due to induction of fecal bile acid secretion followed by increased degradation of hepatic cholesterol into bile acids. Studies in humans should confirm the effects in the future.

Keywords: 3-Hydroxy-3-methylglutaryl coenzyme A reductase; Low-density lipoprotein receptor; CYP7A1; Bile acids; Cholesterol

Introduction

Hypercholesterolemia is a major cause of atherosclerosis and cholesterol buildup in the coronary arteries [1], and subsequent formation of atheroma can cause coronary heart disease. Based on a meta-analysis, a 1% decrease in plasma cholesterol levels can lower the risk of coronary events up to 3%. This level of cholesterol decrease could be achieved by appropriate food intake such as low-cholesterol, low-fat diets [2], dietary fiber [3,4], and yogurt containing certain lactic acid bacteria (LAB) [5–7]. Plasma cholesterol can also be lowered by several independent mechanisms, including decreasing hepatic cholesterol biosynthesis, increasing plasma cholesterol removal, decreasing dietary cholesterol uptake, and disruption of bile acid reabsorption [8–11]. The relative activity of each mechanism can be assessed by quantifying appropriate cellular biomarkers. For example, the measurement of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a key rate-limiting enzyme, can provide information about hepatic cholesterol synthesis rate and so forth.

Lactic acid bacteria may be protective against mutagens and carcinogen exposure, may be preventive against
gastrointestinal disease, and have immune function [12], antidiabetic effect [13], and cholesterol-lowering abilities
[14,15]. Hypocholesterolemic effects of LAB have been shown in various animals and in human trials. The health
benefits of LAB are strain specific. The cholesterol-lowering mechanism of LAB has not been clearly demonstrated, but it
appears that LAB contribute to increased fecal excretion of bile acids and this may improve overall hepatic cholesterol
homeostasis.

*Lactobacillus plantarum* is one of the most common LAB found in fermented foods such as *kimchi* [16–18], soy bean
paste [19], and several cheeses [20]. Some strains of *L. plantarum* show potential for cholesterol-lowering abilities in
vitro; however, most LAB cannot survive the digestive process in the gastrointestinal tract after intake. Data have
shown, that of free bacteria ingested, only 1% survive gastrointestinal transit, limiting the overall therapeutic effect of
LAB [21]. Thus, some type of specific formulation is necessary to improve the survival rate of LAB after intake. Previously
we developed a microencapsulation technique to coat LAB cells with protein and carbohydrates to improve their
bioavailability in the large intestine [22]. This method improved acid resistance and bile resistance by 2.1-fold and
3.3-fold, respectively [23].

In the present study, we investigated the effects of the administration of double-coated *L. plantarum KCTC3928*
on plasma cholesterol metabolism in C57BL/6 mice. Because dead cell bodies may have biological effects, we
compared the administration of live *L. plantarum KCTC3928* with the administration of dead *L. plantarum*
cell bodies. We found that the oral administration of double-coated *L. plantarum KCTC3928* showed hypo-
cholesterolemic effects by multiple mechanisms: increasing fecal bile acid excretion and hepatic bile acid
synthesis.

**Materials and methods**

**Double-coated *L. plantarum KCTC3928***

Double-coated *L. plantarum KCTC3928*, live and dead cells, were obtained from the R&D Center of Cellbiotech
Co. Ltd (Gimpo, Korea). The cells were double-coated with carbohydrates and proteins [22,23]. These double-coated bac-
teria have better acid resistance, bile resistance, and stability in accelerated testing than do non-coated lactic acid bacteria.
They are not killed by gastric acid or bile acid on uptake, and they maintain their inherent physiologic activities.

**Reagents**

TRI reagent, reverse transcriptase, and *Taq* DNA polymerase were purchased from Bio-Rad (Seoul, Korea). The
oligodT1 primer and random hexamers were obtained from Promega (Madison, WI, USA). Enhanced chemilumines-
cent immunoblotting detection reagents and enhanced
chemiluminescent Hyper-film were purchased from Amer-
sham-Pharmacia Korea (Seoul, Korea). Primary antibodies
(anti-HMG-CoA reductase, anti-low-density lipoprotein
[LDL] receptor, anti-CYP7A1, and anti-CYP27A1) and
secondary antibodies (anti-rabbit, anti-mouse, and anti-
goa immunoglobulin G) were acquired from Santa Cruz
Biotechnology, Inc. (Santa Cruz, CA, USA). All other
chemicals were purchased from Duksan Pure Chemical
Co (Kyungki-Do, Korea).

**Mice**

Six-week-old C57BL/6 male mice were purchased from Samtako Co. (Kyunggido, Korea). All mice were fed a
commercial chow diet for 1 wk and randomly divided into three groups of seven mice each: the control group was fed
no *L. plantarum KCTC3928*, and the two treatment groups were orally fed with live (PL) or dead (PD) *L. plantarum
KCTC3928* daily. Each mouse in the PL group was fed 10⁹ colony-forming units of live *L. plantarum KCTC3928*, and each mouse in the PD group was fed 10¹⁰ each of dead *L. plantarum KCTC3928* for 4 wk. The mice were housed at
24 ± 2°C in the light from 08:00 to 10:00 h. At 0, 2, and
4 wk, all mice were fasted, after which body weight, plasma cholesterol, and lipid levels were measured. On the last day of
the experiment, the mice were sacrificed and their livers and intestines immediately removed, rinsed with saline solution,
and stored in liquid nitrogen for measurement of lipids and assays of gene expression.

**Determination of cholesterol and lipid levels in serum
and livers**

Mouse plasma was collected in tubes coated with ethylene-
diaminetetra-acetic acid on the first day of the experiment
(week 0), after 2 wk, and at the end of the feeding period
(4 wk). Blood samples were centrifuged at 10 000 rpm for
10 min to collect plasma. These samples were stored at
−80°C before analysis. Total, high-density lipoprotein
(HDL) cholesterol, and triacylglycerols were measured with
a commercial kit from Asan Pharmaceuticals (Seoul, Korea).
LDL cholesterol was measured using a kit from Wako Diag-
nostics (Richmond, VA, USA). Lipid analysis was performed
on a Cobas c111 machine (Roche Korea, Seoul, Korea).

**Determination of aspartate and alanine transaminases**

To confirm the safety of *L. plantarum KCTC3928*, aspar-
tate transaminase (AST) and alanine transaminase (ALT)
activities were measured in the liver using the method of
Reitman and Frankel [24] and a commercial assay kit
(Asan Pharmaceutical, Seoul, Korea). The reaction was pre-
incubated for 5 min at 37°C. Four microliters of serum was
added to the reactant and then incubated for 30 or 60 min
at 37°C for ALT or AST, respectively. After incubation,
20 μL of 2,4-dinitrophenylhydrazine and 200 μL 0.4 N NaOH were added to the reaction and incubated for 10 min at room temperature. Enzyme activities were monitored spectrophotometrically by following changes in absorbance at 490 nm. The activities were expressed as international units per liter of serum based on a standard curve.

**Histology of the liver and intestine**

To investigate changes in liver and intestine, those tissues were stained with Mayer’s hemalum (Merck, Darmstadt, Germany) and alcoholic eosin (Sigma-Aldrich, Munich, Germany). Tissue sections were prepared and soaked in 10% formaldehyde for 24 h and then stained using previously described reagents.

**Determination of fecal bile acids**

The effect of live or dead *L. plantarum* KCTC3928 on fecal bile acid excretion was examined. Mice fecal material was collected for the last 5 d of the experiment. Fecal bile acids were determined using a modified method of Fausa and Skålhegg [25]. To extract bile acids, 0.2 g of fecal material was soaked in 100% methanol for 7 d. Methanol extracts were centrifuged and concentrated to a 0.5-mL final volume. Three milliliters of commercial enzyme solution (0.1 M hydrazine hydrate, 0.4 N NaCl and 0.024 M Na₂ ethylenediamine tetra-acetic acid, pH 7.5, containing one tablet of protease inhibitor (Roche Molecular Biochemicals, Indianapolis, IN). The lysate was centrifuged at 14 000 rpm for 15 min at 4°C. Protein concentrations were determined using a protein assay reagent (Bio-Rad, Daegu, Korea).

For protein analysis, equal amounts of protein were boiled in sample buffer (Bio-Rad), separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis, and transferred onto a nitrocellulose membrane (0.45 μM, PROTRAN Nitrocellulose Transfer Membrane; Schleicher & Schuell BioScience GmbH, Dassel, Germany). Successful transfers were confirmed with Ponceau S (Sigma-Aldrich). Non-specific protein sites were blocked by incubation in phosphate buffered saline (PBS; pH 7.4) with 0.1% Tween 20 and 5% non-fat dried milk. Experimental antibodies (anti-LDL receptor, anti–HMG-CoA reductase, anti-CYP7A1, and anti-CYP27A1) were obtained from Santa Cruz Biotechnology, Inc. Transferred protein was incubated overnight at 4°C or 2 h at room temperature with anti-LDL receptor, anti–HMG-CoA reductase, anti-CYP7A1, or anti-CYP27A1 antibodies. After being washed three times with PBS-T (0.1% Tween 20 in PBS), the membrane was incubated with anti-goat immunoglobulin G (LDL receptor and CYP27A1) or anti-rabbit immunoglobulin G (HMG-CoA reductase and CYP7A1). After incubation, the membrane was again washed with PBS-T. Protein band detection was performed using the enhanced chemiluminescence agent (AmerHAM, Chicago, IL, USA) and the blot exposed to medical blue X-ray film (Agfa, Brussels, Belgium). The density of each protein immunoblot was quantified using Gel-pro Analyzer software (Media Cybernetics, MD, USA).

**Reverse transcriptase polymerase chain reaction analysis**

According to the manufacturer’s protocol, RNA was extracted from mouse liver using a TRI reagent kit (Welgene, Daegu, Korea). RNA was resuspended in diethanol pyrocatechate-treated water. The degree of RNA purification was determined by the A₂₆₀ to A₂₈₀ ratio using a spectrophotometer (Bio-Rad). Two micrograms of extracted RNA was converted to cDNA using 0.5 μL of PowerScript reverse transcriptase (RT), 1 μL of oligo dT₁₅ primer (50 μM), and 2 μL of deoxynucleoside (10 mM). RT polymerase chain reaction (PCR) was performed with 30, 30, 30, 35, and 27 annealing cycles of commercial enzyme solution (0.1 M hydrazine hydrate, 0.3 μmol/mL of nicotinamide adenosine dinucleotide) and 10 μL of the extract were mixed in a test tube. The mixtures were vortex-mixed every 5 min for 40 min. The absorbance of the supernatant was measured at 340 nm with a SmartSpec Plus spectrophotometer (Bio-Rad). Exhausted bile acid was determined by the reduction of oxidized nicotinamide adenosine dinucleotide.

**Statistical analysis**

All data were expressed as mean ± standard error of the mean. Treatments were compared using one-way analysis of variance followed by Tukey’s tests. Student’s t tests were used to confirm comparisons between groups. Statistical significance was considered to be at the *P* < 0.05 level.
Results

Effects of L. plantarum KCTC3928 on plasma cholesterol and triacylglycerol levels in mice

The in vivo hypolipidemic effects of live or dead L. plantarum KCTC3928 were investigated after oral administration of live or dead micro-organisms to mice fed a high-fat diet for 4 wk. Plasma lipid levels were measured at 0, 2, and 4 wk.

At 2 and 4 wk, total cholesterol levels in the PL group were decreased compared with the control group (119 versus 200 mg/dL in the PL versus control group, respectively, at 4 wk; Fig. 1A). Oral administration of live L. plantarum KCTC3928 decreased the plasma cholesterol level by 33%, whereas cholesterol levels in the PD and control groups did not change. Lactobacillus plantarum KCTC3928 feeding resulted in a decrease in LDL cholesterol levels in the PL (13 mg/dL, −42%) and PD (7 mg/dL, −20%) groups (Fig. 1C). Live and dead L. plantarum KCTC3928 increased the HDL cholesterol levels from 84 to 113 mg/dL (+35%) in the PL group and from 90 to 123 mg/dL (+36%) in the PD group (Fig. 1B). After L. plantarum KCTC3928 feeding, plasma triacylglycerol levels were decreased by 24 mg/dL (−32% decrease), especially in the PL group (Fig. 1D).

The atherogenic indices including HDL/total cholesterol, HDL cholesterol/LDL cholesterol, and triacylglycerol/HDL cholesterol were estimated (Table 1). The ratios of HDL cholesterol to total cholesterol in the PL and PD groups were 0.97 and 0.67, respectively. These were greater than the values of the control group (0.55). The ratios of HDL cholesterol to LDL cholesterol in the control, PL, and PD groups were 3.49, 7.18, and 4.77 respectively. The PL and PD groups also showed a positive effect for the ratio of triacylglycerol to HDL cholesterol. Compared with the control group, the ratios in the PL and PD groups were 0.45 and 0.61, respectively. The triacylglycerol/HDL cholesterol ratio was reduced by 47% and 28%, respectively. These results suggest that live and dead L. plantarum KCTC3928 may improve plasma lipid profiles, with findings of slightly better effects for the live L. plantarum KCTC3928.

Effects of L. plantarum KCTC3928 on hepatic cholesterol and triacylglycerol levels

The effects of the live or dead L. plantarum KCTC3928 on hepatic cholesterol and triacylglycerol levels were investigated. As presented in Table 2, hepatic cholesterol levels were increased after the administration of dead L. plantarum
KCTC3928; however, live L. plantarum KCTC3928 lowered hepatic cholesterol levels (−18%, P < 0.05). There were significant differences among the C, PL, and PD groups. The hepatic triacylglycerol levels were also increased after administration of dead L. plantarum KCTC3928; however, live L. plantarum KCTC3928 feeding lowered the triacylglycerol level by 28%.

Effects of L. plantarum KCTC3928 on liver functions in C57BL/6 mice

Because plasma AST and ALT levels are widely used indicators of liver function, we examined the levels of ALT and AST after L. plantarum KCTC3928 feeding. As shown in Figure 2, plasma AST levels were significantly decreased in the PL and PD groups (P < 0.05 and P < 0.01, respectively). Groups fed L. plantarum KCTC3928 showed decreases in AST levels of 13% and 8%, whereas AST levels in the controls increased by 25%. The PL group also showed a significant decrease in ALT activity (P < 0.05) compared with the control group after 4 wk. The PD group did not show significant changes in ALT activity. To confirm the histologic changes in the liver and small intestine after the intake of L. plantarum KCTC3928, hematoxylin and eosin staining was performed. Due to the high-fat diet, all experimental mouse livers showed fatty liver morphology; however, the PL and PD livers exhibited fewer fatty lesions compared with control livers (Fig. 3A). The small intestines of mice in the control group and in the live or dead L. plantarum

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>TC (mg/dL)</th>
<th>TG (mg/dL)</th>
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<tbody>
<tr>
<td>C</td>
<td>84 ± 7</td>
<td>1770 ± 156</td>
</tr>
<tr>
<td>PL</td>
<td>69 ± 11</td>
<td>1269 ± 298</td>
</tr>
<tr>
<td>PD</td>
<td>93 ± 8</td>
<td>2107 ± 161</td>
</tr>
</tbody>
</table>

C, control; PD, dead bacteria; PL, live bacteria; TC, total cholesterol; TG, triacylglycerols

Values are means ± SEMs (n = 4). Mean values within a column that are followed by the same letter are not significantly different (P < 0.05).

KCTC3928 feeding groups showed no significant histologic differences (Fig. 3B). These results suggest that live L. plantarum KCTC3928 does not have harmful effects on liver functions and may have some beneficial effects in mice.

Effect of L. plantarum KCTC3928 on fecal bile acids

The effect of live and dead L. plantarum KCTC3928 feeding on bile acid excretion was examined. Lactobacillus plantarum KCTC3928 feeding groups showed significant increases in fecal bile acids. The PL and PD groups showed 45% and 38% increases in fecal bile acid excretion, respectively, compared with the control group (Fig. 4).

Effect of L. plantarum KCTC3928 feeding on transcription of key genes in hepatic cholesterol metabolism

We examined the influence of 4 wk of L. plantarum KCTC3928 feeding on the transcription of the LDL receptor, HMG-CoA reductase, CYP7A1, and CYP27A1 in mouse liver using RT-PCR. Transcription of the hepatic LDL receptor gene were increased in the PL (+45%) and PD (+27%) groups (Fig. 5). Transcription of HMG-CoA reductase was decreased by 17% in the PL group. Transcription of the CYP7A1 gene was significantly increased in the PL group (P < 0.05, +80%), although CYP27A1 showed little change after feeding in all experimental groups.

Effect of L. plantarum KCTC3928 on protein levels

Expression of the LDL receptor, HMG-CoA reductase, cholesterol 7-α-hydroxylase, and sterol 27-α-hydroxylase genes were examined using immunoblotting (Fig. 6). LDL receptor levels were increased in the PL and PD groups by 19% and 44%, respectively, compared with the control
The expression of HMG-CoA reductase was not altered after feeding. The expression of cholesterol 7-α-hydroxylase, the protein product of the CYP7A1 gene, was significantly elevated (60%) in the PL group in parallel with transcriptional activation of the CYP7A1 gene. However, the PD group did not show this effect. The CYP27A1 protein level was slightly lower in the PL and PD groups.

Discussion

Our results showed that L. plantarum KCTC3928 had hypolipidemic effects. Oral administration of live L. plantarum KCTC3928 significantly decreased LDL cholesterol and triacylglycerol levels. Two atherogenic indices, HDL/total cholesterol and HDL/LDL cholesterol ratios, were significantly improved in the PL group. Hepatic cholesterol and triacylglycerol concentrations were lower in the PL group. Administration of dead L. plantarum KCTC3928 slightly increased HDL cholesterol levels but did not change other lipid levels. The expression of the hepatic LDL receptor and HMG-CoA reductase were marginally affected after L. plantarum KCTC3928 feeding; however, interestingly, transcription of the CYP7A1 gene in the PL group was significantly elevated compared with controls. Protein expression showed similar trends. These gene expression changes, together with the increase in fecal bile acid excretion, may contribute to the decrease of plasma and hepatic cholesterol levels after L. plantarum KCTC3928 feeding. Excess excretion of fecal bile acids could be harmful, however; in our experiment, bile acid excretion was mildly increased by

![Histology of liver (A) and small intestine (B) from C57BL/6 mice. Hematoxylin- and eosin- stained liver and intestine sections (200×). Note the pale hepatocyte cells in the control group. C, control group; PD, group fed dead bacteria; PL, group fed live bacteria.](image)

![Effect of Lactobacillus plantarum administration on fecal bile acid excretion in C57BL/6 mice. Data are mean ± SEM. C, control group; PD, group fed dead bacteria; PL, group fed live bacteria.](image)
45% and we did not find any negative indication of *L. plantarum* feeding; mice did not show any symptom of diarrhea during the feeding; the amount of collected stool samples were similar among groups; and *L. plantarum*, live and dead cells, did not show any toxicity on mouse livers.

The decreases in hepatic cholesterol and triacylglycerol levels and plasma AST and ALT levels suggested *L. plantarum* KCTC3928 does not have harmful effects on liver functions and may have some beneficial effects in mice. The histologic data confirmed that the hepatic fat-droplet accumulation was ameliorated after a high-fat diet in the PL and PD groups.

It is possible that a compound derived from the cell body may be an active hypolipidemic material. If so, dead cells may have shown similar lipid-lowering effects compared with live cells. This should be advantageous in industrial applications because dead cells could be directly used to make a functional product. This possibility was examined by comparing the effects of live cells with those of dead cells. We found that live cells were more effective than dead cells in the decrease of plasma lipid levels, bile acid excretion, and the expression of CYP7A1; however, dead cells also showed marginal effects such as HDL cholesterol and liver enzyme levels. Thus, we suppose that the active compounds may be derived from metabolic activity of live *L. plantarum*.

The serum and/or liver cholesterol and lipid level-lowering effects of *L. plantarum* and related bacteria have been previously shown [26]. The cholesterol-lowering effect of *L. plantarum* isolated from human stool samples was reported. Naruszewicz et al. [27] suggested that *L. plantarum* decreases cardiovascular disease risk factors in smokers, particularly by primary prevention of atherosclerosis, although the mechanism was inconclusive.

Plasma cholesterol lowering could be achieved by several mechanisms such as inhibiting hepatic synthesis, induction of cellular LDL uptake, accelerating degradation into bile acids of cholesterol [28], elevation of bile acid excretion from the intestine, and decreasing dietary cholesterol uptake in the intestine. Currently, inhibition of HMG-CoA reductase is a major pharmacologic strategy to lower plasma cholesterol levels because the enzyme mediates a rate-limiting step in the hepatic cholesterol biosynthesis [29–34]. Moreover, induction of the LDL receptor enhances cellular uptake of LDL cholesterol and thus contributes to the lowering of
plasma cholesterol levels [35,36]. Statins lower LDL cholesterol levels mainly by these two mechanisms. In addition, bile acid formation is a major pathway of cholesterol degradation and excretion from the body [11,37,38]. Bile acids are synthesized by complicated multiple pathways, however; it has been suggested that approximately 50% to 70% of bile acids are synthesized by the classic pathway and the gene CYP7A1 encodes the rate-limiting enzyme cholesterol 7α-hydroxylase of the classic pathway in the liver [39,40].

Induction of bile acid excretion is an additional approach to lower plasma cholesterol levels and several bile acid sequestrants are available in the market. Dietary cholesterol absorption from the intestine can be decreased by inhibition of an intestinal cholesterol transporter called NPC1L1, as in the case of ezetimibe [10].

We showed that oral administration of live L. plantarum decreased total and LDL cholesterol and triacylglycerol levels. HDL/total cholesterol and HDL/LDL cholesterol ratios were positively altered in the PL group. Our results showed that L. plantarum feeding marginally affected the expression of HMG-CoA reductase and the LDL receptor; however, bile acid excretion and the expression of CYP7A1 were substantially altered. Protein expression showed similar trends. Thus we suppose that the increase in fecal bile acid excretion may be the major mechanism to lower LDL cholesterol levels by L. plantarum and that CYP7A1 overexpression compensated for the smaller bile acid pool. Regulating hepatic cholesterol synthesis or cellular cholesterol uptake may not be a functional mechanism operated by L. plantarum.

There is some evidence that LAB could modulate bile acid excretion and this could cause the lowering of plasma cholesterol levels [15,41–44]. Liong and Shah [45] suggested that the cholesterol-decreasing activity of some Lactobacillus spp. resulted from its coprecipitation with deconjugated bile salts. There are some reports on bile-salt hydrolase in different species of Lactobacillus, Enterococcus, Peptostreptococcus, Bifidobacterium, Clostridium, and Bacteroides [46]. Bile-salt hydrolase is the enzyme that transforms deconjugated bile acids into free bile salts. Fecal loss of bile salts may cause an increased requirement for cholesterol as a precursor for new bile-salt synthesis, thereby resulting in a decrease in the level of cholesterol [47]. This suggested that the high bile-salt hydrolase activity of Lactobacillus spp. may lower serum cholesterol levels and bile-salt hydrolase may play an important role in the overall decrease in cholesterol.

![Effect of Lactobacillus plantarum administration on the protein levels of the LDL receptor, HMG-CoA reductase, CYP7A1, and CYP27A1 in C57BL/6 mice. The LDL receptor, HMG-CoA reductase, CYP7A1, and CYP27A1 protein was normalized to α-tubulin. C, control group; HMG-Coa, 3-hydroxy-3-methylglutaryl coenzyme A; LDL, low-density lipoprotein; PD, group fed dead bacteria; PL, group fed live bacteria.](image-url)
**Conclusion**

Our results show that *L. plantarum* has hypocholesterolemic and hepatoprotective effects. Live *L. plantarum* were more effective than dead bacteria in improving various plasma lipid levels. In particular, bile acid excretion, the CYP7A1 gene, and protein expression in the PL group animals were significantly higher than in the control group. Effects on humans should be confirmed in the future.

**References**


