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# Cholesterol Lowering Effect of *Lactobacillus plantarum* Isolated from Human Feces

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**Abstract** The purpose of this study was to isolate probiotic lactic acid bacteria (LAB) that produce bile salt hydrolase (BSH), and to evaluate its effects on serum cholesterol level. One-hundred-twenty bacterial colonies were initially isolated from human feces, and five strains were selected after screening based on their resistance to acids, tolerance against bile salts, and inhibitory activity on *Escherichia coli*. The *Lactobacillus plantarum* strain with the highest level of BSH activity was identified using 16S rRNA sequences, and was named *L. plantarum* CK 102. *L. plantarum* CK 102 at a level of $1.36\times 10^8$ cfu/ml survived in pH 2 buffer for 6 h and exhibited excellent tolerance for bile salt. Coculturing the strain with *E. coli* in MRS broth resulted in strong inhibition against growth of *E. coli* at 18 h. Furthermore, the potential effect of CK 102 on serum cholesterol level was evaluated in rats. Thirty-two rats [Sprague-Dawley (SD) male, 129±1 g, 5 weeks old] were divided into four groups of eight each. For six weeks, Group 1 was fed a normal diet (negative control); Group 2 was fed a cholesterol-enriched diet (positive control); Group 3 was fed a cholesterol-enriched diet plus *L. plantarum* CK 102 at $1.0\times 10^7$ cfu/ml; and Group 4 was fed a cholesterol-enriched diet plus *L. plantarum* CK 102 at $5.0\times 10^7$ cfu/ml. Blood samples were collected, serum lipids were analyzed, and weights of the organs were measured. Total blood cholesterol level, triglyceride, LDL-cholesterol, and free-cholesterol values were lower in rats that were fed *L. plantarum* CK 102 than in those not fed *L. plantarum* CK 102. This cholesterol lowering effect implies that *L. plantarum* CK 102 could be utilized as an additive for health-assistance foods. In conclusion, these results suggest that the *L. plantarum* CK 102 isolated could be used commercially as a probiotic.

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Cardiovascular disease (CVD) is a leading cause of death in many countries around the world. The World Health Organization (WHO) predicts that, by the year 2020, up to 40% of all deaths would be related to CVD [1]. A high level of serum total cholesterol is generally considered to be a risk factor for coronary heart disease and atherosclerosis [3, 18, 31, 43]. Thus, reducing cholesterol level decreases the incidence and mortality of ischemic heart disease (IHD) and atherosclerosis [7, 23].

Numerous drugs that can decrease cholesterol have been used to treat hypercholesterolemic individuals [37]. However, the undesirable side effects of these compounds have caused many concerns about their therapeutic use [6]. Diet has been identified as a means of controlling serum cholesterol concentrations [5]. In particular, the consumption of dairy products containing probiotics lowers serum cholesterol [11]. Probiotics are live microorganisms that, administered in adequate amounts, confer health benefits on the host [12, 19]. Bifidobacteria and lactobacilli are commonly used as probiotics. The health benefits supported by clinical data and promising animal data include treatment of hypercholesterolemia [9].

Mann and Spoerry [24] first reported that consumption of fermented milk was associated with reduced serum cholesterol level in the Maasai people. This research stimulated much interest in the cholesterol lowering effects of fermented milk and lactic acid bacteria. Thereafter, many experimental studies have been performed with animals and humans to elucidate the effect of fermented dairy products on serum

cholesterol, especially with selected strains of lactic acid bacteria [36].

Rao *et al.* [29] reported a hypocholesterolemic effect of milk that had been fermented by *Streptococcus thermophillus* in rats, and Rodas *et al.* [30] found a similar effect in hypercholesterolemic pigs when they were fed *L. acidophilus*. Taranto *et al.* [39] suggested that *L. reuteri* also might influence serum cholesterol concentration, and Been and Prasad [4] compared standard yogurt with bifidus yogurt inoculated with *Bifidobacterium bifidum*. However, the microorganisms have to overcome the acidity of gastric juice, numerous digestive enzymes, bile acid, intestinal peristaltic movement, immune response, and low surface tension in order to bring these beneficial effects [10]. Therefore, to be effective as a probiotic, LAB must survive the low pH of the stomach and arrive at the intestines as live microbes [26].

In this study, we isolated lactobacilli from human feces that had excellent hydrolysis activity of bile acid and evaluated its properties of acid and bile tolerance and its inhibitory effect on *E. coli*. We also evaluated its cholesterol lowering effect in rats.

## Materials and Methods

### Materials

LAB were isolated from feces of healthy Korean adults. *E. coli* KCTC 104C obtained from KCTC (Korea) was used as a standard microbe. Oxgall powder and bile extract were purchased from Sigma Co., Ltd. (St. Louis, MO, U.S.A.). MRS medium (Difco, Detroit, MI, U.S.A.) and LB broth (Difco, Detroit, MI, U.S.A.; 1.0% tryptone, 0.5% yeast extract, and 1.0% NaCl) were used for the LAB cultures. Cell incubation was maintained for one day at 37°C in anaerobic condition.

### Isolation of *Lactobacillus*

The feces collected from 30 healthy Korean adults were placed into pasteurized bottles and transported to the laboratory. A sterilized cotton rod was used to place the samples into 15-ml test tubes containing MRS liquid medium plus 0.02% sodium azide ($NaN_3$). After one-third (1/3) portion of the cotton rod was cut with sterilized scissors, the tubes were incubated at 37°C for 2 days. The strains suspected to be LAB were selected by observing the colony form, catalase reaction, Gram staining test, and lactic acid formation. Those LAB colonies on MRS agar medium were picked up with a platinum collector and re-cultivated in MRS liquid medium. The liquid cultures were centrifuged for 15 min at 7,000 rpm, and the supernatant was replaced with pasteurized 20% skim milk and stored at −70°C for further experiments.

### Bile Acid Deconjugation of LAB

Deconjugation of bile acid by LAB was measured by the agar plate assay of Tannock *et al.* [38]. Bile acids used in this examination were Oxgall powder and bile extract (Sigma, St. Louis, MO, U.S.A.). A mixture of 0.5% Oxgall and bile extract was added into MRS medium (Difco, Detroit, MI, U.S.A.). The agar plates were prepared by adding 1.7% agar to MRS broth, inoculated with LAB, and cultivated at 37°C for three days. The level of hydrolysis was measured by visually detecting the clear zones formed around the colonies, and these zones were divided into three classes by halo size of precipitate around the colonies.

### Analysis of Resistances to Acid and Bile Salt Acidity

The LAB isolated were analyzed for their resistance to acid and bile salt acidity. The reaction mixtures with various acidic pH levels of 2.0, 3.0, and 4.0 using 1/15 M $KH_2PO_4$-HCl buffer were prepared, and MRS broths at concentrations of 0, 0.1, 0.3, 0.5, and 1.0% (w/v) of bile salt were prepared. The reaction mixture and MRS broth were inoculated with $2.0\times10^8$ cfu/ml of LAB strains isolated. After six hours at 37°C, 100-μl samples were inoculated onto MRS agar plate, and the survival ratio (%) of each strain was calculated by the following equation:

Survival rate (%)
=(cell number in MRS containing HCl or bile salt /cell number in MRS)×100.

### Inhibition of *E. coli* Growth

For this experiment, the isolated strains were cultivated in MRS broth whereas *E. coli* was cultivated in LB broth for 12–24 h. One MRS broth was inoculated with $5.0\times10^8$ cfu/ml *E. coli* and the other was inoculated with *E. coli* and LAB strain at $5.0\times10^8$ cfu/ml. The inoculated MRS broths were sampled at 3- or 6-h intervals and diluted with pasteurized saline solution. One-hundred μl of the diluted sample was plated on LB medium at 37°C for 12–24 h, and the presence of *E. coli* was measured by counting the number of colonies on the LB medium. The inhibition of *E. coli* growth by LAB was measured by comparing *E. coli* counts in the presence of LAB against basal *E. coli* counts without LAB.

### Identification of LAB

One *Lactobacillus* strain that showed superior properties as a probiotic among various selected strains was identified by the 16S rRNA sequencing method [22, 33, 44], and sugar fermentation reaction of an API kit (BioMerieux, France) compared with Bergey's manual [35]. The LAB strain identified was named *L. plantarum* CK 102 for use in this study.

### Growth Conditions of LAB

*L. plantarum* CK 102 was cultured anaerobically in MRS medium (Difco Laboratories, Detroit, U.S.A.) at 37°C for 24 h. Upon termination of growth, the culture was maintained at −40°C in 15% glycerol solution, washed with sterile distilled water, and made into aliquots of two bacterial concentrations ($1.0\times10^7$ cfu/ml and $5.0\times10^7$ cfu/ml).

### Animal Experiment

Thirty-two rats [Sprague-Dawley (SD) male, 129±1 g, 5 weeks old] were purchased from the Experimental Animal Center (Korea). These rats were fed individually in plastic cages for seven days. After this adaptation period, they were divided into four groups of eight each. They were housed in an air-conditioned room at 22–24°C with 60–70% humidity and 12-h light-dark cycles (08:00–20:00). The base composition of the experimental diet is presented in Table 1. This diet was supplemented with 0.5% (by weight) cholesterol. Fresh water was available at all times while the experimental diets were given at 20 g/100 g of body weight per day. Group 1 was fed a normal diet as a negative control, Group 2 was fed a cholesterol-enriched diet as a positive control, Group 3 was fed a cholesterol-enriched diet plus CK102 at $1.0\times10^7$ cfu/ml concentration, and Group 4 was fed a cholesterol-enriched diet plus CK 102 at $5.0\times10^7$ cfu/ml concentration.

The assigned diets were given to the rats for six weeks. Their food intake was measured daily, and their body weight was recorded weekly. At the end of six weeks, the rats were deprived of diet for a minimum of 24 h and anesthetized with ether. Blood samples were collected from the aorta, placed in sterile tubes, and centrifuged at 2,000 ×*g* for 15 min at 4°C. The obtained serum samples were analyzed for total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, total lipid, free cholesterol, and phospholipids, using Cholesterol E-Test Wako, HDL-Cholesterol Test Wako, Triglyceride G-Test Wako, and Phospholipid C-Test Wako test kits (Wako Pure Chemical Industries Ltd., Tokyo, Japan), respectively. Low-density lipoprotein (LDL) cholesterol was calculated using the equations of Friedewald *et al.* [8]:

LDL cholesterol=total cholesterol
−HDL cholesterol−(triglyceride/5).

The AI (Atherogenic Index) was calculated as

AI=(total cholesterol
−HDL cholesterol)/HDL cholesterol.

We also excised and weighed liver, heart, kidney, lung, spleen, epididymal adipose tissue, and perirenal adipose tissue.

**Table 1.** Compositions of high-cholesterol diets for SD rats.

| Ingredients | Compositions (%) |
|---|---|
| Casein | 20.0 |
| L-Methionine | 0.3 |
| Beef tallow | 10.0 |
| Choline chloride | 0.2 |
| Corn starch | 44.3 |
| Sucrose | 20.0 |
| Cholic acid | 0.2 |
| Vitamin mixture (AIN 76) | 1.0 |
| Mineral mixture (AIN 76) | 3.5 |
| Cholesterol | 0.5 |
| Total | 100.0 |

**Table 2.** Deconjugation of bile salt by lactic acid bacteria under anaerobic condition.

| Bile salts | Deconjugation | | | | |
|---|---|---|---|---|---|
| | CK101 | CK102 | CK103 | CK104 | CK105 |
| Oxgall | − | ++ | − | + | + |
| Bile extract | + | +++ | + | ++ | + |

−: No halos of precipitate around colonies.
+: Halos of precipitate around colonies.
++: Big halos of precipitate around colonies.
+++: Very big halos of precipitate around colonies.

### Statistical Analysis

Data were collected and statistically analyzed with Windows MS Excel and SAS programs (Statistic Analytical System, U.S.A., V.8.01). The experimental data were presented as the means±standard deviation (SD). Analysis of variance, analysis of covariance, post hoc comparison of Tuckey, and regression analysis were applied to determine the statistical significance of the difference in lipids among the four groups, with the significance level set at $p<0.05$.

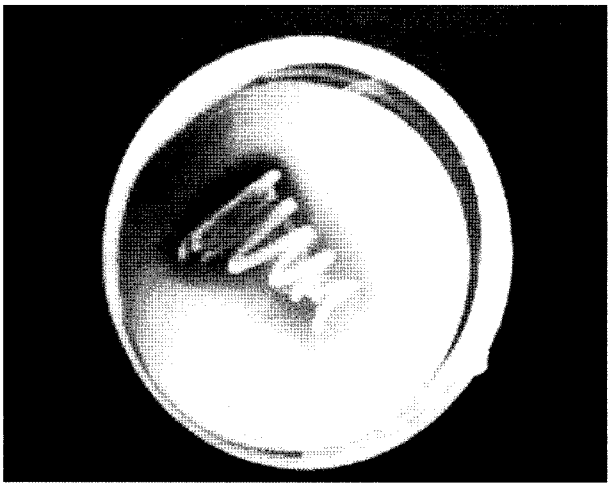

**Fig. 1.** Deconjugation of conjugated bile salts by *Lactobacillus* sp. CK 102 grown on MRS agar plates containing bile salts.

**Table 3.** Hydrochloric acid tolerance of various isolates of *Lactobacillus* sp. at different initial pH.

| pH | | CK 101 | CK 102 | CK 103 | CK 104 | CK 105 |
|---|---|---|---|---|---|---|
| pH 2.0 | Viable cell (cfu/ml) | 0 | $1.36\times10^8$ | 0 | $6.92\times10^8$ | 0 |
| | Viability (%)[a] | 0 | 68.2±3.4 | 0 | 34.6±1.7 | 0 |
| pH 3.0 | Viable cell (cfu/ml) | $3.08\times10^7$ | $1.65\times10^8$ | 0 | $1.52\times10^8$ | $6.64\times10^7$ |
| | Viability (%) | 15.4±0.8 | 82.7±4.1 | 0 | 75.8±3.8 | 33.2±1.7 |
| pH 4.0 | Viable cell (cfu/ml) | $1.90\times10^8$ | $1.98\times10^8$ | $8.32\times10^7$ | $1.98\times10^8$ | $1.89\times10^8$ |
| | Viability (%) | 95.1±4.8 | 99.0±5.0 | 41.6±2.1 | 99.0±5.0 | 94.6±4.7 |
| pH 5.0 | Viable cell (cfu/ml) | $1.94\times10^8$ | $1.98\times10^8$ | $1.54\times10^8$ | $1.98\times10^8$ | $1.92\times10^8$ |
| | Viability (%) | 97.2±4.9 | 99.0±5.0 | 77.2±3.9 | 99.0±5.0 | 96.2±4.8 |

[a]Viability (%)=(cell number in MRS containing HCl or bile salt÷cell number in MRS)×100. Inoculated various *Lactobacillus* sp. were approximately $2.0\times10^8$ cfu/ml at initial time and regarded as 100%.

## RESULTS

### *Lactobacillus* Isolation

Initially, 120 microbial colonies were obtained from human feces as raw material. Thirty colonies were selected based on criteria such as morphological shape, catalysis negativity, Gram positivity, and lactic acid formation, and they were considered to be LAB. Among these 30 colonies, five strains (*L. plantarum* CK 101, CK 102, CK 103, CK 104, and CK 105) were found to form clear round zones in solid MRS medium containing 0.5% bile salt. The clear zones around the colonies showed hydrolysis reaction by bile salt dehydrogenase, and their size indicated proportionality of bile salt dehydrogenase activity. These five strains were selected and kept for the next experiment.

### Bile Salt Deconjugation of *Lactobacillus*

After being subjected to hydrolysis of bile salt acid, two strains (CK 102 and CK 104) were found to be superior for bile salt deconjugation (Table 2). The strain CK 102 formed the largest clear zone (Fig. 1).

### Resistance to Acid and Bile Acidity

We investigated the survival rate of LAB at various conditions, especially low pH (pH 2.0–5.0) (Table 3). In general, each strain survived the exposure to low pH, although the microbial count in each case decreased markedly after 6 h. As LAB can reproduce rapidly, survival of some cells is sufficient for their viability. Therefore, we assumed 6 h to be sufficient to detect and determine any apparent difference in survival ratio. The survival ratio was tested by smearing the sample on the plate and counting the number of cells after six hours of incubation [20]. The CK 102 strain exhibited the highest survival ratio of 68.2%, even at pH 2.0. In order to test resistance against the bile, which is secreted from the duodenum, the LAB survival ratio was investigated under various concentrations of bile salt (0.1–1.0%). As revealed in Table 4, CK 102 exhibited the highest survival ratio of 92.2%, even in 1% bile acid, and CK 104 also showed a high survival ratio of 65.2%. The strains CK 102 and CK 104 were the most bile acid resistant strains among those tested.

### Inhibition of *E. coli* Growth

LAB are known to have an antibacterial effect against harmful bacteria. Such an inhibitory function is achieved by bacteriocin, a substance that LAB produce. The growth of *E. coli* in the presence of the LAB strain was gradually inhibited for 6–9 h, but the growth rapidly decreased after 12 h. *E. coli* was not detected after 18 h (Fig. 2). Other

**Table 4.** Bile salt tolerance of various isolates of *Lactobacillus* sp. at different concentrations of bile salts.

| Bile salt (%) | | CK 101 | CK 102 | CK 103 | CK 104 | CK 105 |
|---|---|---|---|---|---|---|
| 0.1 | Viable cell (cfu/ml) | $1.98\times10^8$ | $1.98\times10^8$ | $1.52\times10^8$ | $1.98\times10^8$ | $1.32\times10^8$ |
| | Viability (%)[a] | 99.0±5.0 | 99.0±5.0 | 76.2±3.8 | 99.0±5.0 | 66.1±3.3 |
| 0.3 | Viable cell (cfu/ml) | $1.10\times10^8$ | $1.98\times10^8$ | $9.02\times10^7$ | $1.98\times10^8$ | $6.24\times10^7$ |
| | Viability (%) | 55.1±2.8 | 99.0±5.0 | 45.1±2.3 | 99.0±5.0 | 31.2±1.6 |
| 0.5 | Viable cell (cfu/ml) | $7.06\times10^7$ | $1.98\times10^8$ | $2.38\times10^7$ | $1.98\times10^8$ | 0 |
| | Viability (%) | 35.3±1.8 | 99.0±5.0 | 11.9±0.6 | 99.0±5.0 | 0 |
| 1.0 | Viable cell (cfu/ml) | $2.42\times10^7$ | $1.84\times10^8$ | 0 | $1.30\times10^8$ | 0 |
| | Viability (%) | 12.1±0.6 | 92.2±4.6 | 0 | 65.2±3.3 | 0 |

[a]Viability (%)=(cell number in MRS containing HCl or bile salt÷cell number in MRS)×100. Inoculated various *Lactobacillus* sp. were approximately $2.0\times10^8$ cfu/ml at initial time and regarded as 100%.

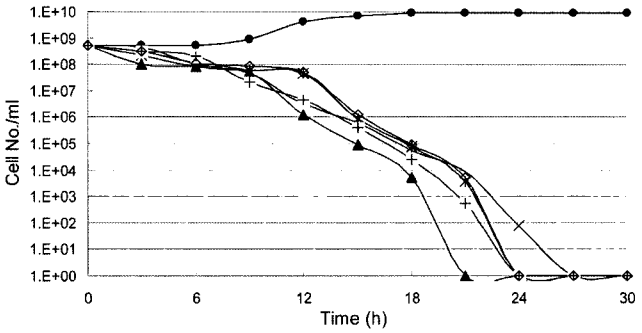


**Fig. 2.** Growth inhibition of *E. coli* KCTC 1041 by *Lactobacillus* sp. CK 101, 102, 103, 104, and 105 in MRS broth.
●, *E. coli* only; *, *E. coli* and *Lactobacillus* sp. CK 101; ▲, *E. coli* and *Lactobacillus* sp. CK 102; ×, *E. coli* and *Lactobacillus* sp. CK 103; +, *E. coli* and *Lactobacillus* sp. CK 104; □, *E. coli* and *Lactobacillus* sp. CK 105.

LAB strains exhibited similar inhibition of *E. coli* culture within 30 h; however, a difference in death hours was observed among them (Fig. 2).

## Identification of Lactobacillus

The *Lactobacillus* sp. CK 102 that showed superior experimental results was identified as *L. plantarum*, using 16S rRNA analysis. To confirm the result, the isolated CK 102 was amplified, and its RNA sequence was determined. The 16S rRNA sequence of CK 102 matched perfectly with that of *L. plantarum* WCFS1 (Fig. 3). Therefore, it was named *L. plantarum* CK 102 for use in this study.

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Query  22      ACAGTTACTCTCAGATATGTTCTTCTTTAACAACAGAGTTTTACGAGCCGAAACCCTTCT  81
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  163167  ACAGTTACTCTCAGATATGTTCTTCTTTAACAACAGAGTTTTACGAGCCGAAACCCT-CT  163226
Query  82      TCACTCACGCCGGCGTTGCTCCATCAGACTTTCGTCCATTGTGGAAGATTCCCTACTGCTG  141
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  163227  TCACTCACGCCGGCGTTGCTCCATCAGACTTTCGTCCATTGTGGAAGATTCCCTACTGCTG  163286
Query  142     CCTCCCGTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATTACCCTCTCAGGTC  201
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  163287  CCTCCCGTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATTACCCTCTCAGGTC  163346
Query  202     GGCTACGTATCATTGCCATGGTGAGCCGTTACCCCACCATCTAGCTAATACGCCGCGGGA  261
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  163347  GGCTACGTATCATTGCCATGGTGAGCCGTTACCCCACCATCTAGCTAATACGCCGCGGGA  163406
Query  262     CCATCCAAAAGTGATAGCCGAAGCCATCTTTCAAACTCGGACCATGCGGTCCAAGTTGTT  321
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Sbjct  163467  ATGCGGTATTAGCATCTGTTTCCAGGTGTTATCCCCCGCTTCTGGGCAGGTTTCCCACGT  163526
Query  382     GTTACTCACCAGTTCGCCACTCACTCAAATGTAAATCATGATGCAAGCACCAATCAATAC  441
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  163527  GTTACTCACCAGTTCGCCACTCACTCAAATGTAAATCATGATGCAAGCACCAATCAATAC  163586
Query  442     CAGAGTTCGTTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCCTGAGC  495
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  163587  CAGAGTTCGTTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCCTGAGC  163640
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**Fig. 3.** Sequence of *Lactobacillus plantarum* WCFS1. Query, *Lactobacillus plantarum* CK 102; subject 16S rRNA *Lactobacillus plantarum* WCFS1.

**Table 5.** The change of body weight and food intake after 6 weeks (mean±SD).

| Group* | Body weight (g) | | | Food intake (g/day)[#] |
|---|---|---|---|---|
| | Initial[#] | Final | Weight gain[@] | |
| 1 | 107.9±2.9[NS] | 297.3±31.2 | 189.4±31.6[a] | 32.7±7.2[NS] |
| 2 | 112.4±6.0 | 365.3±42.1 | 252.9±45.4[b] | 32.5±8.8 |
| 3 | 111.4±5.6 | 352.4±38.7 | 241.0±41.4[b] | 29.9±6.7 |
| 4 | 106.1±6.6 | 325.5±23.3 | 219.4±22.5[a] | 30.4±5.5 |

*Group 1, normal diet (negative control) rats; Group 2, cholesterol-enriched diet (positive control) rats; Group 3, cholesterol-enriched diet plus *Lactobacillus plantarum* CK 102 ($1.0\times10^7$ cfu/ml) rats; Group 4, cholesterol-enriched diet plus *Lactobacillus plantarum* CK 102 ($5.0\times10^7$ cfu/ml) rats.
[#]Analysis of variance.
[@]Analysis of covariance.
NS, Statistically insignificant.
[a,b]Statistically different group by post hoc comparison of Tuckey.

## Animal Experiments

The weight gain and food intake of rats during the experimental six-week period are presented in Table 5. The initial weights among the four groups did not differ [Analysis of Variance (ANOVA), $p>0.05$]. The mean body weight gains of the group that was fed cholesterol-enriched diet were slightly higher than those of the group fed *L. plantarum* CK 102, but the difference was statistically significant in only Group 4 (*L. plantarum* CK 102 $5.0\times10^7$ cfu/ml, Analysis of Covariance, $p<0.05$). The mean body weight gains of the cholesterol-enriched diet group were higher than those of the normal diet group. The mean food intakes among the groups did not differ (ANOVA, $p>0.05$).

The effects of *L. plantarum* CK 102 on serum lipids are presented in Tables 6 and 7. The mean serum concentration of triglyceride was reduced by *L. plantarum* CK 102 (ANOVA and post hoc comparison of Tuckey, $p<0.05$). Total cholesterol and LDL cholesterol were more reduced in the group fed $5.0\times10^7$ cfu/ml *L. plantarum* CK 102 than in the group fed a cholesterol-enriched diet only (ANOVA and post hoc comparison of Tuckey, $p<0.05$). As indicated in Table 7, total cholesterol in the group fed $5.0\times10^7$ cfu/ml *L. plantarum* CK 102 was 27.7% lower than that in the cholesterol-enriched diet group. The free-cholesterol values of the group fed *L. plantarum* CK 102 was lower than that of the cholesterol-enriched diet group (ANOVA and post

**Table 6.** The serum lipids level in rats (mean±SD) after 6 weeks.

| Group | Triglyceride* | Total lipid* | Phospholipid* |
|---|---|---|---|
| Group 1 | 44.6±18.2[a] | 240.4±92.4[NS] | 69.4±32.9[NS] |
| Group 2 | 49.8±28.6[a] | 347.6±193.6 | 85.5±29.9 |
| Group 3 | 22.0±23.0[b] | 254.0±148.0 | 70.8±27.4 |
| Group 4 | 19.1±11.3[b] | 236.0±111.7 | 64.8±33.5 |

*Analysis of variance.
[a,b]Statistically different group by post hoc comparison of Tuckey.
NS, Statistically insignificant.

**Table 7.** The cholesterol level and atherogenic index of serum in rats.

| Group | Total cholesterol* | HDL cholesterol* | LDL cholesterol* | Free cholesterol* | AI* |
|---|---|---|---|---|---|
| Group1 | 65.6±12.69[a] | 15.9±7.2[NS] | 41.1±5.9[a] | 3.43±0.98[a] | 2.62±1.39[NS] |
| Group2 | 100.6±20.6[b] | 18.8±7.3 | 71.9±16.2[b] | 7.75±3.11[b] | 4.32±2.12 |
| Group3 | 82.8±18.7[a,b] | 19.1±7.4 | 60.5±13.6[a,b] | 3.63±0.92[a] | 3.43±1.51 |
| Group4 | 72.5±12.6[a] | 16.8±8.5 | 51.3±10.9[a] | 3.50±1.20[a] | 3.34±1.21 |

*Analysis of variance.
[ab]Statistically different group by post hoc comparison of Tuckey.
NS, Statistically insignificant.

hoc comparison of Tuckey, $p<0.05$). HDL cholesterol, total lipid, phospholipids, and atherogenic indices did not differ (ANOVA and post hoc comparison of Tuckey, $p>0.05$). In order to control weight difference among the groups, we compared serum lipids by regression analysis, controlling the weight of the 6th week. However, the results did not change.

The weights of organs are presented in Table 8. Differences in weights of heart, liver, kidney, spleen, perirenal adipose tissue, and epididymal adipose tissue among the groups were statistically not significant (ANOVA, $p>0.05$). Specifically, in the case of perirenal adipose tissue and epididymal adipose tissue weights, those of the cholesterol-enriched diet group were higher than those of the *L. plantarum* CK 102 group, but statistically insignificant (ANOVA, $p>0.05$).

## Discussion

LAB, including *Streptococcus*, *Lactobacillus*, and *Bifidobacterium*, are normal components of the intestinal microflora in both humans and animals, and have been associated with various health-promoting properties. For these reasons, there has been much interest in developing food products containing these bacteria as dairy adjuncts [27, 45]. Lactobacilli are frequently used in products for human consumption and can be found as probiotics in infant foods, cultured milks, and various pharmaceutical preparations [32]. One beneficial effect that has been suggested to result from human consumption of LAB is a reduction of serum cholesterol levels, as suggested by the results of several human and animal studies [28].

*L. plantarum*, a normal resident of the human gut microflora, is able to adhere to the epithelial cells, with a preference for the small intestine [25]. A few reports have suggested its cholesterol lowering effect [16, 25]. In the present study, we isolated *L. plantarum* CK 102 that produced BSH, and then purified it and characterized its BSH activity. Furthermore, we evaluated the hypocholesterolemic effect of *L. plantarum* CK 102 in rats.

The precise mechanism of the cholesterol lowering activity of LAB has been difficult to explain. First, bacteria in the large intestine ferment unabsorbed carbohydrates and endogenous polysaccharides to produce short chain fatty acids (SCFA) (*e.g.*, acetate, propionate, and butyrate) [41]. When SCFAs are present in the gut, they are quickly absorbed in the large intestine and then metabolized by the liver [14]. Wolever *et al.* [42] concluded that acetate increases total cholesterol and decreases fatty acids, and that propionate increases blood glucose and lowers the hypercholesterolemic response caused by acetate, a precursor of cholesterol. An increase in bacterial count or a change in the composition of the bacterial population in the large intestine would result in increased fermentation and SCFA production. Depending on the proportion of each fatty acid produced, plasma cholesterol concentrations may thus be altered through this mechanism [36].

Another mechanism is that cholesterol would also bind to bile acids [9]. This assimilation of cholesterol by bacteria would make cholesterol unavailable for absorption into the circulation. It has also been proposed that the hypocholesterolemic effect seems to be related to the hydrolase activity of bile salts of the cells [21]. Bile

**Table 8.** The weights of organs in rats (mean±SD) after 6 weeks.

| Group | Heart* | Liver* | Kidney* | Spleen* | Perirenal adipose tissue* | Epididymal adipose tissue* |
|---|---|---|---|---|---|---|
| Group1 | 349.1±19.6[NS] | 3.64±0.43[NS] | 395.8±38.6[NS] | 174.8±17.1[NS] | 442.0±171.3[NS] | 505.6±109.3[NS] |
| Group2 | 321.6±23.3 | 3.84±0.43 | 383.1±30.5 | 190.1±34.9 | 605.0±118.8 | 619.5±66.9 |
| Group3 | 328.1±27.2 | 3.86±0.54 | 403.5±64.8 | 189.9±42.4 | 381.9±177.9 | 511.8±158.1 |
| Group4 | 355.0±30.3 | 3.65±0.23 | 398.0±39.9 | 214.3±33.9 | 410.5±103.8 | 538.0±63.1 |

*Analysis of variance.
Unit: mg/100 g BW.
NS, Statistically insignificant.

acids produced from cholesterol by hepatocytes are conjugated with glycine and taurine, and these conjugated bile acids are exposed to the intestinal microflora, where bacteria such as Bacteroides species, bifidobacteria, fusobacteria, clostridia, peptostreptococci, lactobacilli, and streptococci hydrolyze conjugated bile acids [13]. Because deconjugated bile acids are not reabsorbed in the large intestine, they are excreted through feces and urine. To maintain bile salt homeostasis, the bile acids have to be newly synthesized in the liver, thus increasing the demand for cholesterol as a precursor for bile acids [15, 40]. According to this theory, the hypocholesterolemic effect of bacteria is due to their hydrolysis of bile salts.

Bile acid is secreted from the duodenum and suppresses the bacterial growth. It is known that the microbes that are not native to intestinal organs cannot live in a medium containing bile acid, owing to the acidity [34]. In this study, LAB exhibited an inverse correlation between growth and bile salt acid concentration, as shown from agar plate growth tests using varying levels of bile acid. However, we noted that colonies became a pinpoint shape at high bile salt concentrations (above 0.5%). This result suggests that the isolated strains, although hindered in growth, were not killed by the acid.

Kim [17] investigated the effects of *L. plantarum* MUL-2, MUL-4, and MUL-18 isolated from *Mul-Kimchi* on the growth of *E. coli* and *Salmonella typhimurium*. After growing for 30 h, MUL-2 inhibited the growth of both *E. coli* and *S. typhimurium* by 50%, but *L. acidophilus* and *L. casei* showed low inhibitory effects, where *E. coli* cell concentration decreased from $10^7$ cfu/ml to only $10^5$–$10^6$ cfu/ml even after 40 h. However, in our study where CK 102 strain was used, no *E. coli* cells were visible after 18 h. The strain CK 102 seemed to exhibit a far stronger inhibition on *E. coli* than the microbes tested by Kim [17], possibly suggesting a better candidate as a probiotic.

In this study, *L. plantarum* CK 102, producing the highest activity for bile salts hydrolase, lowered serum lipids in rats. Specifically, the total cholesterol, triglyceride, LDL-cholesterol, and free-cholesterol values were lower in the *L. plantarum* CK 102-fed group than those of the cholesterol-enriched diet group. This finding is similar to data from other studies on LAB [2, 4] and *L. plantarum* [16, 25]. Because weight also can affect serum lipids, the effect of weight on lipids was adjusted by regression analysis. Nevertheless, the results did not change. Therefore, it was concluded that the difference in serum lipids among the groups was due to *L. plantarum* CK 102. The blood sample from rats could not be collected initially, so changes of serum lipids level were not compared during the six-week period among the groups. However the initial body weight and the amount of food intake were not different among the groups, thus possibly confirming our results. This result is encouraging for CK 102's potential application as a probiotic.

In summary, this study was focused on the cholesterol lowering effect of a *L. plantarum* genetic variant in Korean adults. Our results suggest that *L. plantarum* CK 102 supplement in the diet of rats significantly reduced serum cholesterol, triglycerides, free cholesterol, and LDL cholesterol without affecting serum HDL cholesterol. Thus, further efforts should be made to evaluate the use of *L. plantarum* CK 102 to reduce serum cholesterol in humans.

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