***Lactobacillus plantarum* strains as potential probiotic cultures with cholesterol-lowering activity**

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ABSTRACT

Elevated blood cholesterol is an important risk factor associated with atherosclerosis and coronary heart disease. The search for mediators that fine-tune cholesterol homeostasis has revealed lactic acid bacteria (LAB) to be potentially beneficial. The aim of the present study was to identify and characterize probiotic strains with bile salt hydrolase activity from kefir grains and evaluate their potential use as cholesterol-reducing probiotics in rats. Two isolates, Lp09 and Lp45, obtained from kefir grains were identified as *Lactobacillus plantarum* via molecular typing methods. *Lactobacillus plantarum* Lp09 and Lp45 exhibited excellent tolerance to low pH levels and high bile salt concentrations and showed potential bile salt hydrolase activity, bile salt deconjugation activity, and cholesterol coprecipitation ability. Additionally, the potential effect of *Lb. plantarum* Lp09 and Lp45 on plasma cholesterol levels was evaluated in Sprague-Dawley rats. Rats in 3 treatment groups were fed different experimental diets: a high-cholesterol diet, a high-cholesterol diet plus *Lb. plantarum* Lp09, or a high-cholesterol diet plus *Lb. plantarum* Lp45 for 4 wk. Total cholesterol, triglyceride, and low-density lipoprotein cholesterol levels in serum as well as cholesterol and triglyceride levels in liver were significantly decreased in the LAB-treated rats compared with rats fed a high-cholesterol diet without LAB supplementation. Also, both fecal cholesterol and bile acid levels were significantly increased after LAB administration. No significant changes were detected in high-density lipoprotein cholesterol levels. These results suggest that the *Lb. plantarum* Lp09 and Lp45 strains present the potential to be explored as probiotic agents for the management of hypercholesterolemia.

Key words: *Lactobacillus plantarum*, cholesterol-lowering activity, kefir grains

INTRODUCTION

Hypercholesterolemia is one of the major risk factors for cardiovascular disease, which is the leading cause of death in many countries (Law et al., 1994). It has been reported that even a 1% decrease in total serum cholesterol could reduce the risk of coronary heart disease by 2 to 3% (Manson et al., 1992). Current drug therapies, with their high relative costs and associated side effects, are not considered to be optimal long-term solutions for this condition. The development of new dietary supplements to reduce serum cholesterol is, therefore, of increasing interest. Recently, lactic acid bacteria (LAB) have attracted attention regarding their use as potential cholesterol-lowering milk additives (Chandan, 1999; de Roos and Katan, 2000). Reduction of cholesterol by LAB has been demonstrated in studies in humans, mice, and pigs (Kawase et al., 2000; Haberer et al., 2003; Sadrzadeh-Yeganeh et al., 2010). These cholesterol-lowering effects may be due in part to the deconjugation of bile salts by strains of bacteria that produce the enzyme bile salt hydrolase (BSH; Pereira et al., 2003; Ha et al., 2006). As deconjugated bile salts are more readily excreted in the feces than conjugated bile salts (De Smet et al., 1998; Jeun et al., 2010; Kumar et al., 2011), bacteria showing BSH activity may effectively reduce serum cholesterol by enhancing the excretion of bile salts, with a consequent increase in the synthesis of bile salts from serum cholesterol, or by decreasing the solubility of cholesterol and, thus, reducing its uptake from the gut.

Kefir is an alcoholic, fermented milk beverage produced by the fermentation of kefir grains, which contain LAB, acetic acid bacteria, and yeast (Farnworth and Mainville, 2003). Kefir possesses many different health-promoting abilities and has antitumor (Liu et al., 2002), antimutagenic (Liu et al., 2005), antioxidant (Liu et al., 2005), and hypocholesterolemic effects (Liu et al., 2006). Kefir is considered to be a good source of potentially beneficial and useful LAB. Various LAB present in kefir grains or products have been isolated and include *Lactobacillus acidophilus* (Angulo et al., 1993), *Lactobacillus plantarum* (Garrote et al., 2001), and *Lactobacillus kefirifaciens* (Chen et al., 2012).

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These studies have demonstrated the diversity of LAB in several different kefir grain starters. The aim of the current study was to identify and characterize probiotic strains with BSH activity from kefir grains and evaluate their potential use as cholesterol-reducing probiotics in rats.

MATERIALS AND METHODS

Screening for LAB with BSH Activity

Ten grams of kefir grains was suspended in 90 g of sterile saline buffer (0.85%) with a Stomacher blender (Stomacher 400 laboratory blender; Seward Ltd., West Sussex, UK) for 20 min. Serial dilutions of the suspended samples were used to perform microbial quantification and isolation using de Man, Rogosa, and Sharpe (MRS) agar (Difco Laboratories Inc., Detroit, MI) under anaerobic conditions. In addition, 200 µg/mL of cycloheximide (Sigma, St. Louis, MO) was added to both of the plates containing MRS agar to inhibit the growth of yeast. The plates were incubated at 30°C for 7 d. After 7 d, representative pure-culture colonies of LAB were collected and preliminary characterization was performed by colonial morphology and catalase testing.

The qualitative BSH activity of each of the LAB was evaluated using the procedure as previously described (du Toit et al., 1998). Sterile filter disks were impregnated in an overnight culture of the test strains and placed on MRS agar plates supplemented with 0.5% (wt/vol) taurodeoxycholic acid sodium salt (TDCA; Sigma) and 0.37 g/L of CaCl₂ (Merck, Darmstadt, Germany), whereas MRS agar plates without TDCA and CaCl₂ were used as controls. The plates were incubated anaerobically at 37°C for 72 h. The presence of precipitated bile acids around colonies (resulting in an opaque halo) or the formation of opaque granular white colonies with a silver shine was considered to be a positive reaction. Each strain was tested in triplicate. The 2 strains that showed the largest precipitation zone were selected for further characterization. Semiquantitative BSH activity assay was performed by measuring the release of amino acids from hydrolysis of conjugated bile salts by *Lactobacillus* strains used in the study as previously described (Kumar et al., 2011).

Identification of *Lactobacillus* Isolates by PCR

Genomic DNA was extracted from cultures grown at 37°C for 16 to 18 h in MRS broth and purified using the FastDNA Spin kit (Qbiogene Inc., Carlsbad, CA) according to the manufacturer's instructions. The DNA obtained was quantified using a Qubit fluorom-

eter (Invitrogen Detection Technologies, Eugene, OR). Polymerase chain reaction amplifications were carried out in a total volume of 20 µL, including a 0.5 µM concentration of each primer, 2.5 U of Taq DNA polymerase/100 µL, 20 ng of DNA template, a 200 µM concentration of each deoxyribonucleotide triphosphate (dNTP), and PCR buffer containing Tris-HCl, KCl, and MgCl₂. The amplification conditions were as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing for 30 s at 55°C, and a final extension of 10 min at 72°C.

The PCR-amplified DNA fragments were resolved by agarose gel electrophoresis and stained with ethidium bromide (0.5 mg/mL) and then visualized under UV light. Sequencing of the 16S rRNA gene was carried out to validate the phenotypic characterization of the *Lactobacillus* strains. The 16S recombinant DNA (rDNA) genes of the selected strains were amplified via PCR using 2 universal primers: 27f and 1492r (Weisburg et al., 1991). The obtained PCR products were cloned and sequenced and then compared with the NCBI BLAST sequence database (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) to identify the species of the strain. Sequencing was performed by Bio-Asia Diagnostics Co. Ltd. (Shanghai, China).

Bile and pH Tolerance

Bile salts (oxgall; Sigma) were added to a final concentration of 1% to MRS broth for the determination of bile tolerance of *Lactobacillus* strains. Cultures grown overnight were pelleted, washed, resuspended in peptone saline, and inoculated (approximately 10⁹ cfu/mL) into MRS broth either with bile salt (1%) or without bile salt, and their viability was determined using the plate count method. The cultures were incubated in the medium at 37°C for 0, 1, 2, or 3 h. The samples were collected, pelleted, and washed, and the number of colony-forming units in the samples was determined by performing serial dilutions in peptone saline water, followed by plating onto MRS agar medium and incubation at 37°C under anaerobic conditions for 24 h.

Initially, cell suspensions were prepared from the *Lactobacillus plantarum* Lp09 (**Lp09**) and *Lb. plantarum* Lp45 (**Lp45**) test cultures by pelleting cultures grown overnight (37°C), which was followed by washing and resuspension in peptone saline at a rate of 10⁹ cfu/mL. de Man, Rogosa, and Sharpe medium preadjusted to pH 3 or 6.8 was inoculated with approximately 10⁹ cfu/mL of Lp09 or Lp45 and then incubated at 37°C for 3 h. Viability was determined using the plate count method. A 1-mL aliquot was drawn from each tube at 0, 1, 2, and 3 h, pelleted, and washed, and 10-fold serial dilutions were performed using peptone saline diluent,

which was followed by plating onto MRS agar and anaerobic incubation of the plates at 37°C for 24 h.

Bile Salt Deconjugation

de Man, Rogosa, and Sharpe broth supplemented with 6 mM sodium glycocholate was inoculated with an overnight growth culture at a concentration of 1% and then incubated anaerobically at 37°C for 20 h. The bile salt deconjugation ability of the bacteria was measured following the protocol as previously described (Liong and Shah, 2005), based on the release of free cholic acid. After incubation, a 10-mL sample from the cultures of each organism was adjusted to pH 7.0 with NaOH (1 M) and centrifuged at $10,000 \times g$ at 4°C for 10 min. The pH of the obtained supernatant was adjusted to 1.0 with HCl (10 N). The assay reaction was set up by adding 1 mL of the supernatant to 2 mL of ethyl acetate, followed by vortexing for 1 min. Then, 2 mL of the ethyl acetate layer was transferred to a glass tube and evaporated under N₂ at 60°C. The residue was immediately dissolved in 1 mL of NaOH (0.01 M). After complete mixing, 1 mL of furfuraldehyde (1%) and 1 mL of H₂SO₄ (16 N) were added, and the mixture was vortexed for 1 min before heating at 65°C in a water bath for 10 min. After cooling, 2 mL of glacial acetic acid was added, and the mixture was vortexed for 1 min. The absorbance was read at 660 nm. The amount of cholic acid released was determined from the cholic acid standard curve (Sigma).

Coprecipitation of Cholesterol with Deconjugated Bile

Freshly prepared sterile MRS broth was supplemented with 6 mM sodium glycocholate. Filter-sterilized, water-soluble cholesterol was added to the broth at a final concentration 100 µg/mL. Coprecipitation of cholesterol with formed cholic acid was determined based on the difference between the cholesterol levels in the control (inoculated MRS broth without bile) before and after the inoculation of MRS broth supplemented with sodium glycocholate.

Animal Groups and Diets

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (National Institutes of Health, 1996). All animal studies were conducted according to the experimental practices and standards approved by the Animal Welfare and Research Ethics Committee at Jilin University (Changchun, P.R. China; permit number 20090719-1).

All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering of the rats. Forty male Sprague-Dawley rats, aged 4 wk and weighing 126.9 ± 3.7 g, were purchased from the National Animal Breeding and Research Centre (Beijing, China). The rats were fed a commercial diet (Kangqiao Inc., Beijing, China) that contained 32% protein, 5% fat, 2% fiber, 1.8% Ca, 1.2% P, and 59% N-free extract for 1 wk. After this adaptation period, the rats were randomly selected and assigned to 3 groups of 10 rats each. The initial average BW were similar among the 3 groups. The 4 groups were assigned diets according to the following regimen: (1) high-cholesterol diet (**HC diet**), (2) HC diet + *Lb. plantarum* Lp09 (**HC-Lp09**), or (3) HC diet + *Lb. plantarum* Lp45 (**HC-Lp45**). The HC diet contained 1% (wt/wt) cholesterol, 10% lard, 10% sucrose, 0.3% sodium cholate, 0.2% propylthiouracil, and a commercial diet mix (Aoboxing Biotech Co. Ltd., Beijing, China). All rats were housed individually in metal cages under controlled room temperature ($23 \pm 2^\circ\text{C}$) and humidity ($54 \pm 5\%$) and maintained on a 12-h light-dark cycle. The rats had free access to water and their group-specific diet. Each day, the HC-Lp09 group and HC-Lp45 group received 2 mL (10^9 cfu/mL) of a *Lb. plantarum* Lp09 or *Lb. plantarum* Lp45 solution, respectively, intragastrically for the 4-wk study period. The HC diet groups received an equivalent amount of normal saline. Body weights were recorded weekly and food consumption was monitored daily. After the feeding period, the rats were fasted for 12 h and euthanized. The weight of the liver was measured.

Assay for Serum Lipid Measurement

The overnight-fasted rats were bled from the tail vein under sodium pentobarbital anesthesia at d 28. Approximately 1 mL of blood was taken from each rat, transferred to nonheparinized vacuum collection tubes, and kept on ice for 30 min. The tubes were then centrifuged at $2,000 \times g$ for 20 min at 4°C. Serum total cholesterol (**TC**), high-density lipoprotein cholesterol (**HDL-C**), low-density lipoprotein cholesterol (**LDL-C**), and triglycerides (**TG**) were measured with commercial kits (BioSino Biotechnology and Science Inc., Beijing, China). Atherogenic indexes (TC/HDL-C and TG/HDL-C ratios) were then calculated.

Assays for Liver TC, TG, and Fecal Cholic Acid Measurement

After euthanasia, the rat livers were removed, rinsed with a physiological saline solution, blotted dry, and weighed. The liver TC and TG levels were determined

Table 1. Probiotic attributes of selected *Lactobacillus* strains¹

Probiotic attribute	Time (h)	Strain ²		
		Lp09	Lp45	ATCC 8014
Viable counts (log cfu/mL) at pH 3	0	9.12 ± 0.32	9.14 ± 0.26	9.10 ± 0.28
	1	7.44 ± 0.23	6.81 ± 0.41	7.53 ± 0.26
	2	6.35 ± 0.18 ^a	5.88 ± 0.12 ^b	5.73 ± 0.22 ^b
	3	5.69 ± 0.13 ^a	4.79 ± 0.27 ^b	4.62 ± 0.15 ^b
Viable counts (log cfu/mL) at 1% bile	0	9.08 ± 0.45	9.11 ± 0.32	9.06 ± 0.34
	1	8.21 ± 0.31	7.89 ± 0.18	8.26 ± 0.27
	2	7.65 ± 0.27 ^a	7.19 ± 0.21 ^b	7.12 ± 0.23 ^b
	3	6.30 ± 0.19 ^a	5.08 ± 0.13 ^b	5.01 ± 0.18 ^b
Bile salt deconjugation (mM)		4.31 ± 0.17 ^a	4.25 ± 0.19 ^a	3.11 ± 0.16 ^b
Cholesterol coprecipitation (µg/mL)		3.97 ± 0.15 ^a	3.68 ± 0.13 ^a	2.34 ± 0.16 ^b
Bile salt hydrolase activity (nmol/mL)		92.15 ± 6.48 ^a	89.63 ± 5.04 ^a	65.27 ± 3.21 ^b

^{a,b}Mean values within a row with different superscript letters differ significantly ($P < 0.05$).

¹The data are shown as means ± standard deviation.

²Lp09 = *Lactobacillus plantarum* Lp09; Lp45 = *Lb. plantarum* Lp45; ATCC 8014 = *Lb. plantarum* ATCC 8014.

according to the method of Chiu et al. (2006). The fecal droppings of the experimental animals were collected over a 2-d period (d 27 and 28) using disposable wooden chopsticks to avoid contamination with feed or rodent hair. The samples were dried in an oven (55°C) for 2 d and any remaining hair or dust contaminants were blown away using an air blower; the remaining material was finely ground using an analytical mill for further analysis. Fecal cholic acid levels were measured using published methods (Locket and Gallaher, 1989).

Statistical Analysis

Data analysis was carried out with SPSS Inc. software (version 10.0). A one-way ANOVA was used to check for any significant differences between means with a significance level of $P < 0.05$. Critical difference values were used to perform multiple comparisons between means. All data are presented as means with standard deviations. All experiments were replicated 3 times.

RESULTS

Screening and Identification of *Lactobacillus* Isolates

A total of 57 typical *Lactobacillus* isolates recovered from kefir grains on MRS plates were initially selected to screen for potential probiotic *Lactobacillus* isolates. Typical colonies of these *Lactobacillus* isolates were randomly selected from MRS plates based on their morphological characteristics under microscopic examination following Gram staining and a catalase-negative reaction.

Of the 26 strains screened for qualitative BSH activity on plates, only 6 returned positive results (data not

shown), with the observed precipitation zones differing in size. The 2 isolates displaying the largest precipitation zones were selected for further study.

The 2 isolates and the reference strain *Lb. plantarum* ATCC 8014, when subjected to the semiquantitative BSH activity assay (Table 1), showed that the BSH activity was 92.15 ± 6.48 , 89.63 ± 5.04 , and 65.27 ± 3.21 nmol/mL (for Lp09, Lp45, and ATCC 8014, respectively). The 2 isolates possessed significantly higher BSH activity compared with ATCC 8014. By expressing strong BSH activity, the 2 isolates were expected to play a positive health-promoting role, specifically by lowering serum cholesterol levels.

For 16S rRNA sequencing, genomic DNA was isolated, and an approximately 1.5-kb DNA fragment was amplified via PCR and then bidirectionally sequenced. The sequences of the 2 isolates were identical to that of *Lb. plantarum* LS7 (accession number JQ236617.1 in the NCBI GenBank database). Therefore, the 2 isolates were confirmed as strains of the species *Lb. plantarum* and were designated *Lb. plantarum* Lp09 and Lp45 (Figure 1).

Survival of *Lactobacillus* Strains at a Low pH and High Bile Concentration

The effect of an acidic pH (3.0) on the survival of the *Lactobacillus* strains over different incubation periods was recorded in Table 1. The 2 strains and the reference strain *Lb. plantarum* ATCC 8014 exhibited adequate survival at a pH of 3.0 for 3 h, despite marginal variations in the degree of viability. However, *Lb. plantarum* Lp09 exhibited the significantly higher survival rate after 2 h compared with *Lb. plantarum* Lp45 and ATCC 8014. The largest decrease in survival appeared after 3 h of incubation in *Lb. plantarum* Lp09, Lp45, and

Figure 1. Effects of the *Lactobacillus plantarum* Lp09 (Lp09) and *Lb. plantarum* Lp45 (Lp45) isolates on lipid metabolism in rats fed experimental diets. HC diet = high-cholesterol diet; HC-Lp09 = HC diet + Lp09; HC-Lp45 = HC diet + Lp45. (A) serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) contents; (B) atherogenic indexes of rats. The data are shown as means $\pm$ standard deviation. Mean values with different letters (a–c) within each clustered group differ significantly ($P < 0.05$).

ATCC 8014 examined in the present study. In general, *Lb. plantarum* Lp09 was significantly more acid tolerant than *Lb. plantarum* Lp45 and ATCC 8014 ($P < 0.05$).

The concentration of bile salts used in this experiment was high, representing a strong selective factor, whereas the in vivo bile concentration in the intestine was much lower. The data presented in Table 1 indicate that the more bile-tolerant strain was *Lb. plantarum* Lp09, which survived after 0, 1, 2, and 3 h of incubation in 1% bile at rates of up to 10^9 , 10^8 , 10^7 , and 10^6 cfu/mL, respectively. On the other hand, *Lb. plantarum* Lp45 and ATCC 8014 survived bile treatment at a rate of only approximately 10^5 cfu/mL after 3 h. *Lactobacil-*

lus plantarum Lp09 grew significantly ($P < 0.05$) better than *Lb. plantarum* Lp45 and ATCC 8014 in the presence of 1% bile salts.

Bile Salt Deconjugation

The bile salt deconjugation activity in the examined strains of lactobacilli is shown in Table 1. Bile salt deconjugation was determined by the amount of cholic acid released. *Lactobacillus plantarum* Lp09, Lp45, and ATCC 8014 demonstrated deconjugation activity at different levels. *Lactobacillus plantarum* Lp09 and Lp45 deconjugated significantly greater amounts of sodium glycocholate, as indicated by the release of 4.31 ± 0.17 mM and 4.25 ± 0.19 mM cholic acid compared with 3.11 ± 0.16 mM cholic acid released by ATCC 8014.

Coprecipitation of Cholesterol with Deconjugated Bile

The coprecipitation of cholesterol with cholic acid liberated from the deconjugation of sodium glycocholate by the lactobacilli is shown in Table 1. The amount of sodium glycocholate deconjugated by *Lb. plantarum* Lp09 and Lp45 was significantly greater than that of ATCC 8014.

BW and Feeding Efficiency

All of the rats appeared healthy throughout the feeding period. No significant differences in the total food intake ($P > 0.05$) were observed among the 3 groups (Table 2). The HC-Lp09 group showed a lower weight gain and food efficiency than the HC diet group ($P < 0.05$), whereas the HC-Lp45 group exhibited no obvious difference in weight gain or food efficiency compared with the model group.

Blood Lipid Analysis

The serum TC, TG, HDL-C, and LDL-C levels in the 4 groups are shown in Figure 1A. The TC, TG, and LDL-C levels differed significantly among the 3 groups. Compared with the HC diet group, the HC-Lp09 group achieved a maximal TC reduction of 30.5% ($P < 0.05$), TG reduction of 23.7% ($P < 0.05$), and LDL-C reduction of 47.9% ($P < 0.05$), whereas the HC-Lp45 group achieved a TC reduction of 18.4% ($P < 0.05$), a TG reduction of 12.3% ($P < 0.05$), and an LDL-C reduction of 21.5% ($P < 0.05$). The 2 LAB strains did not demonstrate obvious influences on HDL-C levels. Compared with the HC diet group, the atherogenic indexes were significantly lower in the HC-Lp09 group ($P < 0.05$) and the HC-Lp45 group ($P < 0.05$; Figure 1B).

Table 2. Body weight gain, total food intake, and feeding efficiency of rats fed experimental diets after 4 wk¹

Item	Treatment group ²		
	HC diet	HC-Lp09	HC-Lp45
BW gain (g)	172.6 ± 6.7 ^a	145.4 ± 8.5 ^b	169.3 ± 9.2 ^a
Total food intake (g)	467.3 ± 12.2 ^a	475.2 ± 9.5 ^a	471.7 ± 5.9 ^a
Food efficiency ³ (%)	36.9 ± 2.3 ^a	30.6 ± 1.6 ^b	35.9 ± 0.8 ^a

^{a,b}Mean values within a row with different superscript letters differ significantly ($P < 0.05$; $n = 10$).

¹The data are shown as means ± standard deviation.

²HC diet = high-cholesterol diet; HC-Lp09 = HC diet + *Lactobacillus plantarum* Lp09 (Lp09); HC-Lp45 = HC diet + *Lb. plantarum* Lp45 (Lp45).

³Food efficiency = (BW gain/food intake) × 100.

Between the 2 LAB-treated groups, the HC-Lp09 group showed a lower ($P < 0.05$) TC/HDL-C ratio and TG/HDL-C ratio than the HC-Lp45 group.

Liver Lipid Analyses

Table 3 provides the data obtained regarding the weight and lipid content of the liver. The average liver weight was not significantly different among the groups. The liver cholesterol and TG concentrations were significantly lower in the HC-Lp09 and HC-Lp45 groups than in the HC diet group ($P < 0.05$). The HC-Lp09 and HC-Lp45 groups showed no obvious differences in either hepatic TC or TG levels.

Fecal Cholic Acid Excretion

As shown in Figure 2, the fecal cholic acid levels differed significantly among the experimental groups ($P < 0.05$). The HC-Lp09 and HC-Lp45 groups showed considerable increases in the excretion of cholic acid in their feces. After 28 d of feeding, the maximum fecal cholic acid excretion ($6.35 \pm 0.12 \mu\text{mol/g}$ of feces) was recorded in the HC-Lp09 group, whereas in the HC-Lp45 group, the corresponding increase in fecal cholic acid was $6.23 \pm 0.11 \mu\text{mol/g}$.

DISCUSSION

Reduction of total cholesterol and LDL-C in hypercholesterolemic men reduces the incidence of cardiovas-

cular disease (Fradi et al., 2008). One possible strategy that could be promising and cost effective for lowering serum cholesterol levels is modulating diets through probiotic interventions. This approach could be very effective for the management of coronary heart disease.

The present study was initially aimed at investigating the role of 2 BSH-positive *Lb. plantarum* strains, Lp09 and Lp45, regarding their survival under a high bile concentration, along with their cholesterol-lowering ability under in vitro conditions, before assessing their functional efficacy in vivo in a rat model. *Lactobacillus plantarum* Lp09 and Lp45 showed different levels of viability at a pH of 3.0 and in the presence of 1% bile salts after 3 h, indicating that the isolates possess the potential to survive under the harsh conditions present in the GI tract.

The BSH activity of probiotics has often been correlated with their in vivo cholesterol-lowering effects (Ha et al., 2006; Jones et al., 2012). To establish the role of BSH in lowering cholesterol, the effect of the potential BSH-producing *Lb. plantarum* Lp09 and *Lb. plantarum* Lp45 strains was examined in the present study in a rat model. The results showed that BSH-active *Lb. plantarum* strains could reduce plasma TC and LDL-C in rats fed a diet high in cholesterol. Supplementation of the HC diet with Lp09 and Lp45 resulted in significant reductions in plasma TC, LDL-C, and TG in hypercholesterolemic rats.

It has been reported that certain probiotic strains can enhance fecal elimination of bile acids, which may

Table 3. Hepatic cholesterol and triglyceride (TG) levels in rats fed experimental diets¹

Item	Treatment group ²		
	HC diet	HC-Lp09	HC-Lp45
Liver weight (g)	9.08 ± 0.21 ^a	9.06 ± 0.09 ^a	9.01 ± 0.05 ^a
Liver cholesterol (mg/g)	12.27 ± 0.08 ^a	8.18 ± 0.06 ^b	8.11 ± 0.04 ^b
Liver TG (mg/g)	34.32 ± 0.26 ^a	28.54 ± 0.21 ^b	27.89 ± 0.13 ^b

^{a,b}Mean values within a row with different superscript letters differ significantly ($P < 0.05$; $n = 10$).

¹The data are shown as means ± standard deviation.

²HC diet = high-cholesterol diet; HC-Lp09 = HC diet + *Lactobacillus plantarum* Lp09 (Lp09); HC-Lp45 = HC diet + *Lb. plantarum* Lp45 (Lp45).

Figure 2. Fecal cholic acid concentrations in rats fed the experimental diets. HC diet = high-cholesterol diet; HC-Lp09 = HC diet + *Lactobacillus plantarum* Lp09 (Lp09); HC-Lp45 = HC diet + *Lb. plantarum* Lp45 (Lp45). The data are shown as means $\pm$ standard deviation. Mean values with different letters (a and b) differ significantly ($P < 0.05$).

alter cholesterol synthesis pathways and result in a decrease of serum cholesterol concentrations (Park et al., 2007). We found greater amounts of bile acids in the feces of the LAB-treated rats, especially the rats in the HC-Lp09 group. Therefore, one possible mechanism through which our 2 strains lowered cholesterol levels may be that more bile acids in the enterohepatic circulation were deconjugated and precipitated and, thus, excreted in the feces, resulting in reduced serum cholesterol concentrations. This may partly explain why the rats in the HC-Lp09 group exhibited lower serum cholesterol levels than the rats in the HC-Lp45 group.

CONCLUSIONS

The kefir grain-derived *Lb. plantarum* Lp09 strain showed strong bile salt resistance, tolerance of low pH levels, and unique hypocholesterolemic properties in vitro, and this strain exerted significant hypocholesterolemic effect on Sprague-Dawley rats fed an HC diet. Consumption of *Lb. plantarum* Lp09 as a probiotic dietary supplement might be useful for reducing human serum cholesterol levels. However, well-designed placebo-controlled clinical trials need to be conducted to validate the efficacy and safety of this strain and its potential use in the management of high cholesterol in humans.

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