J Korean Soc Appl Biol Chem (2014) 57(4), 597–603
DOI 10.1007/s13765-013-4226-y

Online ISSN 2234-344X
Print ISSN 1738-2203

ARTICLE

Characteristics of Cholesterol-lowering *Lactobacillus casei subsp. casei* strain GL-03 Isolated from Cheese

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Received: 16 September 2013 / Accepted: 9 June 2014 / Published Online: 31 October 2014

Abstract Cholesterol-lowering effect of lactic acid bacteria is well-known. In the present study, nine cholesterol-lowering *Lactobacillus* strains from Chinese traditional cheese, pickle, and yoghurt were screened and characterized for their potential use. The microbial contents of all strains significantly decreased at pH 1.5; however, the residual counts of *Lactobacillus casei subsp. casei* GL-03, *L. plantarum* ZP-Z, *L. plantarum* ZP-05, and *L. brevis* ZP-04 were more than 10^7 CFU/mL after incubation for 6 h. All nine strains of *Lactobacillus* indicated good tolerance to bile at concentration less than 0.2% after incubation for 2 to 6 h. *L. plantarum* ZP-W had maximum hydrophobicity towards xylene, whereas GL-03 strain possessed maximum hydrophobicity for both hexadecane and octane. ZP-05 strain had more effective inhibitory activity against both *Staphylococcus aureus* and *Bacillus subtilis* than other eight strains. Furthermore, GL-03 strain significantly reduced cholesterol TC and TG levels in hyperlipidemia mice fed high-cholesterol diet. The growth of GL-03 strain was promoted by five kinds of Chinese herbal medicines, and the Chinese hawthorn at concentration of 0.0125% showed the highest promoting effect. These results suggest that *L. casei subsp. casei* GL-03 may be effective as a probiotic with cholesterol-lowing activities.

Keywords Chinese herbal medicine · cholesterol-reduction · *Lactobacillus* · *Lactobacillus casei subsp. casei* GL-03

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Introduction

Probiotics are defined as living microorganisms. When administered in adequate amounts, they confer health beneficial effects on the host (Reid et al., 2003). The characteristics of a successful probiotic are acid and bile tolerance, antimicrobial activity against intestinal pathogens and ability to adhere and colonize the intestinal tract (Mishra and Prasad, 2005).

Lactic acid bacteria (LAB) strains are normal intestinal microflora in humans and animals. The ability of the LAB strains to survive from gastric and bile conditions and to adhere to the intestinal epithelium may confer a competitive advantage and is important for bacterial maintenance in the human gastrointestinal tract (Naidu et al., 1999).

Some LAB strains can be used as probiotics for human and animals (Chou and Bart, 1999), in which *Lactobacillus* and *Bifidobacterium* spp., in particular, have the ability to metabolize cholesterol (De Smet et al., 1995). It has been reported that *Lactobacillus* strains reduces blood cholesterol by direct breakdown of cholesterol and deconjugation of bile salt (Gilliland et al., 1985). The deconjugation of bile salts by *Lactobacillus* strains may attribute to the production of bile salt hydrolase (BSH) (Taranto et al., 1997).

In order to improve the growth of LAB, attempt is made by addition of prebiotics, such as non-absorbable starch (Topping et al., 2003), oligosaccharide (Van Loo et al., 1999), and other metabolizable sugars (Corcoran et al., 2005). The importance of the interaction between these indigestible dietary carbohydrates and the large-bowel microflora has been well recognized (Gibson and Roberfroid, 1995). Moreover, it was found that octadecenoic acid and *Trans* fatty acids have strong promotional activities for the *Lactobacillus* growth due to their incorporation into membrane lipids (Endo et al., 2006). However, the effect of Chinese medicinal herbs used as prebiotics has rarely been reported.

In the present study, we evaluated nine *Lactobacillus* strains from Chinese traditional cheese, pickle, and yoghurt by *in vitro*

assessment, and investigated their characteristics including cholesterol reduction, acid and bile tolerance, cell surface hydrophobicity, and antimicrobial effect on common pathogens. The promotional effects of five kinds of Chinese herbal medicine extracts on the growth of *Lactobacillus casei subsp. casei* GL-03 were further investigated.

Materials and Methods

Strains. *Lactobacillus* strains (GL-03, GL-AA, GL-02, ZP-W, ZP-05, ZP-Z, ZP-04, YS-09, and YS-11) were isolated from cheese, pickle, and yoghurt in our laboratory, characterized by physiological features, and identified to species level. The stock culture collection was maintained at −80°C in MRS broth with 40% glycerol. Prior to assays, strains were transferred at least three times at 37°C for 24 h in Lactobacilli MRS broth. The indicator strains including *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Shigella dysenteriae* were obtained from the Microbiology Laboratory (Dalian Polytechnic University, China).

In vitro cholesterol-lowering test. MRS broth containing 100 μg/mL cholesterol (AoBoXing Bio-tech CO., LTD, China) was distributed in test tubes. The inoculation volume of probiotic bacterial culture was 2% of cholesterol-MRS broth. After the tubes were incubated at 37°C for 48 h, the strains were centrifuged (10,000×*g*, 15 min). Uninoculated MRS broth was also incubated at 37°C for 48 h as the control. The cholesterol content assay was performed according to Rudel and Morris (1973) with modification. To measure the amount of cholesterol, the dye layer was observed at 560 nm. The observations were compared with a standard curve prepared by using appropriate concentrations of cholesterol, read at 560 nm, and reduction percent was determined in the spent broth by comparison with the uninoculated control.

Acid tolerance. The acid tolerance of Lactobacillus strains were assessed in MRS broth with different pH, which were prepared by adjusting the hydrochloric acid (HCl) solution to pH levels of 1.5, 2.5, 3.5, and 4.5. The cultures were inoculated (2%, v/v) into MRS broth at different pH values of 1.5, 2.5, 3.5, and 4.5 and incubated at 37°C for 0, 2, and 6 h,. The samples at each interval were serially diluted with double distilled water. Appropriate dilutions were plated in MRS agar and incubated aerobically at 37°C for 48 h.

Bile tolerance. The bile tolerance of lactic acid bacteria strains were studied in MRS broth containing 0.1–0.4% (*w*/*v*) oxgall. MRS broth was sterilized at 121°C for 15 min and stored at 4 until use. Over-night culture was inoculated (2%, *v*/*v*) into MRS broth containing different oxgall and incubated at 37°C for 0, 2, and 6 h. The samples at each interval were serially diluted with double distilled water. Appropriate dilutions were plated in MRS agar and incubated aerobically at 37°C for 48 h.

Cell surface hydrophobicity. The test for bacterial adhesion to hydrocarbons was adopted to screen lactobacilli for cell surface hydrophobicity. Hydrophobicity was determined by the method of Mishra and Prasad (2005) with modification. Briefly, the cultures were grown in MRS broth under aerobic conditions for 16–8 h at 37°C. Cultures were harvested after growth by centrifugation for 15 min at 5,000 rpm, washed twice in PBM buffer, and finally suspended in the same buffer. The initial absorbance (*A*) of the suspension at 600 nm was adjusted to 0.2–0.7. Five milliliters of cell suspension in PUM buffer (pH 7.1, 97.6 K_2HPO_4, 53.6 KH_2PO_4, 30 urea, and 0.81 mM $MgSO_4$) was taken in clean and dry round bottomed test tubes, and in the tubes, 1 mL of different hydrocarbon, including xylene, hexadecane, and octane was added and mixed by vortexing at 2,500 rpm for 2 min. The tubes were left undisturbed for 1 h at 37°C to allow the phase separation. The lower aqueous phase was carefully removed, and final absorbance (A_0) was recorded at 600 nm. The decreased absorbance in aqueous phase was taken as measure of cell surface hydrophobicity (*H*%), calculated using following equation:

$$H\% = \frac{A - A_0}{A} \times 100\%$$

where, *A*= initial absorbance at 600 nm, and; A_0= final absorbance.

Antimicrobial activity. To check the antimicrobial activity, the MRS agar plates were overlaid with soft MRS agar inoculated overnight with active culture of indicator strains (10^{-2} dilution). All wells were filled with 100 μL cell-free MRS broth cultures obtained by centrifuging at 2,400×*g* for 5 min. After incubation at 37°C for 36 h, the zone diameter of inhibition was measured.

In vivo cholesterol-lowering test. *Lactobacillus* strains GL-03 were grown in MRS broth for 20 h at 37°C to reach cell numbers of 10^9 CFU/mL. The cells were pelleted by centrifugation at 10,000 rpm for 5 min, washed twice with sterile saline, then were resuspended in 0.9% NaCl to obtain numbers of approximately 10^7 CFU/25 μL. The suspension was prepared daily for feeding to mice.

A total of 30 male mice were randomly assigned to three groups (n=10). The mice were fed a normal commercial diet as control group, and a high-cholesterol diet containing 12% fat, 1% cholesterol and 0.5% bile salt as hyperlipidemia group. To *Lactobacillus* strains (0.8 mL) GL-03 solution was orally administered to the hyperlipidemia mice once each day as GL-03 group. The mice of the former two groups were also orally administered with 0.8 mL of 0.9% NaCl. After administration of bacteria for 30 days and 12 h fasting, the mice were euthanized with ether. Blood was obtained from the retro orbital, and serum samples were analyzed for total cholesterol and triglycerides using an Express Plus analyzer (Chiron Diagnostics, USA). A p value of <0.05 was considered to be statistically significant by student's *t*-test.

Chinese herbal medicine extracts treatment. Fifty grams of each Chinese herbal medicinal plants (Chinese hawthorn, gambir plant, glossy privet fruit, Acanthopanacis Senticosi, and Codonopsis) were cut into small pieces and soaked in 200–50 mL water for 2 h, followed by boiling for 20–30 min. Each extract was filtered, and the residue was extracted two times. All extracts were concentrated to 100 mL and was centrifuged at 8,300×*g* for 15 min. The

supernatant was sterilized at 115°C for 15 min. Over-night cultures of GL-03 were inoculated (2%, *v*/*v*) into MRS broth containing 0.10, 0.05, 0.025, and 0.0125% (w/v) of Chinese herbal medicine extracts. After incubation at 37°C for 36 h, the OD value was measured at 620 nm.

Results

Screening and identification of Lactobacillus. A total of 50 cholesterol-lowering *Lactobacillus* strains were isolated from cheese, pickle, and yoghurt by determination of cholesterol content *in vitro*. Nine strains showed cholesterol reduction rate higher than 40%. According to the results of carbohydrates fermentation to produce acid in combination with physiological and biochemical tests, the species of nine strains were also identified, namely *L. casei subsp. casei* (GL-03), *L. murinus* (GL-AA and GL-02), *L. plantarum* (ZP-W, ZP-05, and ZP-Z), *L. brevis* (ZP-04) and *L. acidlophilus* (YS-09 and YS-11) (Table 1). ZP-05 showed lowest cholesterol reduction rate among the nine strains. There were no significant differences among GL-03, GL-AA, ZP-W, ZP-Z, ZP-04, and YS-09 with cholesterol reduction rate higher than 50% (p >0.05).

Acid and bile tolerance of cholesterol-lowering Lactobacillus. The survival of *Lactobacillus* strains at pH 1.5, 2.5, 3.5, and 4.5 was observed for 0, 2, and 6 h (Table 2). The microbial contents of all strains were significantly decreased at pH 1.5 after incubation for 2 h (p <0.05), and strains including GL-03, ZP-Z, ZP-05, and ZP-04 were relatively acid insensitive with the residual counts of more than 10^7 CFU/mL. All strains showed acid tolerance without losing viability at pH 2.5 and 3.5 after incubation for 2 h (p >0.05); however, all of the viabilities were significantly decreased at pH 2.5 up to 6 h (p <0.05). GL-02 and ZP-W were the most acid-sensitive of all strains tested with less than 10^7 and 10^9 CFU/mL at pH 2.5 and 3.5, respectively, after incubation for 6 h. All strains were tolerant in pH 4.5, retaining around 100% viability or a little growth up to 6 h.

The results of bile salt tolerance are shown in Table 3. All nine *Lactobacillus* strains indicated good tolerance to bile at concentration less than 0.2% without losing viability after incubation for 2 to 6 h (p >0.05). The strains except GL-AA and YS-09 were significantly decreased by 0.3% bile after incubation for 2 h (p <0.05). All residual counts of strains were significantly decreased during incubation with 0.4% bile (p <0.05), and the reduction degree became greater as time progressed.

Hydrophobicity of Lactobacillus strains. The hydrophobicity of selected *Lactobacillus* strains was also investigated (Table 4). Among the nine strains, ZP-W had maximum hydrophobicity towards xylene, whereas GL-03 possessed maximum hydrophobicity for both hexadecane and octane. Strains ZP-Z, ZP-04, and YS-09 showed similar levels of hydrophobicity towards xylene (p >0.05), as did the strains GL-AA, GL-02, and ZP-Z towards both hexadecane and octane (p >0.05). Compared comprehensively, YS-11 had relatively lower hydrophobicity towards xylene, hexadecane, and octane. The maximum hydrophobicity was observed for GL-03 against octane with a value of 52.00±3.56 %.

Table 1 Cholesterol reduction by different *Lactobacillus* strains

Strains No.	Cholesterol reduction rate (%)	Species	Source
GL-03	56.11±3.58^{a}	*L. casei subsp. casei*	Cheese
GL-AA	55.85±5.06^{a}	*L.murinus*	Cheese
GL-02	48.79±3.91^{ac}	*L.murinus*	Cheese
ZP-W	54.64±4.60^{a}	*L. plantarum*	Pickle
ZP-05	42.30±2.13^{bc}	*L. plantarum*	Pickle
ZP-Z	56.17±5.18^{a}	*L. plantarum*	Pickle
ZP-04	54.26±5.52^{a}	*L. brevis*	Pickle
YS-09	53.88±2.71^{a}	*L. acidlophilus*	Yoghurt
YS-11	47.58±4.23^{ac}	*L. acidlophilus*	Yoghurt

Data are expressed as means ± SD from triplicate determinations. For each cholesterol reduction rate (%), different lowercase letters indicate significant differences (p <0.05).

Antimicrobial activity of Lactobacillus strains. The inhibitory activities of the selected *Lactobacillus* strains against common pathogens are shown in Table 5. The results indicated that all strains showed inhibitory activity against the tested pathogens with the zones of diameter greater than 16.0 mm. Strain ZP-Z produced maximum zone of inhibition against *Escherichia coli*, and similar effects were found in ZP-W, ZP-04, YS-09, and YS-11 (p >0.05). Strain ZP-05 had more effectively inhibitory effect than the other strains against both *Staphylococcus aureus* and *Bacillus subtilis.* Moreover, strains GL-AA, GL-02, and ZP-04 had relatively higher inhibitory effect than the other strains against *Shigella dysenteriae.*

Cholesterol reduction of L. casei subsp. casei GL-03. Among the nine selected *Lactobacillus* strains, the *L. casei subsp. casei* GL-03 showed the highest cholesterol reduction activity and hydrophobicity, and also possessed relative higher acid and bile tolerance and antimicrobial abilities. Therefore, we further studied the cholesterol reduction activity of *L. casei subsp. casei* GL-03 *in vivo*. Fig. 1 showed changes in mice serum lipids after the 4-week feeding period. The mice serum total cholesterol (TC) and triglyceride (TG) concentrations of the control group were 1.93 and 1.33 mmol/L, respectively, whereas those of the high-cholesterol diet group had significantly increased to 2.88 and 1.78 mmol/L (p <0.05), respectively. This indicated that the 12% fat, 1% cholesterol, and 0.5% bile salt diets successfully induced hyperlipidemia in the control mice. The hyperlipidemia groups treated with GL-03 showed significant differences (p <0.05) in both TC and TG levels as compared to the high-cholesterol diet group.

Growth-promoting effect of Chinese herbal medicines on L. casei subsp. casei GL-03. We selected fourteen kinds of potential cholesterol-reducing Chinese herbal medicines, and investigated their growth-promoting effects on GL-03 strain. The growth of GL-03 strain was promoted by five kinds of Chinese herbal

Table 2 Acid tolerance of different *Lactobacillus* strains (log cfu/mL)

Strains No.	Time (h)	Acid tolerance			
		pH			
		1.5	2.5	3.5	4.5
GL-03	0	10.17±0.03^{a}	10.17±0.03^{a}	10.17±0.03^{a}	10.17±0.03^{a}
	2	9.00±0.08^{b}	10.12±0.10^{a}	10.18±0.03^{a}	10.19±0.04^{a}
	6	7.06±0.06^{c}	8.04±0.04^{d}	10.28±0.08^{ae}	10.36±0.09^{e}
GL-AA	0	10.18±0.04^{a}	10.18±0.04^{a}	10.18±0.04^{a}	10.18±0.04^{a}
	2	9.05±0.10^{b}	10.15±0.07^{a}	10.19±0.06^{a}	10.18±0.07^{a}
	6	6.33±0.18^{c}	8.08±0.13^{d}	10.34±0.18^{ae}	10.52±0.21^{e}
GL-02	0	10.18±0.03^{a}	10.18±0.03^{a}	10.18±0.03^{a}	10.18±0.03^{a}
	2	9.14±0.10^{b}	10.15±0.08^{a}	10.07±0.09^{a}	10.18±0.00^{a}
	6	5.28±0.12^{c}	5.75±0.06^{d}	8.50±0.10^{e}	10.23±0.13^{a}
ZP-W	0	10.17±0.01^{a}	10.17±0.01^{a}	10.17±0.01^{a}	10.17±0.01^{a}
	2	9.12±0.05^{b}	10.27±0.09^{a}	10.12±0.13^{a}	10.18±0.01^{a}
	6	5.40±0.22^{c}	6.05±0.14^{d}	7.14±0.14^{e}	10.33±0.11^{a}
ZP-05	0	10.18±0.03^{a}	10.18±0.03^{a}	10.18±0.03^{a}	10.18±0.03^{a}
	2	9.17±0.10^{b}	10.15±0.12^{a}	10.17±0.05^{a}	10.18±0.08^{a}
	6	7.55±0.12^{c}	8.33±0.12^{d}	10.19±0.09^{a}	10.46±0.12^{e}
ZP-Z	0	10.18±0.03^{a}	10.18±0.03^{a}	10.18±0.03^{a}	10.18±0.03^{a}
	2	9.25±0.19^{b}	10.14±0.10^{a}	10.17±0.03^{a}	10.18±0.09^{a}
	6	7.55±0.11^{c}	8.12±0.14^{d}	10.20±0.05^{a}	10.34±0.14^{a}
ZP-04	0	10.18±0.06^{a}	10.18±0.06^{a}	10.18±0.06^{a}	10.18±0.06^{a}
	2	9.39±0.10^{b}	10.12±0.10^{a}	10.18±0.02^{a}	10.19±0.04^{a}
	6	7.51±0.08^{c}	8.30±0.08^{d}	10.22±0.05^{ae}	10.29±0.03^{e}
YS-09	0	10.18±0.07^{a}	10.18±0.07^{a}	10.18±0.07^{a}	10.18±0.07^{a}
	2	9.16±0.07^{b}	10.17±0.12^{ae}	10.18±0.03^{a}	10.18±0.02^{a}
	6	6.44±0.04^{c}	8.32±0.09^{d}	10.23±0.04^{ae}	10.28±0.04^{e}
YS-11	0	10.18±0.03^{a}	10.18±0.03^{a}	10.18±0.03^{a}	10.18±0.03^{a}
	2	9.12±0.04^{b}	10.14±0.07^{a}	10.18±0.04^{a}	10.18±0.01^{a}
	6	6.19±0.07^{c}	8.13±0.04^{d}	10.21±0.03^{a}	10.28±0.03^{e}

Data are expressed as means ± SD from triplicate determinations. For each *Lactobacillus* strain, different lowercase letters indicate significant differences (p <0.05).

medicines, including Chinese hawthorn, gambir plant, glossy privet fruit, Acanthopanacis Senticosi and Codonopsis (Fig. 2). The Chinese hawthorn at 0.0125% showed the highest promoting effect on the growth of GL-03 strain.

Discussion

Recent studies have demonstrated that *Lactobacillus* strains exhibit the beneficial effects of removal and reduction of cholesterol. This is important, because a small reduction in serum cholesterol of 1% could reduce the risk of coronary heart disease by 2–3% (Liong and Shah, 2005). It was found that hypercholesterolaemic rats fed with *L. acidophilus* ATCC 43121 reduced total serum cholesterol after 21 days of supplementation (Park et al., 2007). In addition, the consumption of yoghurt containing *L. acidophilus* has also been reported to decrease serum total cholesterol levels in mildly to moderately hypercholesterolaemic subjects with two treatment periods each lasting 6 weeks (Ataie-Jafari et al., 2009). Our results demonstrated that *L. casei subsp. casei* GL-03 reduced not only total serum cholesterol but also triglyceride after 4 weeks, suggesting this strain could be applied as a potential probiotic.

Although hypocholesterolaemic properties of *Lactobacillus* strains have been reported, the exact mechanisms involved in this effect remain unclear. *Lactobacillus* strains could remove cholesterol *in vitro* via various mechanisms, and may exert such hypocholesterolaemic effects in vivo (Lye et al., 2010). It has been suggested that LAB could assimilate cholesterol from media during growth due to the binding of cholesterol with the bacterial cell wall. The assimilation of cholesterol by growing cells could reduce the amount of cholesterol available for absorption from the intestine (Pigeon et al., 2002). *Lactobacillus* strains with BSH activity may effectively reduce serum cholesterol by enhancing the excretion of bile salts, consequently increasing in the synthesis

Table 3 Bile salt tolerance of different *Lactobacillus* strains (log cfu/mL)

Strains No.	Time (h)	Bile tolerance			
		Bile Concentration (%)			
		0.10	0.20	0.30	0.40
GL-03	0	9.10±0.02^{a}	9.10±0.02^{a}	9.10±0.02^{a}	9.10±0.02^{a}
	2	9.11±0.08^{a}	9.10±0.07^{a}	8.01±0.02^{b}	7.46±0.12^{c}
	6	9.32±0.17^{a}	9.27±0.14^{a}	6.55±0.19^{d}	5.92±0.18^{e}
GL-AA	0	9.08±0.06^{a}	9.08±0.06^{a}	9.08±0.06^{a}	9.08±0.06^{a}
	2	9.08±0.04^{a}	9.07±0.07^{a}	8.98±0.21^{a}	7.94±0.24^{b}
	6	9.11±0.08^{a}	9.10±0.05^{a}	6.86±0.08^{c}	5.81±0.13^{d}
GL-02	0	9.09±0.05^{a}	9.09±0.05^{a}	9.09±0.05^{a}	9.09±0.05^{a}
	2	9.09±0.06^{a}	9.08±0.04^{a}	8.14±0.03^{b}	7.00±0.23^{c}
	6	9.12±0.04^{a}	9.11±0.03^{a}	6.76±0.06^{c}	5.76±0.19^{d}
ZP-W	0	9.11±0.05^{a}	9.11±0.05^{a}	9.11±0.05^{a}	9.11±0.05^{a}
	2	9.12±0.08^{a}	9.11±0.05^{a}	8.58±0.21^{b}	7.63±0.15^{c}
	6	9.13±0.03^{a}	9.12±0.02^{a}	6.30±0.09^{d}	5.41±0.16^{e}
ZP-05	0	9.12±0.01^{a}	9.12±0.01^{a}	9.12±0.01^{a}	9.12±0.01^{a}
	2	9.12±0.06^{a}	9.11±0.05^{a}	8.41±0.04^{b}	7.26±0.08^{c}
	6	9.13±0.05^{a}	9.13±0.04^{a}	6.41±0.09^{d}	5.45±0.09^{e}
ZP-Z	0	9.12±0.04^{a}	9.12±0.04^{a}	9.12±0.04^{a}	9.12±0.04^{a}
	2	9.11±0.05^{a}	9.11±0.06^{a}	8.73±0.10^{b}	7.89±0.18^{c}
	6	9.14±0.03^{a}	9.13±0.04^{a}	6.93±0.11^{d}	5.89±0.04^{e}
ZP-04	0	9.10±0.07^{a}	9.10±0.07^{a}	9.10±0.07^{a}	9.10±0.07^{a}
	2	9.11±0.06^{a}	9.10±0.02^{a}	8.74±0.10^{b}	7.98±0.15^{c}
	6	9.12±0.07^{a}	9.12±0.06^{a}	6.72±0.02^{d}	5.65±0.18^{e}
YS-09	0	9.07±0.12^{ab}	9.07±0.12^{ab}	9.07±0.12^{ab}	9.07±0.12^{ab}
	2	9.08±0.07^{a}	9.07±0.05^{a}	8.88±0.06^{b}	7.83±0.08^{c}
	6	9.09±0.05^{ab}	9.08±0.09^{ab}	6.75±0.12^{d}	5.89±0.09^{e}
YS-11	0	9.08±0.03^{a}	9.08±0.03^{a}	9.08±0.03^{a}	9.08±0.03^{a}
	2	9.09±0.06^{a}	9.08±0.09^{a}	8.93±0.02^{b}	7.98±0.11^{c}
	6	9.10±0.04^{a}	9.09±0.08^{a}	6.80±0.14^{d}	5.88±0.11^{e}

Data are expressed as means ± SD from triplicate determinations. For each *Lactobacillus* strain, different lowercase letters indicate significant differences (p <0.05).

Table 4 Cell surface hydrophobicity of different *Lactobacillus* strains

Strains No.	Cell surface hydrophobicity (%)		
	Xylene	Hexadecane	Octane
GL-03	20.23±3.42^{a}	36.12±2.91^{a}	52.00±3.56^{a}
GL-AA	16.35±1.74^{ab}	17.90±2.47^{bc}	28.56±2.80^{b}
GL-02	12.20±2.45^{bd}	20.32±2.42^{bd}	30.12±3.09^{b}
ZP-W	42.30±1.89^{c}	18.50±2.24^{bc}	22.00±2.53^{c}
ZP-05	7.95±1.79^{df}	18.52±2.22^{bc}	14.32±1.46^{d}
ZP-Z	30.32±2.59^{e}	17.52±2.01^{bc}	32.20±2.08^{b}
ZP-04	31.20±2.24^{e}	24.50±1.72^{d}	17.52±3.22^{cd}
YS-09	31.20±1.58^{e}	14.30±2.05^{c}	9.02±1.85^{e}
YS-11	5.60±1.95^{f}	4.52±0.60^{f}	10.26±3.17^{de}

Data are expressed as means ± SD from triplicate determinations. For each hydrocarbon, different lowercase letters indicate significant differences among different *Lactobacillus* strain (p <0.05).

of bile salts from serum cholesterol or decreasing the solubility of cholesterol.

Characterization of nine *Lactobacillus* strains including *L. casei subsp. casei*, *L. murinus*, *L. plantarum*, *L. brevis*, and *L. acidlophilus* showed that they were bile- and acid-tolerant, and possessed cell surface hydrophobicity. These characteristics are important in potential probiotics. The bile- and acid-tolerant characteristics are required for survival in the small intestine (Lee and Salminen, 1995) and passage through the stomach (Henriksson et al., 1999). The cell surface of hydrophobicity for microorganisms is related to the attachment of bacteria to host tissue in some cases (Schillinger et al., 2005), although Conway and Adams (1989) reported lack of correlation between the capacity for adhesion and hydrophobicity.

It has been demonstrated that binding of dietary cholesterol by

Table 5 Antimicrobial activity of different *Lactobacillus* strains against common pathogens

Strains No.	Zone diameter (mm)			
	Escherichia coli	*Staphylococcus aureus*	*Bacillus subtilis*	*Shigella dysenteriae*
GL-03	16.7±1.2^{a}	27.8±1.5^{ae}	22.0±1.7^{a}	22.5±1.0^{ac}
GL-AA	18.1±0.6^{ab}	29.4±1.2^{ad}	18.6±1.1^{cd}	25.0±1.4^{ad}
GL-02	17.0±1.7^{ab}	26.0±2.0^{ace}	31.0±0.9^{b}	24.1±1.1^{ad}
ZP-W	21.0±1.0^{df}	23.0±1.6^{c}	29.6±1.2^{b}	16.4±1.3^{b}
ZP-05	23.1±1.6^{de}	30.9±0.7^{d}	30.9±1.8^{b}	20.9±1.2^{cef}
ZP-Z	27.4±1.3^{c}	16.0±0.9^{b}	19.0±1.0^{acd}	20.1±1.2^{ce}
ZP-04	19.0±1.9^{af}	28.0±1.2^{ae}	28.5±1.6^{b}	26.4±1.7^{d}
YS-09	19.4±0.7^{bf}	18.5±1.4^{b}	17.4±1.2^{d}	18.5±1.2^{be}
YS-11	19.6±2.4^{aef}	25.6±1.2^{ce}	20.3±0.9^{ac}	22.5±1.1^{acf}

Data are expressed as means ± SD from triplicate determinations. For each pathogen, different lowercase letters indicate significant differences among different *Lactobacillus* strain (p <0.05).

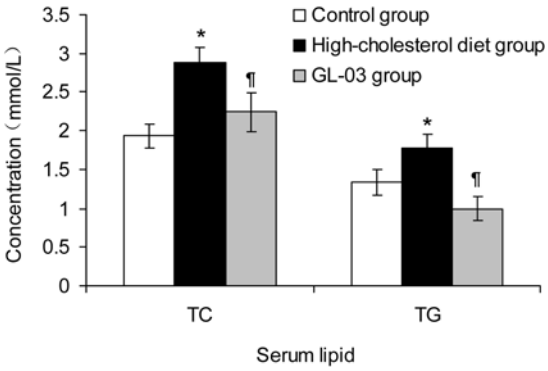

Fig. 1 Cholesterol-reduction effect of *L. casei subsp. casei* GL-03 *in vivo*. A total of 30 male mice were randomly assigned to three groups (n=10). The mice were fed a normal commercial diet as control group, a high-cholesterol diet containing 12% fat, 1% cholesterol, and 0.5% bile salt as hyperlipidemia group. To 0.8 mL of *Lactobacillus* strains GL-03 solution (approximately 10^{7} CFU/25 μL in 0.9% NaCl) was orally administered to the hyperlipidemia mice once a day as GL-03 group. The mice of the former two groups were orally administered with 0.9% NaCl. After administration of bacteria for 30 days and 12 h fast, the serum samples were analyzed for total cholesterol and triglycerides. *p <0.05 as compared with control group. ¶p <0.05 as compared with hyperlipidemia group.

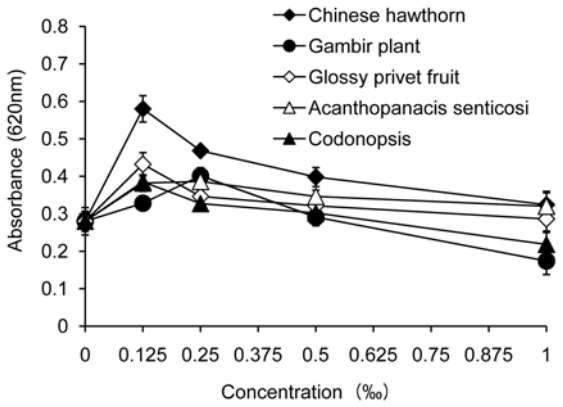

Fig. 2 Growth-promoting effect of Chinese herbal medicines on *L. casei subsp. casei* GL-03. Over-night cultures of GL-03 were inoculated (2%, v/v) into MRS broth containing 0.10, 0.05, 0.025, and 0.0125% (w/v) of Chinese herbal medicine extracts. After incubation at 37°C for 36 h, the OD value was measured at 620 nm.

LAB cells in different fermented milk products varies among strains and species (Hosono and Tono-oka, 1995). We selected nine *Lactobacillus* strains including *L. casei subsp. casei*, *L. murinus*, *L. plantarum*, *L. brevis,* and *L. acidlophilus* with similar cholesterol-reducing abilities (42–56%) from 50 strains in the present study. The results also showed that they had similar bile- and acid-tolerance, but with obvious variations in cell surface hydrophobicity and antimicrobial activity. *L. casei subsp. casei* GL-03 has the highest reduction of cholesterol reducing activity, which is in agreement with earlier studies on *L. casei* that demonstrated it possessed high cholesterol-reducing as well as other abilities (Mishra and Prasad, 2005; Jain et al., 2009). The cell surface hydrophobicity of *L. casei subsp. casei* GL-03 is similar with those of other *L. casei* strains (Mishra and Prasad, 2005), but lower than other *Lactobacillus* spp. (Mathara et al., 2008). *L. casei subsp. casei* GL-03 possesses higher antimicrobial activity against the common pathogens as compared with other *L. casei* strains (Mishra and Prasad, 2005). The organic acids, hydrogen peroxide, and bacteriocins as antimicrobial substances produced by GL-03 may contributed to this effect.

Moreover, among five kinds of Chinese herbal medicine extracts, Chinese hawthorn showed the strongest effect on promotion of GL-03 growth. It has been reported that fructose, glucose, and sucrose were detected in Chinese hawthorn (Liu et al., 2010). These sugars may accelerate the growth of microorganism. Because the studies on Chinese hawthorn are limited, it is also indicates that other functional compositions may be attributed to this promotion effect. Further studies will be required to investigate the mechanism underlying the growth promotion of Chinese hawthorn on GL-03, and will also be necessary to study the application of GL-03 combining with Chinese hawthorn to cholesterol-lowering foods.

Acknowledgment This work was financially supported by "Liaoning Provincial Natural Science Foundation of China (No. 2014026014)".

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