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ORIGINAL ARTICLE

Diversity of bile salt hydrolase activities in different lactobacilli toward human bile salts

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Abstract This study was conducted to evaluate the diversity of bile salt hydrolase (BSH) activities in eight species of lactobacilli. BSH activities were quantified based on the amount of taurine or glycine liberated from six main human bile sodium salts [glycocholic, glycodeoxycholic, glycochenodeoxycholic, taurocholic (TC), taurochenodeoxycholic, taurodeoxycholic] and a mixture of bile salts that resembled human bile. The eight species differed in their BSH activities. Specifically, *Lactobacillus helveticus*, *Lactobacillus fermentum* and *Lactobacillus gallinarum* had the ability to deconjugate taurine-conjugated bile salts, but not glycine-conjugated bile salts, which suggested that microbial BSHs recognize bile salts on both the cholate steroid nucleus and the amino acid moiety. Of the eight species evaluated, *Lactobacillus acidophilus* strains exhibited the highest specific BSH activity toward human bile salts, with the exception of TC. In addition, the *L. acidophilus* specific BSH activity toward glycine-conjugated bile salts was ten times higher than that toward taurine-conjugated bile salts. Moreover, the specific BSH activity of *Lactobacillus plantarum* did not vary significantly toward different bile salts, and *Lactobacillus gasseri* Am1 exhibited higher specific BSH activity toward TC than other lactobacilli. A comparison of *bsh* genes indicated that the LA-*bshA*, LA-*bshB*, LG-*bsh* and LP-*bsh1* genes that encode the BSH enzymes are highly homologous (higher than 45%), while the LP-*bsh2*, LP-*bsh3* and LP-*bsh*4 genes, which might not encode BSH enzymes, had lower similarity (lower than 26.3%).

Keywords Lactobacillus · Bile salt hydrolase · Human bile salts · Secondary bile acid

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Introduction

Bile salts play an essential role in fat digestion and confer potent antimicrobial activity to intestinal bacteria via their detergent properties. However, when acting on microbes, bile salts can also be modified extensively by indigenous intestinal bacteria. One of the most important transformations of bile salts by intestinal bacteria is deconjugation, which is the first step prior to further modification. Deconjugation is catalyzed by bile salt hydrolase (BSH) enzymes (EC 3.5.1.24), which hydrolyze the amide bond and separate the bile salts into a glycine/taurine moiety and free bile acids. Recently, BSH activity has often been considered a desirable trait of probiotics. Previous studies have suggested that the deconjugation of bile salts is functional in bile detoxification (De Smet et al. 1995; Grill et al. 2000; Begley et al. 2005b), and that in doing so the intestinal persistence and survival of the strains capable of producing BSH (Bateup et al. 1995; Dussurget et al. 2002) may be increased considerably. In addition, supplementation of strains with BSH activity has been reported to reduce serum cholesterol level in humans, rats and dogs (De Smet et al. 1994; Pereira and Gibson 2002; Ha et al. 2006b; Strompfov et al. 2006).

However, there is a lack of concordance between the positive evaluation of host health and microbial BSH

activity. The precise function of microbial BSHs toward intestinal bacteria and the host is currently unknown (Begley et al. 2006); indeed, it has been reported that BSH can be indirectly detrimental to the host. After deconjugation, free bile acids and amino acids are modified into a wide variety of metabolites including secondary bile acids and hydrogen sulfide (Begley et al. 2005a). Secondary bile acids, which are produced solely by intestinal bacteria, can accumulate to high levels in the enterohepatic circulation of some individuals (Ridlon et al. 2006). High concentrations of these secondary bile acids in the feces, blood, and bile have been linked to the pathogenesis of colon cancer, gallstones, and other gastrointestinal (GI) diseases (McGarr et al. 2005). Hydrogen sulfide is highly toxic and has been shown to increase colonocyte turnover (Christl et al. 1996). Overall, deconjugation is the first step in bile salt modification by gut bacteria and likely increases secondary bile acids and hydrogen sulfide in the large intestine.

There are six main bile salts sodium (approximately 26% glycocholic, 22% glycodeoxycholic, 26% glycochenodeoxycholic, 9% taurocholic, 9% taurodeoxycholic and 8% taurochenodeoxycholic) in human bile (Hofmann 1994; Ridlon et al. 2006). So, in addition to total BSH activity, the detrimental potential caused by deconjugation of different bile salts should also be considered when selecting probiotics. *Lactobacillus* and *Bifidobacterium* are the most popular probiotics and have long been considered key commensals involved in the promotion of host health. The BSHs of *Bifidobacterium* can be separated into three types, which is helpful for strain selection (Kim et al. 2004). However, BSH groups in lactobacilli are still not clear. In a previous study, the BSH activities of *Lactobacillus plantarum* CK102 and *Lactobacillus acidophilus* NCFM toward six human bile salts were investigated, but the BSH activities of *L. acidophilus* NCFM were defined only as relative activities (McAuliffe et al. 2005; Ha et al. 2006a). Other experiments have usually used only one or two bile salts to detect BSH activity or to select probiotics with high BSH activity (Lundeen and Savage 1990; De Boever and Verstraete 1999; Ha et al. 2006b; Zhang et al. 2008). Furthermore, the BSH activities of some species, such as *Lactobacillus salivarius*, *Lactobacillus helveticus* and *Lactobacillus gallinarum*, have not been studied to date.

In this study, we quantified the BSH activities of 16 strains (8 species) of *Lactobacillus* toward six main human bile salts and bile mixtures that resemble human bile. In addition, we examined the changes in BSH activity in MRS and MRSB (MRS supplied with 0.15% oxgall). We also sequenced seven *bsh* genes of *L. plantarum*, *L. acidophilus* and *L. gasseri* that showed greater BSH activities than other species.

Materials and methods

Materials

Sodium glycocholic (GC), sodium glycodeoxycholic (GCD), sodium glycochenodeoxycholic (GCDC), sodium taurocholic (TC), sodium taurochenodeoxycholic (TCD), sodium taurodeoxycholic (TCDC), sodium thioglycolate and oxgall were obtained from Sigma (St. Louis, MO). Bile mixtures resembling human bile were made up using the six bile salts (26% GC, 22% GDC, 26% GCDC, 9% TC, 9% TCDC, and 8% TDC; Hofmann 1994; Ridlon et al. 2006). An anaerobic gas generating kit was obtained from Oxoid (Basingstoke, UK). Bovine serum albumin, glycine, and taurine were obtained from Shanghai Sangon, China. An agarose gel DNA purification kit, pMD19-T vector, competent cells of *Escherichia coli*, primers and other molecular biology agents were obtained from TaKaRa (Kyoto, Japan).

Bacterial strains and culture conditions

Sixteen strains of *Lactobacillus* were used in this study. Strains of *L. plantarum* Lp529, *L. plantarum* Lp501, *L. salivarius* La5 and *L. helveticus* ZL51 were isolated from human intestine in our lab. Strains of *L. helveticus* Lh1, *L. plantarum* Lp-onlly, *L. acidophilus* LA11, *L. acidophilus* LA4, *L. acidophilus* LA12, *L. acidophilus* SA1, *L. casei* Lc2, *L. casei* Zh2, *L. gasseri* Am1, *L. fermentum* ZL4, *L. fermentum* L545, *L. galllinarum* LA8 were obtained from Shanghai Jiao Da Onlly (Shanghai, China). Stock cultures were stored in 40% glycerol at −70 C. All organisms were subcultured three times before use in sterile de Man, Rogosa, Sharpe (MRS) or MRSB (MRS supplied with 0.15% oxgall) using 1% inoculum at 37°C under anaerobic conditions.

Escherichia coli cells were propagated at 37°C in Luria-Bertani (LB) broth with vigorous shaking or on LB medium solidified with 2% agar. When appropriate, ampicillin (100 μg/ml) was added.

BSH assay

BSH activity was measured by determining the amount of amino acids released from conjugated bile salts as previously described (Liong and Shah 2005), with several modifications. Briefly, 4 ml stationary phase cells were centrifuged at 4,000 *g* and 4°C for 20 min. The cell pellet was then washed twice, and resuspended in 3.5 ml 0.1 M phosphate buffer (pH 6.0). Next, 0.5 ml 2% sodium thioglycolate was added to the cell suspension and the mixture was sonicated for 4 min while constantly cooling on ice. The samples were then centrifuged at 13,000 *g* and

4°C for 20 min. Next, 0.1 ml supernatant was mixed with 0.8 ml 0.1 M sodium phosphate buffer (pH 6.0) and 0.1 ml 50 mmol/l conjugated bile salt. The mixture was then incubated at 37°C for 30 min, after which 0.75 ml aliquots were mixed immediately with 0.75 ml 15% (w/v) trichloroacetic acid. Next, the samples were centrifuged at 13,000 *g* and 4°C for 10 min, and then 1 ml of the supernatant was mixed with 2 ml ninhydrin reagent [0.5 ml 1% ninhydrin in 0.5 M citrate buffer (pH 5.5), 1.2 ml 30% glycerol, 0.2 ml 0.5 M citrate buffer pH 5.5). The mixture was then boiled for 30 min and subsequently cooled for 3 min in ice water. Finally, the absorption was recorded at 570 nm and the amount of product formed was estimated from a calibration curve produced using glycine or taurine separately. One unit of BSH activity was defined as the amount of enzyme that liberated 1 μmol amino acid from the substrate per minute. The specific activity was defined as the number of units of activity per milligram of protein.

The protein concentrations of the supernatant were determined by the Lowry method (Oosta et al. 1978) using bovine serum albumin as the standard. All experiments in this study were repeated three times.

DNA extraction and manipulation

Lactobacillus genomic DNA was isolated using the method described by Rowan et al. (2003) with some modifications. The modified step involved the addition of 50 μl proteinase K during the lyzing step, after which the mixture was incubated for 30 min at 37°C. Then, 600 μl phenol/chloroform/isoamyl alcohol (25:24:1) was added. The extracted DNA was checked by 1% agarose electrophoresis using ethidium bromide as the staining dye, quantified spectrophotometrically at 260 nm and diluted to 50 mg ml^{-1} for PCR amplification.

PCR amplification of *bsh* genes

The primers (shown in Table 1) used for amplifying *bsh* gene fragments were derived from the whole genome sequences of *L. acidophilus* NCFM, *L. plantarum* WCFS1, *L. casei* ATCC 334, *L. gasseri* ATCC 33323, and *L. salivarius* UCC118.

The PCR reactions were carried out in a 50 μl volume containing 2 μl DNA template solution, 5 μl 10X PCR buffer [100 mM Tris-HCl (pH 8.3), 500 mM KCl, 15 mM $MgCl_2$], 2.5 U *Taq* DNA Polymerase (TaKaRa), 20 nmol of each deoxyribonucleoside triphosphate and 10 pmol of each primer. PCR was conducted using a GeneAmp2400 thermal cycler (Applied Biosystems, Foster City, CA).

Cloning and sequence analysis

PCR products of each reaction were purified with an agarose gel DNA purification kit, ligated into the vector pMD19-T and cloned in competent cells of *Escherichia coli* according to the manufacturer's instructions. Clones were selected randomly, and cloned inserts were identified using primers M13-47 and RV-M, which targeted the vector

Table 1 Primers used for amplification of the *bsh* gene fragments used in this study

Primer	Nucleotide sequence(5′–3′)	Use
bsh-Lp1f	TGTATTTTAGTAGGTATTTCAAGCATCTC	Clone *Lactobacillus plantarum bsh1*
bsh-Lp1r	CAATGAAATGGTTACGATTACGC	Clone *L. plantarum bsh1*
bsh-Lp2f	GCTTTTTTGAGTTACTGCTTTTCTG	Clone *L. plantarum bsh2*
bsh-Lp2r	GATGAGTTTCCCCAGCTTGTT	Clone *L. plantarum bsh2*
bsh-Lp3f	ATCATTGAAAGTGCTATTCTGCC	Clone *L. plantarum bsh3*
bsh-Lp3r	CGATGACGTTACGATTAAAAACT	Clone *L. plantarum bsh3*
bsh-Lp4f	ATGAGTTCCATCCGACCATAAAT	Clone *L. plantarum bsh4*
bsh-Lp4r	AGGTCTTGTTCGGCTATTTGC	Clone *L. plantarum bsh4*
bsh-Lgf	ATTTTTACTCGCGATCGTGAACCTA	Clone *Lactobacillus gasseri bsh*
bsh-Lgr	AGCTATTGCAGATGGTGACCTTGGT	Clone *L. gasseri bsh*
bsh-laAf	TACAACTATTCATTTAGACGCAATATCC	Clone *Lactobacillus acidophilus bshA*
bsh-laAr	CACTCTGCCAACACTCCATAACG	Clone *L. acidophilus bshA*
bsh-laBf	CAAAAGCCATTTATTCCGACTGA	Clone *L. acidophilus bshB*
bsh -laBr	CATAATTTATTACTTCCTTTGTTAGACAGC	Clone *L. acidophilus bshB*
bsh-lsf	ATGAAATGTGTACAAGTGTTAGTGT	Clone *Lactobacillus salivarius bsh*
bsh-lsr	TTTATTAACTTAACATTTGTGGA	Clone *L. salivarius bsh*
bsh-lcf	GCCATTAAGCAATTCGGGTTATA	Clone *Lactobacillus casei bsh*
bsh-lcr	CCAATGATTGGTCTCTCGTTCA	Clone *L. casei bsh*

sequences. Nucleotide sequences were determined using AmpliTaq FS DNA polymerase fluorescent dye terminator reactions and an Applied Biosystems 3730 automated sequencer.

Nucleotide sequence accession numbers

The *bsh* gene nucleotide sequences have been deposited in the GenBank database under accession numbers FJ356243, and FJ439770–FJ439781.

Statistical analysis

Data analysis was conducted using SPSS software (version 13.0; http://www.spss.com). Differences among groups were identified by one-way analysis of variance (ANOVA) at α=0.05. Tukey's test was used to perform multiple comparisons of the means.

Results

BSH activity

The specific BSH activities obtained from cell extracts of lactobacilli strains is shown in Table 2. Of the 16 strains, 13 showed BSH activity at varying levels and 3 (*L. casei* Lc2, *L. casei Zh2* and *L. salivarius* La5) did not exhibit any BSH activity. Strains of *L. helveticus*, *L. fermentum* and *L. galllinarum* were found to be capable only of deconjugating taurine-conjugated bile salts and their specific BSH activity was very low, ranging from 8.7 to 32.3 U mg^{-1}. Strains of *L. acidophilus*, *L. plantarum* and *L. gasseri* were able to deconjugate both glycine-conjugated bile salts and taurine-conjugated bile salts, with activities ranging from 14.1 to 773.4 U mg^{-1}.

When tested against a bile salt mixture that resembled human bile, *L. acidophilus* LA11 showed the highest bile

Table 2 Specific bile salt hydrolase (BSH) activity of *Lactobacillus* strains toward six bile salts and a bile salt mixture. Values are means of triplicates repeats ± standard error of means. Means within a column with different lowercase letters are significantly different (P<0.05). Means within a row with different uppercase letters are significantly different (P<0.05). *TC* Sodium taurocholic, *TCD* sodium taurochenodeoxycholic, *TCDC* sodium taurodeoxycholic, *GC* Sodium glycocholic, *GCD* sodium glycodeoxycholic, *GCDC* sodium glycochenodeoxycholic

Lactobacillus strains	Specific BSH activity (U mg^{-1})						
	TC	TCD	TCDC	GC	GCD	GCDC	Mixture
L. plantarum Lp-onlly	27.1±2.7 bc,B	35.6±4.0 abcd,AB	23.6±2.0 abc,B	43.6±1.0 d,A	33±5.0 cd,AB	40±3.9 bcd,A	35.6±1.5 cd,AB
L. plantarum Lp529	23.4±3.4 bc,B	24.9±2.7 bcd,B	24.4±2.1 abc,B	54.5±6.2 cd,A	27.6±2.3 cd, B	41.2±1.9 bcd,AB	39.1±1.7 cd,AB
L. plantarum Lp501	26±4.9 bc,AB	30.2±4.7 abcd,AB	22.1±1.7 abc,B	40±4.3 d,A	29.5±2.6 cd,AB	40.2±0.2 bcd,A	41.7±3.0 cd,A
L. acidophilus LA4	58.8±8.3 abc,ABC	61±9.5 ab,ABC	49.8±3.3 ab,BC	515±10.8 abc,A	450.6±11.4 ab,AB	430.9±5.4 abc,AB	533.7±10.4 ab,AB
L. acidophilus LA11	81.3±9.5 ab,BC	86.7±1.7 a,ABC	81.9±5.0 a,BC	773.4±14.5 a,A	763.3±11.4 a,A	725.3±22.5 a,AB	776.4±2.6 a,A
L. acidophilus LA12	24.8±4.8 bc, C	43.5±0.9 abc, BC	24.9±1.6 abc, C	706.0±33.4 ab,A	405.8±21.6 abc,ABC	475.0±21.3 ab,AB	453±17.9 abc,AB
L. acidophilus SA1	42.7±2.8 abc,C	55±8.9 ab,BC	45.3±2.7 ab,C	448.0±3.5 abcd,A	210.0±9.1 abcd,ABC	290.5±5.4 abcd,AB	336.5±14.4 abcd,AB
L. gasseri Am1	141.5±2.7 a,AB	30.8±0.3 abcd,BC	22.1±1.0 abc,C	223.2±4.7 bcd,A	85.9±3.5 bcd,ABC	19.0±0.8 cd,C	138.8±7.1 bcd,AB
L. helveticus Lh1	22.5±5.3 bc,AB	32.3±5.3 abcd,A	23.6±1.1 abc,AB	0	0	0	18.8±1.3 d,B
L. helveticus ZL51	16±2.2 c,A	19.7±3.0 cd,A	12±0.9 c,B	0	0	0	15.5±0.8 d,A
L. fermentum ZL4	11.5±2.5 c,B	15.2±0.8 cd,A	13.4±0.4 c,B	0	0	0	16.4±2.4 d,A
L. fermentum L545	21±3.4 bc,B	27.2±2.9 bcd,A	22.9±0.7 abc,B	0	0	0	23.8±2.8 d,B
L. galllinarum LA8	11.8±1.6 c,B	22.6±2.0 bcd,A	16.1±0.5 bc,AB	0	0	0	10.5±0.6 d,B
L. casei ZH2	0	0	0	0	0	0	0
L. casei Lc2	0	0	0	0	0	0	0
L. salivarius La5	0	0	0	0	0	0	0

salts deconjugation activity (776.4 U mg^{-1}), while *L. galllinarum* LA8 exhibited the lowest activity (10.5 U mg^{-1}). The activity of *L. plantarum* strains ranged from 35.6 to 41.7 U mg^{-1}, while those of *L. acidophilus*, *L. helveticus*, and *L. fermentum* strains ranged from 336.5 to 776.4, 15.5 to 18.8, and 16.4 to 23.8 U mg^{-1}, respectively. When the BSH activities toward each human bile salts was evaluated, *L. acidophilus* LA11 had the greatest deconjugation abilities toward GC, GDC, GCDC, TDC and TCDC, at 773.4, 763.3, 725.3, 86.7, and 81.9 U mg^{-1}, respectively. *L. gasseri* Am1 deconjugated the highest level of TC (141.5 U mg^{-1}). For *L. acidophilus*, the specific BSH activity toward taurine-conjugated bile salts ranged from 24.8 to 86.7 U mg^{-1}, which was much lower than the activities toward glycine-conjugated bile salts, which ranged from 210.0 to 773.4 U mg^{-1}. Finally, the BSH activities of *L. gasseri* Am1 toward GC, GDC and GCDC, were 223.2, 85.9, and 19.0 U mg^{-1}, respectively. The BSH activities of *L. gasseri* Am1 toward TC, TDC and TCDC were 141.5, 30.8, and 22.1 U mg^{-1}, respectively.

Comparison of BSH activity of *lactobacilli* strains incubated in MRSB and in MRS

The BSH activity of the different *Lactobacillus* strains incubated in MRS and MRSB is shown in Table 3. Strains of *L. salivarius* and *L. casei* did not exhibit any BSH activity in MRS and MRSB medium. In addition, the BSH activity of *L. plantarum*, *L. helveticus*, *L. fermentum* and *L. galllinarum* did not change much when cultivated in both media. When compared with cells cultivated in MRS, the BSH activity of *L. gasseri* Am1 cultured in MRSB toward the six bile salts decreased by 79, 66, 60, 56, 45, and 66%, respectively. The BSH activity of *L. acidophilus* also decreased in MRSB, with important differences between strains. For example, the BSH activity of *L. acidophilus* LA4 toward TC, TDC and TCDC decreased by 50, 49, and 46%, respectively, while its activity toward GC, GDC, and GCDC decreased by 78, 80, and 75%, respectively. For *L. acidophilus* LA11, BSH activity toward TC, TDC and

Table 3 Comparison of BSH activity in de Man, Rogosa, Sharpe (MRS) medium or MRSB (MRS supplied with 0.15% oxgall)

Lactobacillus strains	Culture	Specific BSH activity(U mg^{-1})					
		TC	TDC	TCDC	GC	GDC	GCDC
L. acidophilus LA4	MRS	58.8	61.0	49.8	515	450.6	430.9
	MRSB	25.6 (44%)[a]	37.3 (49%)	20.6 (58.6%)	112.0 (78%)	92.0 (80%)	107.0 (75%)
L. acidophilus LA11	MRS	81.3	86.7	81.9	773.4	763.3	725.3
	MRSB	61.6 (24%)	63.7 (27%)	57.0 (30.4%)	653.1 (16%)	678.3 (11%)	569.8 (21%)
L. acidophilus LA12	MRS	24.8	43.5	24.9	706	405.8	475.0
	MRSB	24.5 (2%)	42.6 (2%)	23.8 (4%)	533 (25%)	284.5 (30%)	377.9 (20%)
L. acidophilus SA1	MRS	42.7	55	45.3	448	210	290.5
	MRSB	39.9 (6%)	54.3 (1%)	43.9 (3%)	268.4 (40%)	131 (38%)	161.5 (44%)
L. gasseri Am1	MRS	141.5	30.8	22.1	223.2	85.9	19
	MRSB	30.0 (79%)	10.5 (66%)	9.0 (59%)	97.9 (56%)	47.2 (45%)	6.5 (66%)
L. plantarum Lp-onlly	MRS	27.1	35.6	23.6	43.6	33	40
	MRSB	26	40.2	21.6	40	29.5	40.2
L. plantarum Lp529	MRS	23.4	24.9	24.4	54.5	27.6	41.2
	MRSB	21.4	25.5	22.2	55.5	29.3	41.3
L. plantarum Lp501	MRS	26	30.2	22.1	40	29.5	40.2
	MRSB	25.4	31.5	22.9	40.8	29.1	41.1
L. helveticus Lh1	MRS	22.5	32.3	23.6	0	0	0
	MRSB	22.2	35.7	20.6	0	0	0
L. fermentum L545	MRS	21	27.2	22.9	0	0	0
	MRSB	21.3	26.8	22.0	0	0	0
L. gallinarum LA8	MRS	11.8	22.6	16.1	0	0	0
	MRSB	14.2	23.9	16.9	0	0	0
L. salivarius La5	MRS	0	0		0	0	0
	MRSB	0	0		0	0	0
L. casei Lc2	MRS	0	0		0	0	0
	MRSB	0	0		0	0	0

[a] The percent in parenthesis means the decrease of BSH activity in MRSB compared with that in MRS

TCDC was more pronounced (24, 27, and 25%, respectively) than that toward GC, GDC and GCDC (16, 11, and 21%, respectively). For *L. acidophilus* LA12 and SA1, the BSH activity toward taurine-conjugated bile salts did not decrease, while the activity toward glycine-conjugated bile was reduced by 20–44%.

Cloning of *bsh* genes from different species of lactobacilli

The *bsh* genes of *L. plantarum* Lp529, *L. plantarum* Lp-onlly, *L. plantarum* Lp501, *L. acidophilus* LA11, *L. acidophilus* LA4, *L. acidophilus* LA12, *L. acidophilus* SA1 and *L. gasseri* Am1 were amplified using primers derived from the relevant whole genome sequences. No *bsh* genes were amplified from *L. casei* Lc2, *L. casei* ZH2 and *L. salivarius* La5 using primers derived from the whole genome sequences of *L. casei* ATCC 334 and *L. salivarius* UCC118. The three strains also did not show any BSH activity toward the six bile salts. The *bsh* gene of *L. helveticus* Lh1, *L. helveticus* ZL51, *L. fermentum* L545, *L. fermentum* ZL4 and *L. galllinarum* LA8 could not be amplified because no genome sequences were available to design the PCR primers. Furthermore, *bsh* genes from *L. plantarum* Lp529, *L. plantarum* Lp-onlly, *L. acidophilus* LA11, *L. acidophilus* LA4, and *L. gasseri* Am1 were sequenced. The similarity of the *bsh* genes among strains of the same species was greater than 99% (data not shown). *Lactobacillus plantarum* contains four *bsh* genes (Lp-*bsh1*, Lp-*bsh2*, Lp-*bsh3*, Lp-*bsh4*), while *L. acidophilus* has two *bsh* genes (LA-*bshA*, LA-*bshB*) and *L. gasseri* has only one *bsh* gene (LG-*bsh*). The similarity of *bsh* genes among species is listed in Table 4. The four *bsh* genes from *L. plantarum* had low similarity (all about 20–25%), except for *bsh2* and *bsh3*, which had a similarity of 43.8%. In addition, LP-*bsh2*, LP-*bsh3* and LP-*bsh4* had low similarities (lower than 26.3%) to the other *bsh* genes (LA-*bshA*, LA-*bshB*, LG-*bsh* and LP-*bsh1*). However, the LA-*bshA*, LA-*bshB*, LG-*bsh* and LP-*bsh1* genes had high similarity (higher than 45%) to each other.

Table 4 Percentage identity of the different *bsh* genes. *LA L. acidophilus* LA11, *LG L. gasseri* Am1, *LP L. plantarum* Lp-onlly

	LA-*bshA*	LA-*bshB*	LG-*bsh*	LP-*bsh1*	LP-*bsh2*	LP-*bsh3*	LP-*bsh4*
LA-*bsh*A	100	57.0	56.0	49.8	20.8	20.1	24.1
LA-*bsh*B		100	66.1	45.8	21.4	20.2	22.3
LG-*bsh*			100	46.6	19.4	20.3	23.0
LP-*bsh1*				100	20.0	18.8	26.3
LP-*bsh2*					100	43.8	21.2
LP-*bsh3*						100	21.0
LP-*bsh4*							100

Discussion

Among the 16 strains evaluated in this experiment, 10 (Lp529, La5, ZL51, Lh1, Lp-onlly, LA11, LA4, LA12, SA1, Lc2) have been studied previously for their probiotic abilities (Zhang et al. 2007, 2008), and were found to be capable of removing cholesterol in vitro and possessing other useful probiotic characteristics. Many other studies have also indicated that various strains of lactobacilli could reduce host cholesterol levels (Liong and Shah 2005; Ha et al. 2006b; Nguyen et al. 2007), and that their ability to remove cholesterol was probably due to their BSH activity (Begley et al. 2006). However, few studies have investigated the diversity of BSH activity in lactobacilli toward the six main human bile salts. Therefore, in this study, we examined the BSH activities of 16 strains toward a bile mixture that resembled human bile as well as the six main human bile salts individually.

In humans, the ratio of taurine-conjugated bile salts to glycine-conjugated bile salts is approximately 1:3 (Hofmann 1994), although this ratio is affected greatly by diet. For example, native black Africans tend to have ratios (1:9), lower than individuals who consume a "western diet" (Sjövall 1959). Supplementation with probiotics with BSH activity toward taurine-conjugated bile salts will increase the hydrolysis of bile salts into taurine and results in hydrogen sulfide generation, especially in individuals with a "western diet." Therefore, strains with a high BSH activity toward glycine-conjugated bile salts and low or no activity toward taurine-conjugated bile salts are desirable as probiotics. The four *L. acidophilus* strains evaluated in this study were found to have a BSH activity that was ten times higher toward glycine-conjugated bile salts than taurine-conjugated bile salts.

In the present study, eight strains with both glycine-conjugated bile salts and taurine-conjugated bile salts deconjugation ability showed substrate preference toward glycine-conjugated bile salts, which was consistent with the results of other studies conducted to evaluate *Lactobacillus* strains (Taranto et al. 1999; Liong and Shah 2005). In a review, Begley, Hill and Gahan deduced that BSHs possibly recognized bile salts on both the amino acid moiety and the cholate steroid nucleus (Begley et al. 2006). Recognition of the cholate group has been reported previously in a study that revealed that *L. buchneri* JCM1069 expressed BSH activity toward TDC, but not TC (Moser and Savage 2001). The present study is the first to identify five lactobacilli strains that had the ability to deconjugate only taurine-conjugated bile salts, while not being able to deconjugate glycine-conjugated bile salts. These findings indicate that the BSHs of the five strains recognized the amino acid moiety of bile salts and confirm Hill's deduction that the BSHs in lactobacilli recognize bile salts on both the amino acid moiety and the cholate steroid nucleus.

It is known that there are four *bsh* genes (LP-*bsh1*, LP-*bsh2*, LP-*bsh3* and LP-*bsh4*) produced by *L. plantarum*. Over-expression and deletion of one or more *bsh* genes suggested that only LP-*bsh1* was responsible for the majority of BSH activity in *L. plantarum* (Lambert et al. 2008). In addition, the only purified BSH produced by *L. plantarum* CK102 that had enzymatic activity was identified as BSH1 (Ha et al. 2006a). Comparison of the BSH genes evaluated in the present study (Table 4) indicated that the LA-*bshA*, LA-*bshB*, LG-*bsh* and LP-*bsh1* genes encoding BSH enzymes are highly homologous (higher than 45%), while the LP-*bsh2*, LP-*bsh3* and LP-*bsh4* genes, which might not encode BSH enzymes, are less similar (lower than 26.3% identity).

When the BSH activity of different strains cultivated in MRS and MRSB were compared, different species showed unique characteristics. In our study, specific BSH activity of *L. planatarum*, *L. helveticus*, *L. fermentum* and *L. gallinarum* strains did not change greatly when cultivated in MRSB and MRS. However, strains of *L. gasseri* and *L. acidophilus* exhibited much lower specific BSH activity when cultivated in MRSB than when grown in MRS. More interesting was that the great drop in BSH activity occurred only in strains such as *L. gasseri* and *L. acidophilus*, which had a higher specific BSH activity. Some studies have found that 1 mM cholic acid could cause growth inhibition of bacteria, while a higher concentration of 5 mM could cause bacteriotoxicity (De Boever and Verstraete 1999). Strains having high BSH activity would produce more cholic acid in MRSB, and the higher burdening effects of cholic acid may lead to a lower BSH activity because of self-protection. It has been estimated that the bile salt concentration in the human intestine is between 2.4 mM and 4.0 mM (Hofmann 1977); therefore, the effects of bile salt concentration on BSH activity should be considered when selecting probiotic strains, especially for species such as *L. acidophilus*, for which the specific BSH activity changes greatly in the presence of bile salts.

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