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Evaluation of lactic acid bacteria isolated from fermented mustards in lowering hypercholesterol by using bile salt hydrolase (BSH) activity and bile salt de-conjugation

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This work evaluates lactic acid bacteria (LAB) obtained from fermented mustards for lowering cholesterol. The LAB strains' ability to reduce cholesterol *in vitro* was determined using activity of bile salt hydrolase (BSH) and deconjugation of bile salt *in vitro*. The LAB strains were examined for their ability to resist acid and bile salt. Strains that can reduce cholesterol adhere to Caco-2 cells and they were identified. 9 LAB isolates had higher total BSH activity (1.07 to 1.92 U/mL) than the reference strains. BSH specific activity range from 1.33 to 3.13 U/mg. Due to different protein contents in the cell extracts, there was no good correlation between the exact BSH activity and total BSH activity by most of the isolates. All of the isolates deconjugated sodium glycocholate, freeing cholic acid, from 1.02 to 2.64 mM. The tested isolates deconjugated sodium glycocholate at different levels. All tested strains exhibited acid tolerance and bile tolerance; they can survive well at pH 3.0 for 3 h and bile tolerance for 24 h. Adhesion of the tested strains to the Caco-2 cell lines, strain B0007 and B0008 strongly adhere in comparison to the control strains and other isolated strains. These results demonstrated positive effect of isolates on BSH activity, deconjugation and thereby leading to the probiotic potential of lowering cholesterol.

Key words: Lactic acid bacteria, acid- and bile-tolerant, bile salt hydrolase activity, deconjugation, adhesion activity.

INTRODUCTION

Hypercholesterol is a risk factor that causes cardiovascular disease. It is the number one cause of death in a lot of nations (Guo et al., 2019). The results from several epidemiological and clinical studies indicate a positive correlation between elevated total serum cholesterol levels and the occurrence of coronary heart

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disease (Pereira and Gibson, 2002). Increase in cholesterol levels causes the functional injury to the endothelium before morphological lesions develop (Sridevi et al., 2009). Cholesterol is a key substance causing coronary artery disease. The primary strategy for lowering cholesterol plasma levels and hindering the deposition of plaque inside the artery wall was studied (Raza et al., 2019). Drug therapy for hypercholesterolemic includes fibrate, statins and bile acid sequestrants, however the undesirable side effects of these compounds have caused concerns about their therapeutic use (Ward et al., 2019). Hence, there is requirement of a more natural method to decrease serum cholesterol concentration in humans. Milk fermented with lactobacilli or bifidobacteria was first demonstrated to exhibit the hypocholesterolemic effects in humans (Liong and Shal, 2005a).

The reduction of serum cholesterol could be an important health benefit of lactic acid bacteria (LAB). Various studies have shown that some lactobacilli could lower total cholesterol and low-density lipoprotein (LDL) cholesterol (Lang and Binggeli, 2002; Zhang et al., 2008). The exact mechanisms of serum cholesterol reduction by probiotic bacteria are not completely clear. Different hypothesis, such as assimilation (Wang et al., 2014; Reis et al., 2017), enzymatic hydrolysis and cholesterol co-precipitation properties (Lye et al., 2010) have been advanced to explain the cholesterol removal effect. Bile salt is one of the major routes of eliminating cholesterol from the body (Zhang et al., 2008). The bile salts are deconjugated in the small intestine, the solubility and emulsifying capacity decrease. Thus, the excretion of the free bile acids can reduce serum cholesterol level by increasing the formation of new bile salts from cholesterol (Liong and Shal, 2005b). Deconjugation has been included as one of the main activities of intestinal microorganisms that increase the synthesis of bile salts from serum cholesterol and thus reducing its uptake from the gut (Nguyen et al., 2007). BSH activity was detected in several bacterial species of the gastrointestinal tract, such as *Lactobacillus*, *Bifidobacterium*, *Clostridium*, *Enterococcoccus* and *Bacteroides* spp (Shehata et al., 2016). *Lactobacilli*, Bifidobacteria are probiotic strains and were found to excrete bile salt hydrolase (BSH) (Liong and Shal, 2005b; Zhang et al., 2008), which catalyzes the hydrolysis of glycine or taurine-conjugated bile salts into amino acid residues and free bile salts. *L. plantarum* PH04 from infant feces was evaluated as a potential probiotic with cholesterol-lowering effect (Nguyen et al., 2007). *Bifidobacterium infantis* 17930 showed a high deconjugation ability to 3.22 mM and *B. longum* 536 showed high BSH activity of 1.37 U/ml (Liong and Shal, 2005b). Liong and Shah (2005a) found *L. acidophilus* ATCC33200, 4356 and 4962 and *L. casei* ASCC1521 showed higher deconjugation ability and BSH activity. Five *L. plantarum* and seven *L. pentosus* strains isolated from fermented olive, exhibited partial bile salt hydrolase activity (Argyri et al., 2013). Five strains of lactic acid bacteria with BSH activity were preliminarily selected from swine origins (Guo et al., 2010). Probiotics are live microorganisms that improved its intestinal microbial balance (Vivek et al., 2019). Probiotics are normal components of the intestinal microflora in both humans and animals (Arepally and Goswami, 2019). They must have the ability to survive passage through the stomach and small intestine. Therefore, resistance to the low pH of the gastric juice and the bile salt in the small intestine and to prevent the attachment of pathogens and stimulate their removal from the infected intestinal tract is one of the important selection criteria for probiotic (Shehata et al., 2016; Delcarlo et al., 2019).

Green mustard is used to make fermented mustard (picked mustard green). It produced by fermentation through a mixed microbial population comprising LAB (Wang et al., 2014). This work aimed to examine the isolated lactic acid bacteria (LAB) from fermented mustard, and to show the ability of the screened Lactobacillus isolates to reduce cholesterol with BSH activity and to deconjugate bile salt. The isolates were used to test for acid tolerance, bile salt tolerance and adherence assay with Caco-2 cells.

MATERIALS AND METHODS

Bactria strains

Microbial colonies were got from fermented mustard and considered as LAB (Wang et al., 2014). Liquid fermented mustards were obtained from central and southern Taiwan farms. The liquid samples diluted were placed on the De Man, Rogosa and Sharpe (MRS) agar surface composed of 5 g/L calcium carbonate. This was followed by their incubation for 1-2 days at 37°C. There was a random selection of colonies of clear zones on MRS agar plates, followed by their purification. Gram-positive and catalase-negative strains were considered to be speculative LAB; they were kept in MRS agar plate at 4°C. BCRC 17010, BCRC 17474, BCRC 910236 are collected from Bioresource Collection and Research Center (BCRC), Hsinchu, Taiwan. All isolated strains were stored at -80℃. Prior to experimental use, BCRC 17010, BCRC 17474, BCRC 910236 obtained as reference strains, all strains were subcultured three times in MRS broth.

Bile salt hydrolase (BSH) assay activity

Sridevi et al. (2009)'s ninhydrin method was used for the BSH assay. The culture was grown shortly for 72 h at 37°C in MRS media and centrifuged at 12,000 *g* and 4°C for 2 min. Potassium phosphate buffer (pH 6.5) was used to wash the cell pellet, followed by centrifugation. Next was the determination of the pellets' wet weight. Cell pellet was suspended in 100 µL reaction mixture having 10 mM potassium phosphate buffer pH 6.5, 10 mM dithiothreitol (DTT), and 10 mM sodium glycodeoxycholate. The mixture was incubated at 37°C for 30 min; the reaction ended with the addition of 100 µL of trichloroacetic acid (15% w/v). The mixture was centrifuged and 50 µL of the supernatant was mixed with 50 µL of 2% ninhydrin reagent. Then, it was well mixed and heated for 14 min. The absorbance of the cooled sample was recorded at 570 nm. One unit of bile salt hydrolase activity was taken as the amount

of enzyme that freed 1 mole of amino acid from the substrate every minute. The benchmark curve was made using glycine. The same method above was used to determine the specific BSH activity for cell free extract. Lowry's technique (Liong and Shal, 2005a) was used to determine the concentrations of proteins in cell free extract. All experiments were duplicated.

Deconjugation of sodium glycocholate

Bile salt deconjugation was related to the deconjugated bile released and Liong and Shah (2005b)'s modified method was utilized for measuring the amount of free cholic acid by each culture released. 10 mL of culture after being incubated was modified to pH 7.0 with NaOH (1 N). Cells were centrifuged at 10,000 *g* (KUBOTA, KM-15200, Japan) and 4°C for 10 min. The supernatant was modified to pH1.0 with HCl (10 N). One milliliter of the supernatant was added in 2 mL of ethyl acetate and the mixture was vortexed for 1 min. Two milliliter of the ethyl acetate layer was moved to a glass tube and evaporated under nitrogen at 60°C. The residue was instantly melted in 1 mL of NaOH (0.01 N). After thorough mixing, 1 mL of furfuraldehyde (1%) and 1 mL of H_2SO_4 (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 glacialacetic 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 using cholic acid standard (Sigma Chemical Co., St. Louis, MO, USA). All experiments were duplicated.

Acid tolerance

The cultures acid tolerance was examined by incubation of the organisms in MRS broth with 0.30% oxigall supplement. The pH was modified in 3.0 and 2.0 with HCl and cultures were incubated at 37°C for 3 h. All the isolates were subcultured three times prior to the experiment; next they were centrifuged after the last subculture, and inoculated (1% v/v) into the broth. Plate count technique was used to monitor their growth (Liong and Shal, 2005c). The experiments were duplicated.

Bile tolerance

The resistance of LAB isolates to bile salt was evaluated. The MRS broths (0, 0.5, and 1.0% (w/v) of oxigall) were made, placed in 10 mL volumes and purified by heating 121°C for 15 min. Each of the LAB isolate was subcultured 3 times prior to the use of the experiment; next they were centrifuged after the last subculture, and inoculated (1% v/v) into the broth. The reaction mixture and MRS broth were incubated at 37°C for 24 h. Their growth was monitored with the plate count technique (Liong and Shal, 2005c). All the experiments were duplicated.

Adherence assay

The Caco-2cell-lines derived from a colon carcinoma were bought from the Bioresources Collection and Research Center (BCRC), Hsin-Chu, Taiwan. They grew normally in Dulbeco's modified Eagle's minimal essential medium (DMEM; GIBCOBRL Laboratories, NY, USA) having 1.0 mmol/L sodium pyruvate and 10% (v/v) fetal bovine serum and 50 unit/mL Penicillin-Streptomycin (GIBCO) supplements. For the assay, monolayers of Caco-2 cells were made on glass cover slips fixed in six-well tissue-culture plates (NUNC products, supplied by the Life Technologies, Auckland, New Zealand). The cells and all experiments were maintained with cell-lines at 37°C in 5% CO_2 atmosphere. Before the test, PBS (phosphate buffered saline) was used to wash all LAB twice and followed by centrifugation at 2100 r/min for 5 min. Bacterial cells were suspended again in 1 mL DMEM. One hundred microliters of the suspension ($1x10^8$ CFU/mL) was moved to a washed monolayer of cells, and incubated for 2 h at 37°C in 5% CO_2. PBS buffer (pH 7.4) was used to wash the monolayers four times; they were placed in methanol, Gram stained (Baxter Scientific Products, McGraw Park, Miami, FL, USA) and microscope was used to examine them under an oil immersion lens. Gopal et al. (2001)'s method was used to count the numbers of LAB cells adhering to the cultured cell lines.

Identification of strains

API 50 CHL fermentation assays (BioMerieux, S.A., Marcy l'Etoile, France) and 16S rDNA sequence analysis was used to identify LAB isolates that are tolerance to acid and bile salt and with lowering cholesterol through physiological tests. Those mainly verified via API 50 CHL fermentation assays were based on the instruction method. In 16S rDNA sequence analysis, the PCR primers were designed from the 16S rDNA genes primers 27F/1492F (Tanner et al., 2000). For the PCR assay, the method of Michael et al. (2000) was followed. DNA purification kit (Promega, Madision, WI, USA) was used to purify the amplification products and Nucleic acid Synthesis and Analysis Core Laboratory (Cheng Kung University College of Medicine, Tainan, Taiwan) was used to sequence them. Sequence homologies were analyzed by making a comparison of the sequence got with those in the database of the DNA (http//www.ncbi.nim.nih.gov/BLAST).

Statistical analysis

All data were calculated as means (means ± SD). Statistical Analysis System software package was used for the Statistical analysis. ANOVA methods were used for the analysis of variance. Duncan's multiple range tests at a level of $P < 0.05$ were used to determine the significant differences between means.

RESULTS

Table 1 shows the bile salt hydrolase (BSH) activity of LAB isolates. All the isolates have different levels of BSH effects on sodium glycocholate. 9 isolates had higher total activity of BSH (1.07 to 1.92 U/mL) in comparison to reference strains, BCRC 10747, BCRC 17010 and BCRC 910236; BCRC 17010 and BCRC 910236 had the lowest total activity of BSH than the 9 isolated strains. BSH specific activity from 1.33 to 3.13 U/mg (Table 1). There was no good correlation between the precise BSH activity with total BSH activity in most of the isolates. B0106, B0024 and reference strains with the lowest total activity had lowest exact activity.

Deconjugation of LAB isolates

Sodium glycocholate was deconjugated by all the isolates freeing the cholic acid, from 1.02-2.64 mM (Table 2). In general, BCRC17010, BCRC 17474, B0007, B0011, B0106, B0113 showed a better deconjugation ability

Table 1. Bile salt hydroxylase activity of lactic acid bacteria.

Strain	Total activity (U /mL)	Total protein (mg/ mL)	Specific activity (U /mg protein)
BCRC 17010	0.56±0.00^{e}	0.60±0.01	0.93±0.01^{b}
BCRC 17474	1.02±0.02^{b}	0.92±0.04	1.11±0.02^{b}
BCRC910236	0.19±0.06^{d}	0.47±0.05	0.38±0.03^{c}
B0007	1.71±0.08^{c}	0.60±0.02	2.84±0.08^{a}
B0008	1.35±0.29^{a}	0.61±0.01	2.22±0.08^{f}
B0010	1.37±0.01^{a}	0.47±0.015	2.91±0.06^{a}
B0011	1.65±0.08^{c}	0.53±0.00	3.13±0.18^{h}
B0024	1.23±0.02^{ab}	0.92±0.02	1.33±0.04^{d}
B0032	1.92±0.07^{f}	0.52±0.00	3.73±0.04^{i}
B0077	1.36±0.05^{a}	0.46±0.01	2.96±0.03^{a}
B0106	1.07±0.12^{b}	0.67±0.04	1.62±0.18^{e}
B0113	1.28±0.05^{a}	0.52±0.00	2.46±0.04^{g}

*Values are means ± SD for seven rats per group. Values in the same column with different superscript letter $^{(a,b)}$ are significantly different at $p < 0.05$ analyzed by Duncan's test.

Table 2. Deconjugation of sodium glycodeoxycholate by lactic acid bacteria.

Strain	Cholic acid released (mM)
BCRC17010	2.35±0.06^{a}
BCRC17474	2.64±0.0 ^{h}
BCRC910236	1.02±0.16^{b}
B0007	2.51±0.06^{g}
B0008	1.54±0.09^{e}
B0010	1.37±0.03^{c}
B0011	2.23±0.03^{a}
B0024	1.02±0.02^{b}
B0032	1.35±0.05^{cd}
B0077	1.46±0.10^{de}
B0106	2.31±0.05^{a}
B0113	1.97±0.11^{f}

*Values are means ± SD for seven rats per group. Values in the same column with different superscript letter $^{(a,b)}$ are significantly different at $p < 0.05$ analyzed by Duncan's test.

which liberated more than 1.90 mM of cholic acid, while BCRC 910236 and B0024 deconjugated very poorly, releasing just 1.02 mM.

Acid tolerance

In this work, all the LAB isolates could withstand the simulated gastric juice at pH3 after incubating for 3 h (Table 3). These findings suggest that all tested strains tolerated acid at pH 3.0.

Bile salt tolerance

The impacts of diverse amounts of bile salt on the capability of the isolates are shown in Table 4. Lactobacilli grown in MRS broth lacking bile salt were the control. All the strains grew well in MRS broth lacking bile salt, but they had different levels of tolerance to bile from 10^{7}-10^{8} CFU/mL after being incubated at 0.5% and 1% oxigall for 24 h. Among the tested LAB isolates B0024 had the highest tolerance to bile salt than other isolates.

Table 3. Effects of pH3.0 solution on viability of lactic acid bacteria

Strain	MRS broth (og CFU/ml)	Treatment with pH3.0 for 3 h (og CFU/ml)
BCRC17010	8.21±0.21	7.76±0.05
BCRC17474	9.64±0.26	7.56±0.03
BCRC910236	9.40±0.02	6.36±0.02
B0007*	9.73±0.08	8.80±0.02
B0008	9.50±0.06	8.76±0.01
B0010	9.32±0.05	8.75±0.04
B0011	9.89±0.04	8.55±0.03
B0024	9.45±0.05	8.77±0.01
B0032	9.62±0.14	9.72±0.02
B0077	9.54±0.12	9.54±0.01
B0106	9.52±0.20	9.46±0.01
B0113	9.20±0.06	9.22±0.09

*The viability of B0007 is from Wang et al. (2014).

Table 4. Effects of different concentration of bile salt on the viability of lactic acid bacteria.

Strain	MRS broth (log CFU/ml)	0.5% Bile-MRS (log CFU/ml)	1.0% Bile-MRS (log CFU/ml)
BCRC17010	8.21±0.21	8.27±0.02	8.16±0.12
BCRC17474	9.64±0.26	8.24±0.04	8.24±0.24
BCRC910236	9.40±0.02	8.36±0.01	8.43±0.13
B0007*	9.73±0.08	8.07±0.01	7.90±0.10
B0008	9.593±0.01	8.07±0.08	7.79±0.07
B0010	9.68±0.07	7.63±0.03	7.91±0.03
B0011	9.89±0.04	7.90±0.02	7.73±0.03
B0024	9.86±0.03	8.70±0.04	8.40±0.01
B0032	9.62±0.14	7.93±0.06	7.58±0.06
B0077	9.54±0.12	8.11±0.05	7.40±0.03
B0106	9.52±0.20	7.53±0.05	7.60±0.11
B0113	9.20±0.06	7.53±0.04	7.48±0.09

*The viability of B0007 is from Wang et al. (2014).

Strain identification

The physiological and biochemical characteristics using API 50 CHL for Lactobacilli identified all tested strains as LAB species. The sequences of the selected strains tallied with the 16S r-DNA sequences from the Gen-Bank database (website) and used to identify the LAB strains. According to the experimental results, B0010 was identified as *Lactobacillus brevis*, other isolates were identified as *Lactobacillus plantarum* using 16S rDNA analysis (Table 5). The data got to show that 16S rDNA sequence of these strains matched well with that of 16S rDNA nucleotide sequence of these strains were 99 to 100% similarity.

Adherence ability

Table 6 shows the adherence ability of the test strains on the Caco-2 cell lines. B0007 and B0008 revealed powerful ability to adhere unlike the control strains and other isolated strains. However, B0010, B0024, B0077, B0106 and B0113 revealed the inability of the system to adhere.

DISCUSSION

Different works have revealed that some lactobacilli can reduce total plasma cholesterol and low-density lipoprotein (LDL) cholesterol (Guo et al., 2019). Our previous studies showed that these isolates can eliminate cholesterol *in vitro* through the mechanisms of assimilation (Wang et al., 2014), and we want to investigate the deconjugation features of bile salt and these isolates' BSH activities. Of the 50 isolates from fermented mustard, 9 isolates displayed over 1.0 U/ml

Table 5. Identification of LAB isolates based on 16S rDNA sequence similarity

Isolate of LAB	Strain	GeneBank acc. no.	Sequence similarity (%)
B0008	*Lactobacillus plantarum*	EU807752	99
B0010	*Lactobacillus brevis*	CP000416	99
B0011	*Lactobacillus plantarum*	HM130542	99
B0024	*Lactobacillus plantarum*	EU419597	99
B0032	*Lactobacillus plantarum*	HM462423	100
B0077	*Lactobacillus plantarum*	GU125597	99
B0106	*Lactobacillus plantarum*	HM058789	99
B0113	*Lactobacillus plantarum*	FJ763580	99

Table 6. Adhesion of LAB strains to Caco-2 cell-line at normal state

Strain	Adhesion index[a]
BCRC17010[b]	51±7
BCRC17474[c]	17±11
BCRC910236[d]	81±17
B0007[b]	223±21
B0008	211±34
B0010	N.D.
B0011	4±3
B0024	N.D.
B0032	68±45
B0077	N.D.
B0106	N.D.
B0113	N.D.

[a]Expressed as the average number of LAB adhering to human intestine cells, counts is carried out on 20 randomized microscopic fields. [b]The adhesion index of B0007 is from Wang et al. (2014).

BSH activity. Guo et al. (2010) showed that of the 14 *Lactobacillus* strains isolated from the samples of swine origins, only 2 (14%) showed BSH activity; out of the 16 *Lactobacillus* strains isolated from the samples of koumiss, 7 (44%) had BSH activity. Several studies showed that elimination of cholesterol *in vitro* is connected to the activity of bile salt hydrolase (Shehata et al., 2016; Guo et al., 2019). Seven isolates reduced over 20% cholesterol and showed activity of bile salt hydrolase (BSH) (Shehata et al., 2016). Guo et al. (2019) used 5 fifferent bile salts to evaluate the *Lactobacillus plantrum* KLDS 1.0344 of BSH activity, the BSH activity of the strain KLDS was 1.04 U/mg towards sodium taurodeoxycholate, and 0.47 U/mg toward sodium glycodeoxycholate. Six isolated probiotics bacteria from human origin are involved in the production of bile salt hydrolase (BSH) that assists in the reduction of serum cholesterol (Miremadi et al., 2014). The enzyme, bile salt hydrolase is involved in the catalysis of bile salt deconjugation for the liberation of primary bile acids (Shehata et al., 2016). The highest total BSH activity (1.72 U/ml) and specificity activity (3.73 U/mg) was displayed by B0032; some isolates had lower total BSH activity, but had higher specificity activity unlike the rest LAB strains (Table 1). There was no good correlation between exact BSH activity and total BSH activity by most of the strains of LAB because the cell extracts had different protein contents. Liong and Shah (2005a) and Guo et al. (2019) had the same results for lactobacillus isolates on varying bile salts.

Two strains of *Bifidobacteria* strains could deconjugate sodium glycocholate to lower serum cholesterol levels (Liong and Shal, 2005b). It is shown that all the strains were able to deconjugate sodium glycocholate bile salt very well at different levels. These results are like others (Miremadi et al., 2014; Ramasamy et al., 2010), in which *Lactobacillus* strains had higher deconjugating activity on the conjugates of sodium glycocholate. In this study, all tested isolates deconjugated sodium glycocholate bile salt varying degrees. Similar results were reported by 12 *Lactobacillus* isolates that deconjugated bile salts to different levels (Ramasamy et al., 2010).

pH 3 is normally used for gastric juice while pH 2 for 3 h is utilized as severe situation for the simulation of the stomach conditions (Tsai et al., 2005). *L. fermentum* strain 4-17 was capable of surviving at various conditions such as low pH values, bile salts exposure (Falah et al., 2019). The results showed that pH 2.0 obviously inhibited the strains. It was accepted in general that the strains with full tolerance to pH 3 for 3 h had relatively higher resistance to the low acidity. B0032, B0077, B0106 and B0113 displayed no difference viability before and after pH 3.0 for 3 h. The results show the tolerance to acid was strain-specific, like other studies (Tsai et al., 2005; Mishra and Prasad, 2005). The useful physiology of of human bile concentrations is from 0.3 to 0.5% (Dunne et al., 2001). In order to test the bile salt tolerance, 0.5 and 1.0% bile salt were used in this study. All the tested strains exhibited bile tolerance of 0.5 and 1.0% bile salt with varying degrees. The results suggest the tolerance to bile salt was similar to the report of Falah et al. (2019).

Researchers have given the suggestion that adhesive probiotic bacteria can inhibit pathogens attachment and help to remove them from the infected intestinal tract (Delcarlo et al., 2019). B0007 and B0008 to Caco-2 cell-line had adhesion index of 223 and 221 respectively. Past studies have shown B0007 with good adhesive features (Wang et al., 2014). The commercial probiotic isolate *L.acidophilus* LA-1 with Caco-2 cells has adhesion index of 155 (Bernet et al., 1994). The adhesion level of *L. fermentum* strain 4-17 to human colon adenocarcinoma Caco-2 cells was 8.5% (Falah et al., 2019). The observation that the B0010, B0011, B0024, B0077, B0106 and B0113 showed no adhesion to Caco-2 cells. The observation also obtained from that *L. bulgaricus* LB1 also showed no significant adhesion to either mucus-secreting or non-mucus-secreting cells (Gopal et al., 2001)

In this work, fermented mustard was used to isolate 50 LAB isolates. Nine of them were chosen based on their high activity of BSH. The nine LAB strains resisted gastrointestinal conditions well (pH, 3.0; bile salt, 0.5-1.0%). These isolates can have potential to be utilized in functional food and health products, due to their good probiotic properties mostly where the main aim is to reduce cholesterol in food. It is necessary to do *in vivo* study to confirm the effect of hypocholesterolemia.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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