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Original Article

Probiotic characterization and *in vitro* cholesterol lowering effects of lactic acid bacteria isolated from healthy Thai infants

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Abstract

Hypercholesterolemia is one of the major health problems affecting people worldwide. Some probiotic lactic acid bacteria possess cholesterol lowering ability. One-hundred lactic acid bacteria isolated from the feces of healthy Thai infants were screened for bile salt hydrolase activity and cholesterol assimilation ability. Seven isolates expressed strong bile salt hydrolase activity and 7 isolates exhibited cholesterol assimilation properties. In total, 11 probiotic candidates were characterized for their general probiotic properties including acid and bile tolerance, adherence to Caco-2 cells and genotypic identification using 16S rRNA gene sequencing. Eight isolates were identified as *Enterococcus faecalis* and others were *E. faecium*, *E. durans*, and *Lactococcus garvieae*. All selected isolates survived after incubation at pH 3 and 4, and in 0.3% and 0.8% bile. The strain *E. faecium* MSMC 25-2 exhibited the highest level of adhesion to Caco-2 cells at 8.8% compared to *L. rhamnosus* GG.

Keywords: bile salt hydrolase, probiotics, lactic acid bacteria, cholesterol, infant feces

1. Introduction

Hypercholesterolemia is associated with the development of atherosclerotic cardiovascular diseases including coronary heart disease, peripheral arterial disease, and stroke, which have caused mortality worldwide (Kaestner *et al.*, 2018). Treatment with 3-hydroxy-3-methylglutaryl coenzyme-A reductase inhibitor or the statin group of drugs and modification of lifestyles are recommended by the American Heart Association to prevent the risk of cardiovascular disease (Roy, 2014). However, drug therapy can not be used as long-term treatment because it is relatively expensive and some side effects may develop including muscle pain, fatigue, and weakness (Golomb & Evans, 2008). Thus, a change in lifestyle, such as dietary modification, is regarded as the preferable solution (Sangwan & Singh, 2018).

Probiotics are beneficial microorganisms, which when administered in adequate amounts confer a beneficial health effect on the host (FAO/WHO, 2002). Most probiotics are bacteria similar to those naturally found in the gastro-

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intestinal tract of humans and animals. The probiotics commonly used in foods, especially dairy products, are members of lactic acid bacteria (LAB) (Lourens-Hattingh & Viljoen, 2001). LAB are Gram-positive, cocci or bacilli, non-motile, non-spore forming, non-catalase producing, facultative anaerobic or strictly anaerobic bacteria. Many researchers have discovered the cholesterol-lowering effect *in vitro* and *in vivo* of many LAB species, such as *Lactobacillus*, *Bifidobacterium*, and *Enterococcus* (Guo, Li, Tang, Yang, & Huo, 2016). The proposed mechanisms of lowering the level of serum cholesterol include (1) suppression of bile acid re-absorbtion mediated by bile salt hydrolase (BSH) enzyme from bacteria and (2) assimilation of cholesterol during the growth of LAB, which are indirect and direct mechanisms, respectively (Guo *et al.*, 2016; Guo, Li, Tang, Yang, & Huo, 2016; Liong & Shah, 2005; Tanaka, Doesburg, Iwasaki, & Mierau, 1999).

Since lowering the serum cholesterol is an approach to reduce the risk of hypercholesterolemia and atherosclerotic cardiovascular disease, microbial strains with cholesterol-reducing potential has been screened and selected from many sources in order to introduce new probiotic microorganisms for the application of non-drug alternatives and food products (Lye *et al.*, 2017). However, the cholesterol lowering effect and other probiotic properties are strain dependent and vary even within the same species (Wang *et al.*, 2014). In addition to the health benefits, tolerance in an acidic gastric condition and in high bile salt concentrations in the small intestine, adhesion to intestinal tissues, and colonization in the human gastrointestinal tract are the key criteria for the selection of probiotic candidates (Vinderola, Gueimonde, Gomez-Gallego, Delfederico, & Salminen, 2017).

The aims of this study were to examine potential probiotics with the cholesterol lowering-effect. In this study, 100 isolates of LAB from newborn feces were investigated for their cholesterol lowering properties, BSH activity, and cholesterol assimilation ability. Isolates expressing BSH activity and cholesterol assimilation were selected for studies of the probiotic characteristics, acid and bile tolerance, and adherence to Caco-2 cells along with genotypic identification using 16S rRNA gene sequencing.

2. Materials and Methods

2.1 Selection of lactic acid bacteria

LAB isolates from healthy infant feces were randomly selected from frozen laboratory stock. The criteria for selection of the isolates were diverse Gram morphology, diverse colony morphology, and acid production. Moreover, all selected isolates were from different subjects. The fecal samples were taken when the infants were between 1 and 3 days old from healthy infants of healthy mothers who delivered at HRH Princess Maha Chakri Sirindhorn Medical Center (SWUEC 37/2551). The isolates were examined by Gram-staining, acid production, and catalase test. Gram-positive, catalase-negative, acid production, cocci, cocco-bacilli, short rods, and regular rod shaped bacteria were maintained as frozen cultures in de Man, Rogosa, Sharpe (MRS) broth (Oxoid, Basingstoke, Hampshire, UK) with the addition of 20% glycerol (w/v) at −80 °C for further analyses.

2.2 Bile salt hydrolase activity

Bacterial isolates from infant feces were recovered from −80 °C and tested for BSH activity using qualitative direct plate assay (Ahn, Kim, Lim, Baek, & Kim, 2003; Dashkevicz & Feighner, 1989). Briefly, bacterial cells (10^9 cells/mL) in MRS broth were spotted on MRS agar supplemented with 0.5% (w/v) sodium salt of taurode-oxycholic acid (TDCA; Sigma, USA) and 0.37 g/L of calcium chloride. The MRS agar plates were incubated in an anerobic jar with Gas Pak at 37 ºC for 72 h. The plates without supplementation of TDCA were used as the control. The precipitation zone surrounding the colonies indicated BSH activity.

2.3 Cholesterol assimilation

Each isolate (10^9 cells/mL) was inoculated to MRS broth supplemented with water soluble cholesterol at a final concentration of 100 µg/mL, and incubated in an anaerobic jar with GasPak at 37 °C for 48 h. The MRS broth without inoculation of bacterial cells was used as a control. Samples were then centrifuged at 4,000 rpm at 4 °C for 15 min. The supernatant from each sample was analyzed for cholesterol concentration using the method from Rudel and Morris (1973) with slight modifications. Briefly, 100 µL of supernatants were mixed with 100 µL of 33% (w/v) potassium hydroxide and 200 µL absolute ethanol. The solutions were heated at 60 °C for 15 min, and cooled. Deionized water (200 µL) and 500 µL hexane were added. The solutions were allowed to separate at ambient temperature. The hexane layer was transferred to 96-well plates and evaporated. When the hexane had completely evaporated, o-phthalaldehyde reagent (OPA; Sigma, USA) prepared in acetic acid and concentrated sulfuric acid was added. Samples were incubated at room temperature for 20 min. The absorbance was read at 550 nm using a BioTek® Synergy™ HT (Multi-Detection Microplate Reader, USA). The ability of cholesterol assimilation of bacteria was expressed in percent cholesterol assimilation. LAB samples that expressed a cholesterol assimilation level greater than 20% were selected for general probiotic property evaluation.

2.4 Acid and bile tolerance

MRS broth was adjusted with 1 N HCl to pH 2, 3, and 4 for the acid tolerance test or supplemented with 0.3% and 0.8% bovine bile (Sigma, USA) for the bile tolerance test (Ladda, Theparee, Chimchang, Tanasupawat, & Taweechotipatr, 2015). Non-supplemented MRS broth was used as the control. Overnight cultures of candidate probiotics (10^9 cells/mL) were incubated at 37 °C for 3 h under anaerobic conditions using an anaerobic jar with GasPak. Viable cell counts were assessed and displayed as $\log_{10}$ values of colony-forming units per mL (CFU/mL) and compared to the viable counts of bacteria incubated in non-supplemented MRS. The experiments were performed three times in duplicate.

2.5 Adherence property test

Adenocarcinoma cell lines (Caco-2) (ATCC, HTB-37) in 24-well tissue culture plates were used for the adhesion

assay (Dimitrov, Gotova, & Chorbadjiyska, 2014). The candidate LAB were cultured overnight in MRS broth (10^9 cells/mL) and then centrifuged at 4,000 rpm at 4 ºC for 10 min. The bacteria cells were washed with PBS (pH 7.2), resuspended in non-supplemented Dulbecco's modified Eagle's medium (DMEM; GIBCO Invitrogen, USA), and filled onto the Caco-2 cells. The culture plates were incubated in a 5% CO_2 atmosphere for 1 h. After incubation, each well was washed three times with PBS to remove non-attached bacterial cells and then Caco-2 cells with bacteria residues were lysed by addition of Triton X 100 (0.05% solution) for 10 min to allow the cells with bacteria residues to be detached from the well. The adhesion of bacteria cells onto the Caco-2 cells was calculated as a percentage of the viable bacteria according to their initial population in the DMEM suspension. In this study, *Lactobacillus rhamnosus* GG (LMG 18243) was used as the positive control and the adhesion assay was conducted in duplicate.

2.6 Genotypic identification of selected probiotic LAB

The candidate and comparison isolates were analyzed using 16S rRNA gene sequencing as described by Taweechotipatr, Iyer, Spinler, Versalovic, and Tumwasorn (2009) with slight modifications. The candidate probiotic LAB were cultured in MRS broth with the addition of 0.5% glycine to facilitate cell lysis (Abed, 2013) and centrifuged at 4,000 rpm for 3 min. The cells were washed twice with milliQ water. PCR Master Mix was prepared in a total volume of 100 µL that consisted of 5 U Taq DNA polymerase, 2 mM dNTP, 25 mM $MgCl_2$, 10X Taq Buffer with $(NH_4)_2SO_4$, and dH_2O. Amplification of the 16S rRNA gene was performed by polymerase chain reaction (PCR). The PCR products were purified using a Geneaid Gel/PCR DNA Fragments Extraction Kit (Geneaid Biotech, Bade City, Taiwan). The sequence analysis of the PCR products was performed by U2Bio in Seoul, Korea. The 16S rRNA gene was amplified using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3'), 518F (5'-CCAGCAGCCGCGGTAATAC G-3'), 800R (5'-TACCAGGGTATCTAATCC-3') and 1492R (5'-TACGG YTACCTTGT TACGACTT-3'). The nucleotide sequences were processed and determined using BLAST software and compared to the EzTaxon-e database. The percent identity of bacterial isolates was determined on the basis of the highest scores. The closest relatives of the 16S rRNA gene sequences were evaluated. A similarity of ≥99% to 16S rRNA gene sequences of the type isolates were used as the criterion for identification (Drisko *et al.*, 2005).

2.7 Statistical analysis

One-way analysis of variance was used for the data analyses. Multiple comparisons were performed using Tukey's test for the percentage of cholesterol assimilation and adherence test. Acid and bile tolerance were evaluated using Student's *t*-test. All statistical analyses were performed using GraphPad Prism version 5.01. Statistical significance was considered at $P<0.05$.

3. Results and Discussion

3.1 Selection of LAB

One hundred isolates from healthy newborn feces were randomly selected from the frozen laboratory stock. They were all Gram-positive cocci or bacilli and catalase-negative. Colony morphology was various. Under the microscope, they appeared as cocci, coccobacilli, short bacilli, bacilli, and slender short bacilli.

3.2 Bile salt hydrolase activity

One hundred isolates of LAB were screened for their BSH activity using agar plate assay containing 0.5% TDCA as the substrate. After 72 h incubation, fine precipitated halos around the colonies were observed from 22 isolates (Table 1). The degree of BSH activity was determined by the size of the precipitation zone around the colonies, and expressed as strong (>2 mm), moderate (1−2 mm) and weak (<1 mm) activity. Seven isolates (MSMC 7-1, MSMC 25-2, MSMC 40-2, MSMC 40-7, MSMC 83-1, MSMC 177-1, and MSMC 248-1) expressed strong BSH activity. The precipitated halos indicated that the added bile salt was deconjugated by the activity of bacterial BSH. Previous studies have shown that numerous LAB strains isolated from different sources possessed BSH activity. *Lactococcus lactic* subsp. *lactis* isolated from Boza, a traditional non-dairy fermented drink from Turkey, expressed strong BSH activity (Shehata, El Sohaimy, El-Sahn, & Youssef, 2016). *Enterococcus faecalis* was found to produce highly active BSH activity compared to other BSH enzymes from other bacterial strains (Chand, Panigrahi, Varshney, Ramasamy, & Suresh, 2018).

BSH enzymes act on conjugated bile salts, thus free bile acid and amino acid residues are released. Free bile acids are less effectively absorbed in the intestine than their conjugated counterparts, so they are eliminated with the feces. As a result, the liver increases the *de novo* synthesis of bile salts from endogenous cholesterol, which leads to reduction of serum cholesterol level (Bustos, Font de Valdez, Fadda, & Taranto, 2018). In addition, increased BSH activity results in decreasing the solubility and absorption of dietary lipids in the intestine (Choi, Lew, Yeo, Nair Parvathy, & Liong, 2015).

3.3 Cholesterol assimilation by LAB

One hundred LAB were studied for their cholesterol assimilation. Figure 1 shows the levels of cholesterol assimilation of LAB during 48-h incubation at 37 ºC in MRS supplemented with 100 µg/mL water-soluble cholesterol. Of the 100 isolates, only 7 LAB showed the ability to assimilate cholesterol which ranged from 8.85% to 70.68%. MSMC 25-2 expressed the highest ability to remove cholesterol. Among the 7 LAB, only 6 isolates, including MSMC 25-2, MSMC 28-2, MSMC 40-7, MSMC 296-1, MSMC 300-2, and MSMC 302-1, exhibited levels of cholesterol assimilation greater than 20%. However, it should be noted that MSMC 28-2 and MSMC 302-1 did not show BSH activity. Bordoni *et al.*

Table 1. Bile salt hydrolase activity of 22 isolates using the agar plate assay method.

Isolate no.	Bile salt hydrolase activity	Isolate	Bile salt hydrolase activity
MSMC 7-1	+++	MSMC 241-1	+
MSMC 12-1	+	MSMC 246-3	+
MSMC 22-1	+	MSMC 248-1	+++
MSMC 24-2	+	MSMC 253-1	+
MSMC 25-2	+++	MSMC 258-1	++
MSMC 40-2	+++	MSMC 261-2	+
MSMC 40-7	+++	MSMC 280-1	++
MSMC 83-1	+++	MSMC 281-1	+
MSMC 96-2	++	MSMC 290-1	+
MSMC 177-1	+++	MSMC 296-1	++
MSMC 211-1	++	MSMC 300-2	++

MSMC=HRH Princess Maha Chakri Sirindhorn Medical Center, +++ = Strong bile salt hydrolase activity, ++ = Moderate bile salt hydrolase activity, + = Weak bile salt hydrolase activity.

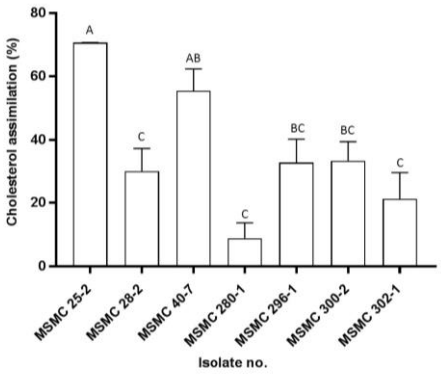

Figure 1. Cholesterol assimilation of the isolates in MRS broth supplemented with 100 µg/mL of cholesterol following 48-h incubation. A-C: Different letters indicate significant difference (P<0.05).

(2013) also demonstrated that two strains of *Bifidobacterium bifidum* (MB 107 and MB 109) expressed higher ability to assimilate cholesterol than other *Bifidobacterium* species, but they were BSH negative.

Apart from BSH activity, the cholesterol lowering effect of probiotics has also been attributed to their ability to bind cholesterol in the small intestines (Kumar *et al.,* 2012). Kimoto, Ohmomo, and Okamoto (2002) found that growing probiotic cells removed more cholesterol than dead cells, but dead cells were still able to remove cholesterol which indicated that part of cholesterol was bound to the bacterial surface. In addition, cholesterol was also removed by probiotics through incorporation of cholesterol to the bacterial cellular membrane during their growth. The ability to assimilate cholesterol was found in many probiotic species including *B. infantis*, *E. durans*, *E. faecalis*, *E. faecium*, *L. fermentum*, *L. plantarum*, *L. reuteri*, and *L. acidophilus* (Pereira & Gibson, 2002).

LAB with strong BSH activity and those with cholesterol assimilation levels greater than 20% were selected to determine the probiotic properties and genotypic identification. Thus, 11 LAB isolates were selected for the studies of acid and bile tolerance, adhesion to intestinal epithelium cells, and identification of bacterial species.

3.4 Acid and bile tolerance

Eleven LAB were tested for their acid and bile tolerance since potential probiotic LAB must be able to survive and function effectively within the acidic gastric environment and tolerate the bile in the small intestine (Jena *et al.*, 2013). The candidate probiotic LAB that were tested were MSMC 7-1, MSMC 25-2, MSMC 28-2, MSMC 40-2, MSMC 40-7, MSMC 83-1, MSMC 177-1, MSMC 248-1, MSMC 296-1, MSMC 300-2, and MSMC 302-1. The results of the acid and bile tolerance tests of the candidate probiotic LAB are shown in Table 2.

The number of MSMC 28-2, MSMC 177-1, MSMC 248-1, and MSMC 300-2 decreased significantly when exposed to pH 3.0 and 4.0, while MSMC 40-7 decreased significantly only at pH 3.0. The other LAB did not show any reduction when incubated at pH 3.0 and 4.0 for 3 h. At pH 2.0, none of the isolates survived in this severe gastric condition. These findings were in agreement with a study by Tulumoglu, Kaya, and Simsek (2014), in which the strain of *L. fermentum* could not survive at pH 2.0. The low pH of the stomach is a severe condition, but it should be noted that the pH levels in the human stomach can increase to pH 4.5 during ingestion of foods (Conway, Gorbach, & Goldin, 1987).

The concentration of bile salts in the human intestine varies over time and within different segments, thus two concentrations of bile salts (0.3% and 0.8%) were used in this study. All candidate probiotic LAB were able to survive in MRS supplemented with bile salts. Viability of the candidate LAB varied among the different isolates. The number of MSMC 25-2 and MSMC 177-1 significantly decreased at bile salt concentrations of 0.3% and 0.8%, while MSMC 28-2 and MSMC 296-1 only decreased when exposed to the high concentration (0.8%) of bile salt. Among these 4 isolates, only MSMC 28-2 did not express BSH activity. Viability of the other LAB, however, was not affected by the bile salt environment. Some studies have suggested that BSH activity might relate to bile tolerance in some Gram-positive bacteria because deconjugated bile salt precipitates and is excreted with the feces, thus the environment in the intestine may be less toxic to bacteria (Bustos, Font de Valdez, Fadda, & Taranto, 2018). However, a study by Vinderola and Reinheimer (2003) showed that bacteria that were negative for BSH activity were still able to tolerate the presence of bile

Table 2. Survival of probiotic candidates after incubation in MRS (Control), after incubation in MRS at pH 2.0 (pH 2.0), after incubation in MRS at pH 3.0 (pH 3.0), after incubation in MRS at pH 4.0 (pH 4.0), after incubation in MRS supplemented with 0.3% bile (0.3% bile), and after incubation in MRS supplemented with 0.8% bile (0.8% bile). All samples were incubated at 37 °C for 3 h. Data are expressed as mean±SD. Significantly different compared to 'Control' (*P<0.05, **P<0.01, ***P<0.001).

Isolate no.	Number of bacteria (log CFU/mL)					
	Control	pH 2.0	pH 3.0	pH 4.0	0.3% Bile	0.8% Bile
MSMC 7-1	7.95±0.06	NG	7.99±0.04	7.99±0.01	7.67±0.88	8.47±0.21
MSMC 25-2	7.78±0.36	NG	6.76±0.59	7.44±0.21	6.10±0.34***	5.93±0.34***
MSMC 28-2	8.09±0.02	NG	7.26±0.04**	7.33±0.02**	8.23±0.01*	7.53±0.08**
MSMC 40-2	7.84±0.01	NG	7.74±0.11	7.74±0.19	7.75±0.03	7.69±0.03*
MSMC 40-7	7.91±0.05	NG	7.12±0.37*	7.74±0.29	8.59±0.12	8.57±0.11
MSMC 83-1	8.21±0.10	NG	7.70±0.17	7.86±0.19	8.71±0.02**	8.63±0.04*
MSMC 177-1	8.59±0.06	NG	7.26±0.52*	7.91±0.00*	8.33±0.02*	8.29±0.02*
MSMC 248-1	8.29±0.02	NG	7.77±0.14*	7.93±0.03*	8.61±0.08	8.33±0.00
MSMC 296-1	7.97±0.03	NG	7.90±0.11	8.22±0.09	7.87±0.11	7.53±0.08*
MSMC 300-2	8.72±0.07	NG	7.84±0.03*	7.88±0.01*	8.60±0.14	8.57±0.10
MSMC 302-1	7.40±0.05	NG	7.30±0.08	7.36±0.00	7.12±0.03	7.10±0.11

MRS=de Man, Rogosa, Sharpe, CFU=Colony-forming units**,** MSMC=HRH Princess Maha Chakri Sirindhorn Medical Center, NG=No growth.

salt. Therefore, we also found in this present study that MSMC 302-1 did not exhibit BSH activity but was able to tolerate the bile salt at both concentrations.

3.5 Adherence property test

Adhesiveness is one of the important criteria for the selection of probiotics. Candidate probiotic LAB were evaluated for their adherence property to Caco-2 cell lines with *L. rhamnosus* GG as the control strain. The adhesion levels of the candidate LAB ranged from 0.8% to 8.8% (Figure 2). The control strain showed an adhesion ability of 1.8%, which was close to the value reported by (Burkholder & Bhunia, 2009), while MSMC 25-2, MSMC 28-2, MSMC 296-1, and MSMC 296-1 had significantly higher adherence properties of 8.8%, 3.5%, 6.1%, and 7.1%, respectively.

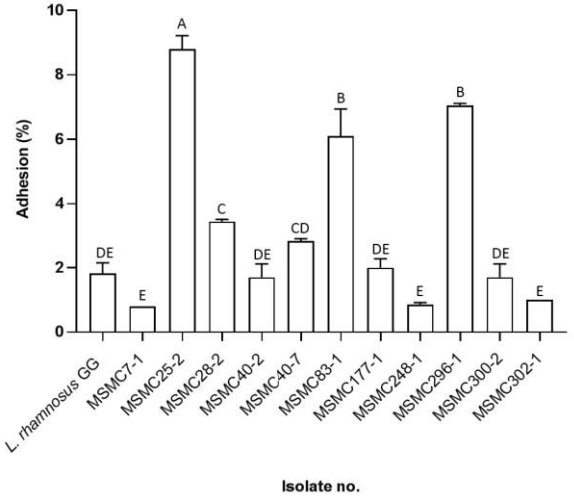

Figure 2. Adherence properties of candidate isolates to Caco-2 cells. *L. rhamnosus* was used as a control strain. A–E: Different letters indicate significant difference (P<0.05).

In general, adhesion to Caco-2 cells is strain dependent (Guo, Li, Tang, Yang, & Huo, 2016). Previous studies reported the adhesion levels of different probiotic LAB. *E. faecium* had 9% adherence (Mansour *et al.*, 2014). Different strains of *L. fermentum* had between 2% and 14% adherence, while *L. rhamnosus* GG used as a reference strain showed an adhesion rate of 7% (Tulumoglu, Kaya, & Simsek, 2014). This discrepancy was probably due to differences in the Caco-2 cell lines used in the experiments (Tulumoglu, Kaya, & Simsek, 2014). However, this adhesion study gave insightful information on the adhesion efficiency of different candidate LAB relative to a standard probiotic strain.

3.6 Genotypic characterization of selected isolates

The candidate probiotic LAB were identified by 16S rRNA gene sequencing. The analyzed base sequence was compared with the base sequences of similar strains registered in a database to examine the correlations between genes.

Identification by 16S rRNA gene sequencing of the 11 selected probiotic LAB showed that 8 isolates including MSMC 7-1, MSMC 28-2, MSMC 40-2, MSMC 83-1, MSMC 177-1, MSMC 248-1, MSMC 296-1, and MSMC 300-2 were closely related to *E. faecalis* with BLAST similarity scores of ≥99% (Table 3). The isolates of MSMC 25-2, MSMC 40-7, and MSMC 302-1 were closely related to *E. faecium*, *E. durans*, and *L. garvieae* with 99.78%, 100%, and 99.87% similarities, respectively (Table 3).

4. Conclusions

This study found that LAB isolated from human origin could be interesting probiotic candidates since they could be of advantage in the ability to compete with the indigenous microflora (Pereira & Gibson, 2002), and human origin probiotics showed superior probiotic characteristics compared to strains from plant and dairy sources (Vemuri *et al.*, 2018). In this present study, the isolates that expressed the ability to lower cholesterol included the strains of *Entero-*

Table 3. Genotypic characterization of 11 selected isolates.

Isolate no.	Nucleotide sequences of 16S rRna gene	Similarity
MSMC 7-1	*Enterococcus faecalis* ATCC 19433T (ASDA01000001)	100%
MSMC 25-2	*Enterococcus faecium* CGMCC 12136T (AJKH01000109)	99.78%
MSMC 28-2	*Enterococcus faecalis* ATCC 19433T (ASDA01000001)	100%
MSMC 40-2	*Enterococcus faecalis* ATCC 19433T (ASDA01000001)	100%
MSMC 40-7	*Enterococcus durans* CECT 411T (AJ420801)	100%
MSMC 83-1	*Enterococcus faecalis* ATCC 19433T (ASDA01000001)	100%
MSMC 177-1	*Enterococcus faecalis* ATCC 19433T (ASDA01000001)	99.24%
MSMC 248-1	*Enterococcus faecalis* ATCC 19433T (ASDA01000001)	100%
MSMC 296-1	*Enterococcus faecalis* ATCC 19433T (ASDA01000001)	100%
MSMC 300-2	*Enterococcus faecalis* ATCC 19433T (ASDA01000001)	99.76%
MSMC 302-1	*Lactococcus garvieae* ATCC 12136T (AP009332)	99.87%

MSMC=HRH Princess Maha Chakri Sirindhorn Medical Center.

coccus and *Lactococcus*. *Enterococcus* exhibited the highest ability to produce BSH and cholesterol assimilation, but *Lactococcus* exhibited only cholesterol assimilation. From this *in vitro* study, it was proven that *Enterococcus* is the dominant species to reduce cholesterol with BSH and cholesterol assimilation mechanisms. Potential probiotics must also be able to withstand the severe conditions of the gastrointestinal tract and adhere to the epithelial cells. All selected isolates showed different degrees of acid tolerance at pH 3.0, bile tolerance, and adhesion properties which demonstrated they are suitable as probiotics to lower serum cholesterol. Among the strains tested, *E. faecium* MSMC 25-2, *E. durans* MSMC 40-7, and *E. faecalis* MSMC 296-1 exhibited interesting probiotic properties. Therefore, more *in vivo* research and applications in probiotic food products as well as determining clinical efficacy are required.

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