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

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<th>Journal:</th>
<th><em>Songklanakarin Journal of Science and Technology</em></th>
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<td>SJST-2018-0363.R1</td>
</tr>
<tr>
<td>Manuscript Type:</td>
<td>Original Article</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>17-Nov-2018</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Sirichotinun, Nutcha; Faculty of Medicine, Srinakharinwirot University, Biomedical science program Pachekrepapol, Ulisa; Faculty of Agricultural Product Innovation and Technology, Srinakharinwirot University, Food Science and Nutrition Nantavisai, Kwannan; Faculty of Medicine, Srinakharinwirot University, Department of Microbiology Taweechotipatr, Malai; Srinakarinwirot University, Microbiology Nilwarangkoon, Sirinun; Srinakharinwirot University Faculty of Medicine, Biochemistry</td>
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<tr>
<td>Keyword:</td>
<td>Bile salt hydrolase, Probiotics, Lactic acid bacteria, Cholesterol, Infant feces</td>
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Original Article

Probiotic characterization and \textit{in vitro} cholesterol lowering effects of lactic acid bacteria isolated from Thai healthy infants

Nutcha Sirichotinun \textsuperscript{1}, Ulisa Pachekrepa\textsuperscript{2}, Kwannan Nantavisai \textsuperscript{3}, Malai Taweechotipatr \textsuperscript{3}, Sirinun Nilwarangkoon \textsuperscript{4,*}

\textsuperscript{1}Biomedical science program, Faculty of Medicine, Srinakharinwirot University, 114 Sukhumvit 23, Wattana, Bangkok 10110, Thailand

\textsuperscript{2}Division of Food Science and Nutrition, Faculty of Agricultural Product Innovation and Technology, Srinakharinwirot University, 63 Moo 7, Rangsit-Nakhon Nayok Road, Nakhon-Nayok 26120, Thailand

\textsuperscript{3}Department of Microbiology, Faculty of Medicine, Srinakharinwirot University, 114 Sukhumvit 23, Wattana, Bangkok 10110, Thailand

\textsuperscript{4}Department of Biochemistry, Faculty of Medicine, Srinakharinwirot University, 114 Sukhumvit 23, Wattana, Bangkok 10110, Thailand

*Corresponding author: Sirinun Nilwarangkoon

Department of Biochemistry, Faculty of Medicine, Srinakharinwirot University

114 Sukhumvit 23, Wattana, Bangkok 10110, Thailand

Email: sirinunkk@gmail.com
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 Thai healthy infant feces 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 property. 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 Enterococcus faecium, Enterococcus 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 Enterococcus 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 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 Statin and modification of lifestyles have been recommended by the American Heart Association for prevention of cardiovascular disease risk (Roy, 2014). However, drug therapy could not be used as a long term treatment because it is relatively expensive and may develop some side effects including muscle pain, fatigue and weakness (Golomb & Evans, 2008). Thus, change of lifestyles, such as dietary modification is regarded as 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 gastrointestinal tract of humans and animals. The probiotics commonly used in foods, especially dairy products are the 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 from many LAB species, such as Lactobacillus, Bifidobacterium and Enterococcus (Guo, Li, Tang, Yang, & Huo, 2016). The mechanisms of cholesterol lowering have been proposed, including suppression of bile acid re-absorption mediated by bile salt hydrolase (BSH) enzyme from bacteria and 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 nondrug alternatives and food products (Lye et al., 2017). However, cholesterol lowering effect and other probiotic properties are strain dependent, and are varied even within the same species (Wang et al., 2014). In addition to health benefits, tolerance in an acidic gastric condition and high concentrations of bile salts 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 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 the studies of probiotic characteristics, acid and bile tolerance and adherence to Caco-2 cells, and genotypic identification using 16S rRNA gene sequencing.

2. Materials and methods

2.1 Selection of lactic acid bacteria

LAB from healthy infant feces were randomly selected from laboratory frozen stock. The criteria for selection of 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, coccobacilli, short rods and regular rods bacteria were maintained as frozen cultures in de Man Rogosa Sharpe (MRS) broth (Oxoid, Basingstoke, Hampshire, UK) with 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\(^{-1}\) in MRS broth were spotted on MRS agar supplemented with 0.5% (w/v) sodium salt of taurodeoxycholic acid (TDCA; Sigma, USA) and 0.37 g/l of calcium chloride (CaCl\(_2\)). The MRS agar plates were incubated in an anaerobic jar with Gas Pak at 37°C for 72 h. The plates without supplementation of TDCA were used as control. The precipitation zone surrounding colonies indicated the BSH activity.

2.3 Cholesterol assimilation

Each isolate \(10^9\) cells ml\(^{-1}\) was inoculated to MRS broth supplemented with water soluble cholesterol at a final concentration of 100 µg ml\(^{-1}\), and incubated in an anaerobic jar with Gas Pak 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. Supernatant from each sample was analyzed for cholesterol concentration using a 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 (KOH) 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. Hexane layer was
transferred to 96 well plates and evaporated. When hexane was completely evaporated, O-
phthalaldehyde reagent (OPA; Sigma, USA) prepared in acetic acid and concentrated sulfuric
acid were 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 % cholesterol
assimilation. LAB expressing 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 acid tolerance test or
supplemented with 0.3% and 0.8% bovine bile (Sigma, USA) for bile tolerance test (Ladda,
Theparee, Chimchang, Tanasupawat, & Taweechotipatr, 2015). Non-supplemented MRS broth
was used as a control. Overnight cultures of candidate probiotics (10⁹ cells ml⁻¹) were incubated
at 37°C for 3 h under anaerobic conditions using anaerobic jar with Gas Pak. Viable cell counts
were assessed and displayed as log-10 values of colony-forming units per ml (CFU ml⁻¹), and
compared to the viable counts of bacteria incubated in non-supplemented MRS. Experiments
were performed three times, in duplicate.

2.5 Adherence property test
The adenocarcinoma cell lines (Caco-2) (ATCC, HTB-37) in 24-well tissue culture
plates were used for the adhesion assay (Dimitrov, Gotova, & Chorbadiyska, 2014).
Candidate LAB cultured overnight in MRS broth (10⁹ cells/ml) were centrifuged at 4,000
rpm at 4 °C for 10 min. Bacterial 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% CO₂ 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 bacterial cells on 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 a positive control and 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 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 consisting of 5 U Taq DNA polymerase, 2 mM dNTP, 25 mM MgCl$_2$ and 10X Taq Buffer with (NH$_4$)$_2$SO$_4$ and dH$_2$O. Amplification of 16S rRNA gene was performed by polymerase chain reaction (PCR). PCR products were purified using a Geneaid Gel/PCR DNA Fragments Extraction Kit (Geneaid Biotech, Bade City, Taiwan). The sequence analysis of PCR products were performed by U2Bio in Seoul, Korea. The 16S rRNA gene was amplified using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3'), 518F (5'-CCAGCAGCAGCGGTAAATC G-3'), 800R (5'-TACCAGGATATCTAATCC-3') and 1492R (5'-TACGGYTACCTTGT TACGACTT-3'). The nucleotide sequences were processed and determined using BLAST software compared to 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 $\geq 99\%$ to 16S rRNA gene sequences of 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 data analyses. Multiple comparisons were performed using Tukey’s test for 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 laboratory frozen stock. They were all Gram-positive cocci or bacilli and catalase-negative. Colony morphology was various. They appeared under microscope 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 substrate. After 72 h incubation, fine precipitated halos around colonies were observed from 22 isolates as shown in 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 added bile salt was deconjugated by activity of bacterial BSH. Previous studies have shown that numerous LAB strains isolated from different sources possessed BSH activity. *Lactococcus lactis* 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 compared to other BSH from other bacterial strains (Chand, Panigrahi, Varshney, Ramasamy, & Suresh, 2018).

BSH enzyme acts on conjugated bile salts, thus free bile acid and amino acid residues are released. Free bile acids are less efficiently to be reabsorbed 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. The levels of cholesterol assimilation of LAB during 48 h incubation at 37°C in MRS supplemented with 100 µg ml⁻¹ water-soluble cholesterol are shown in Figure 1. Out of 100 isolates, only 7 LAB showed cholesterol assimilation ability, which ranged from 8.85% to 70.68% with MSMC 25-2 expressing the highest cholesterol removing ability. Among 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 greater than 20% cholesterol assimilation level. However, it should be noted that MSMC 28-2 and MSMC 302-1 did not show BSH activity. Bordoni et al. (2013) also demonstrated that two strains of *Bifidobacterium bifidum* (MB 107 and MB 109) expressing...
high ability to assimilate cholesterol than other *Bifidobacterium* species, but they were BSH negative.

Apart from BSH activity, 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 indicating that part of cholesterol was bound to the bacterial surface. In addition, cholesterol was also removed by probiotics through incorporation of cholesterol to bacterial cellular membrane during their growth. Cholesterol assimilation ability was found in many probiotic species including *Bifidobacterium infantis*, *Enterococcus durans*, *Enterococcus faecalis*, *Enterococcus faecium*, *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus reuteri*, and *Lactobacillus acidophilus* (Pereira & Gibson, 2002).

LAB with strong BSH activity and those with cholesterol assimilation level greater than 20% were selected for determination of probiotic properties and genotypic identification. Thus, 11 LAB isolates were proceeded 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 gastric acidic environment and bile in the small intestine (Jena et al., 2013). The candidate LAB probiotics 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 acid and bile tolerance test of candidate probiotics 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. Other LAB did not show any reduction when incubated at pH 3.0 and 4.0 for 3 h. At pH 2.0, all isolates did not survive in this severe gastric condition. These findings were in agreement with the study by Tulumoglu, Kaya, and Simsek (2014), in which the strain of *Lactobacillus 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 human stomach could increase to pH 4.5 during ingestion of foods (Conway, Gorbach, & Goldin, 1987).

The concentration of bile salt 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 probiotics were able to survive in MRS supplemented with bile salts. Viability of candidate LAB were varied among different isolates. The number of MSMC 25-2 and MSMC 177-1 significantly decreased at both bile salt contrations (0.3 and 0.8%), while MSMC 28-2 and MSMC 296-1 only decreased when exposed to high concentration of bile salt (0.8%). Among these 4 isolates, only MSMC 28-2 did not express BSH activity. Viability of 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 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 with BSH negative were still able to tolerate in the presence of bile salt, so as we also found in this present study that MSMC 302-1 did not exhibit BSH activity, but was able to tolerate in bile salt at both concentrations.
3.5 Adherence property test

Adhesiveness is one of 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 having been used as a control strain. The adhesion levels of candidate LAB ranged from 0.8 to 8.8% as shown in Figure 2. The control strain showed the 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 property of 8.8%, 3.5%, 6.1% and 7.1%, respectively.

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. *Enterococcus faecium* had 9% adherence (Mansour et al., 2014). Different strains of *L. fermentum* had approximately between 2% and 14% adherence, while *L. rhamnosus* GG used as a reference strain showed the adhesion rate of 7% (Tulumoglu, Kaya, & Simsek, 2014). This discrepancy may probably be due to differences of the Caco-2 cell lines used in the experiments (Tulumoglu, Kaya, & Simsek, 2014). However, this adhesion study gave insightful information of adhesion efficiency of different candidate LAB relative to a standard probiotic strain.

3.6 Genotypic characterization of selected isolates

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

Identification by 16S rRNA gene sequencing of 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 *Enterococcus faecalis*. 

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with BLAST similarity scores of ≥ 99% as shown in table 3. The isolates of MSMC 25-2, MSMC 40-7 and MSMC 302-1 were closely related to *Enterococcus faecium, Enterococcus durans* and *Lactococcus 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 an interesting probiotic candidate as it could be of advantage in its 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 expressing cholesterol lowering ability include the strains of *Enterococcus* and *Lactococcus*. *Enterococcus* exhibited the highest ability to produce BSH and cholesterol assimilation, but *Lactococcus* has only ability for cholesterol assimilation. From this *in vitro* study, it was proven that *Enterococcus* are dominant species for reducing 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 property demonstrating that they are suitable for use as probiotics for cholesterol lowering effect. Among these strains, *Enterococcus faecium* MSMC 25-2, *Enterococcus durans* MSMC 40-7 and *Enterococcus faecalis* MSMC 296-1 exhibited interesting probiotic properties, thus more *in vivo* research and applications in probiotic food products as well as determining clinical efficacy are further required.
Acknowledgements

This work was funded by a research grants (grant no. 222/2558 and 299/2558) from the Faculty of Medicine and HRH Princess Maha Chakri Sirindhorn Medical Center, Srinakharinwirot University. The authors thank the Department of Microbiology, Faculty of Medicine, Srinakharinwirot University for supporting equipment and facilities for our research.

References


Chand, D., Panigrahi, P., Varshney, N., Ramasamy, S., & Suresh, C. G. (2018). Structure and function of a highly active Bile Salt Hydrolase (BSH) from *Enterococcus faecalis* and
post-translational processing of BSH enzymes. *Biochimica et Biophysica Acta.*


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

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<th>Isolate</th>
<th>Bile salt hydrolase activity</th>
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<td>MSMC 7-1</td>
<td>+++</td>
<td>MSMC 241-1</td>
<td>+</td>
</tr>
<tr>
<td>MSMC 12-1</td>
<td>+</td>
<td>MSMC 246-3</td>
<td>+</td>
</tr>
<tr>
<td>MSMC 22-1</td>
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</tr>
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<td>MSMC 253-1</td>
<td>+</td>
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<td>+++</td>
<td>MSMC 258-1</td>
<td>++</td>
</tr>
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<td>MSMC 281-1</td>
<td>+</td>
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<td>MSMC 96-2</td>
<td>++</td>
<td>MSMC 290-1</td>
<td>+</td>
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<tr>
<td>MSMC 177-1</td>
<td>+++</td>
<td>MSMC 296-1</td>
<td>++</td>
</tr>
<tr>
<td>MSMC 211-1</td>
<td>++</td>
<td>MSMC 300-2</td>
<td>++</td>
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(MSMC: HRH Princess Maha Chakri Sirindhorn Medical Center)

+++ Strong bile salt hydrolase activity

++ Moderate bile salt hydrolase activity

+ Weak bile salt hydrolase activity
Table 2 The 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).

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<thead>
<tr>
<th>Isolate no.</th>
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<tr>
<td></td>
<td>Control</td>
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<tr>
<td>MSMC 7-1</td>
<td>7.95 ± 0.06</td>
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<tr>
<td>MSMC 25-2</td>
<td>7.78 ± 0.36</td>
</tr>
<tr>
<td>MSMC 28-2</td>
<td>8.09 ± 0.02</td>
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<tr>
<td>MSMC 40-2</td>
<td>7.84 ± 0.01</td>
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<td>MSMC 40-7</td>
<td>7.91 ± 0.05</td>
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<td>MSMC 83-1</td>
<td>8.21 ± 0.10</td>
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<td>MSMC 177-1</td>
<td>8.59 ± 0.06</td>
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<td>MSMC 248-1</td>
<td>8.29 ± 0.02</td>
</tr>
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<td>MSMC 296-1</td>
<td>7.97 ± 0.03</td>
</tr>
<tr>
<td>MSMC 300-2</td>
<td>8.72 ± 0.07</td>
</tr>
<tr>
<td>MSMC 302-1</td>
<td>7.40 ± 0.05</td>
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NG: No growth
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<th>Isolate no.</th>
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<tr>
<td>MSMC 7-1</td>
<td>Enterococcus faecalis ATCC 19433T (ASDA01000001)</td>
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<td>Enterococcus faecium CGMCC 12136T (AJKH01000109)</td>
<td>99.78%</td>
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<td>MSMC 28-2</td>
<td>Enterococcus faecalis ATCC 19433T (ASDA01000001)</td>
<td>100%</td>
</tr>
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<td>Enterococcus durans CECT 411T (AJ420801)</td>
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<td>99.24%</td>
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<td>Enterococcus faecalis ATCC 19433T (ASDA01000001)</td>
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</tr>
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<td>MSMC 296-1</td>
<td>Enterococcus faecalis ATCC 19433T (ASDA01000001)</td>
<td>100%</td>
</tr>
<tr>
<td>MSMC 300-2</td>
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<td>99.76%</td>
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<td>MSMC 302-1</td>
<td>Lactococcus garvieae ATCC 12136T (AP009332)</td>
<td>99.87%</td>
</tr>
</tbody>
</table>
List of figures

![Figure 1](image)

**Figure 1** Cholesterol assimilation of the isolates in MRS broth supplemented with 100 µg/ml of cholesterol, following 48 h of incubation. A-C Different letters indicate significantly different (p < 0.05).
Figure 2 Adherence properties of candidate isolates to Caco-2 cells. *L. rhamnosus* was used as a control strain. A-E Different letters indicate significantly difference (*p* < 0.05).