SCREENING BILE SALT HYDROLASE ACTIVITY OF LACTOBACILLUS ISOLATED FROM VIETNAMESE HUMAN ORIGINS

Xuan Thach Tran1, Thi Hien Vu1, Thi Thu Ha1, Thi Hoa Nguyen1, The Hung Hoang2,3, Duc Hoang Le1,2, Quyen Van Dong1, Nam Trung Nguyen1, Thi Tuyet Nhung Nguyen4

1Institute of Biotechnology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Cau Giay District, Hanoi, Vietnam
2Graduate University of Science and Technology, 18 Hoang Quoc Viet Road, Cau Giay District, Hanoi, Vietnam
3Military Academy of Logistics, Ngoc Thuy Street, Long Bien District, Hanoi, Vietnam
4R&D Department, Kenubio Company, Duong Noi, Hanoi, Vietnam

*These authors contributed equally to this work

To whom correspondence should be addressed. E-mail: nttnhung@gmail.com

Received: 08.4.2022
Accepted: 04.6.2022

SUMMARY

Hypercholesterolemia is a major cause of cholesterol build-up in the coronary arteries, which can subsequently lead to heart disease or atherosclerosis. Cholesterol levels can be lowered by cholesterol-lowering drugs but some of these drugs may have harmful side effects, while supplementation of Lactobacillus has shown the potential to reduce serum cholesterol levels by virtue of bile salt hydrolase (bsh) activity. In this study, Lactobacillus plantarum VFE-04, L. rhamnosus VFE-08, and L. plantarum VFE-14, had been isolated from Vietnamese healthy adults, were able to deconjugate glycodeoxycholate (GDC) on MRS plates and MRS broth supplemented with GDC. In addition, deconjugating activity of L. plantarum VFE-04, L. rhamnosus VFE-08, and L. plantarum VFE-14 were also found in cell-free extract as expressed by amount of glycine that released in the supernatant. Four bsh genes including bsh1, bsh2, bsh3, and bsh4 have been identified by PCR in these strains. In addition, L. plantarum VFE-04, L. rhamnosus VFE-08, and L. plantarum VFE-14 also showed high ability to resist bile salts and low pH. The results of 16S rRNA gene analyses showed that L. plantarum VFE-04, L. rhamnosus VFE-08, and L. plantarum VFE-14 and had high similarity scores with L. plantarum ZZU 23 (100%), L. rhamnosus JCM 1136 (99%) and L. plantarum S7 (98.65%), respectively. This study suggests that L. plantarum VFE-04, L. rhamnosus VFE-08, and L. plantarum VFE-14 have the potential to be explored as probiotics in the management of hypercholesterolaemia in near future.

Keywords: Lactobacillus, bile salt deconjugation, cholesterol, deconjugate glycodeoxycholate, human origins

INTRODUCTION

Hypercholesterolemia is a major cause of cholesterol build-up in the coronary arteries, which can subsequently lead to heart disease or atherosclerosis (Argyri, Zoumpopoulou et al., 2013) Based on a meta-analysis, a 1% decrease in plasma cholesterol levels can lower the risk of coronary events by up to 3%. Cholesterol levels can be lowered by several independent mechanisms, including decreasing hepatic cholesterol biosynthesis, increasing plasma
cholesterol removal, and disruption of bile acid reabsorption (Duary et al., 2010; Gu et al., 2014). Bile acids synthesized from cholesterol in the liver are important end products of cholesterol metabolism in mammals, and are secreted mostly as conjugated with glycine or taurine by an amide bond. Conjugated and unconjugated bile acids are absorbed by passive diffusion along the entire gut and by active transport in the terminal ileum (Heunis et al., 2014). In the gut, intestinal microbes are capable of hydrolyzing the glycine or taurine conjugated with bile acids using bile salt hydrolase (BSH) enzymes. After the removal of glycine or taurine, deconjugated bile salts are less water-soluble and are more easily excreted via the feces. Enhanced fecal loss of bile acids may result in an increased requirement for cholesterol as a precursor for the de novo synthesis of bile salts in order to maintain bile salt homeostasis. Therefore, this drain on the bile salt pool might be regarded as a ‘biological’ alternative to common therapeutic interventions to treat hypercholesterolemia (Patel et al., 2010; Nie et al., 2015).

Probiotic Lactobacillus are considered as normal components of the intestinal microflora in both humans and animals, and have been invariably associated with various health-promoting functions (Kim, Lee, 2005; Duary et al., 2010, Patel et al., 2010; Kumar et al., 2011; Kumar et al., 2012). Previous studies have demonstrated that consumption of L. plantarum Lp91 and L. plantarum Lp09 (Duary et al., 2012) as probiotic dietary adjuncts are useful in reducing plasma cholesterol levels (Duary et al., 2010; Duary et al., 2012). Besides deconjugation of bile via BSH enzymes, there are several mechanisms for cholesterol removal by probiotics Lactobacillus viz.; assimilation of cholesterol by growing cells, binding of cholesterol to the cellular surface, incorporation of cholesterol into the cellular membrane, co-precipitation of cholesterol with deconjugated bile, binding of bile by fiber, and production of short-chain fatty acids by oligosaccharides (Ridlon et al., 2006; Lambert, 2008, Sédláčková et al., 2015; Shehata et al., 2016). However, in recent years, the possibility of using bile salt deconjugation by Lactobacillus to lower serum cholesterol levels in vivo has received considerable attention. Bile salt hydrolase activity of probiotic Lactobacillus is more often found in strains isolated from the gastrointestinal tract, where bile salts are present, rather than in strains from the non-bile salt environment (Ramasamy et al., 2010; Öner et al., 2014; Tsai et al., 2014; Bustos et al., 2018). The aims of this study were to select Lactobacillus strains with bile salts deconjugate activity from Vietnamese adult origins and screen probiotic potential of selected Lactobacillus that can be used as probiotics in near future to help hypercholesterolaemia patients.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and solvents used in this study were of the highest purity commercially available. Standard bile salts, including glycocholate (GC), glycodeoxycholate (GDC), taurocholate (TC), taurodeoxycholate (TDC) and oxgall powder were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Isolating and screening bile salts deconjugation-positive Lactobacillus

The Lactobacillus strains were isolated as described in previous study (Thu et al., 2020). Lactobacillus colonies were first screened for catalase activity and Gram staining, and only those that were catalase-negative and Gram-positive were used for further investigations.

Deconjugation ability was carried out on the MRS plate supplemented with 0.3% oxgall and 0.37 g/L CaCl₂ (Öner et al., 2014). Once agar plates were solidified, overnight MRS broth cultures were streaked on and incubated anaerobically at 37°C for 48–72 h. MRS agar plates without oxgall were used as controls. The white precipitates around colonies and the clearing of the medium are indicative of deconjugating activity (McAuliffe et al., 2005).
Determination of acid tolerance

Acid tolerance of the Lac.VFE-04, Lac.VFE-08, and Lac.VFE-14 were studied. 10^8 CFU/mL of selected Lactobacillus cultures were inoculated in MRS media pre-adjusted to pH 2.0 and 3.0 using 1 M HCl and incubated at 37°C for 3 h. Viability was determined using the plate count method. Serial tenfold dilutions of the sample were plated onto MRS agar and incubated anaerobically at 37°C for 24-48 h to obtain the colony count (Zheng et al., 2013).

Determination of bile tolerance

Bile tolerance is an important criterion in the selection of probiotic strains. Previous reports have suggested that a concentration of 0.3% oxgall closely approximates the bile levels found in human gastrointestinal tract (McAuliffe et al., 2005). To evaluate the bile tolerance ability of Lactobacillus, isolates were inoculated (2% w/v) into MRS broth with 0.3%, 0.5%, or 1% (w/v) of oxgall. After 24 h cultivation at 37°C, A560 nm was measured and the results were expressed by the percentage of growth (A560 nm) in the presence of oxgall compared to the control culture without oxgall (Isa, Vasavi, 2017).

Substrate specificity

To screen the substrate specificity, oxgall-hydrolyzed Lactobacillus strains were investigated. Agar plates containing 1.5% (w/v) agar and 0.37 g/L CaCl₂ or MRS broth supplemented with either 5 mM GC, 5 mM GDC, 5 mM TC, or 5 mM TDC. The centrifuged and concentrated cultures (10 µL) were spotted on the plates and incubated anaerobically at 37°C for 48-72 h. MRS agar plates without bile salts were used as controls. Each strain was tested in triplicate. The white precipitates around colonies, the clearing of the medium or the broth cloudy are indicative of BSH activity (Begley et al., 2006).

BSH activity was also further monitored by measuring glycine liberation from GDC following the protocol described by Grill. To summarize, cell-free extract was mixed with 5 mM GDC and incubated for 24 h at 37°C. Controls were performed in the absence of GDC. Glycine concentration was measured at 416 nm using a spectrophotometer. A standard curve was established using free glycine (Grill et al., 2000).

Bsh gene investigation

Genomic DNA of selected Lactobacillus was extracted using a Genomic DNA Extraction Kit (Invitrogen Inc.) according to the manufacturer's instruction. bsh genes were PCR-amplified from the genomic DNA of Lac.VFE-04, Lac.VFE-08, and Lac.VFE-14 using the primers (Table 1), which were designed based on the published genome sequence of L. plantarum WCFS1 (NC_004567.2) PCR products were analyzed and compared with the reference sequences available in GenBank (EMBL). The bsh1 of 3 reference strains were submitted to the GenBank database under accession numbers FJ179487, FJ179486, and EU822811, respectively.

Table 1. Nucleotide sequences of 4 pairs of primers.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Nucleotide Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>bsh1-F</td>
<td>GGAATTCATATAGTGAGTGACAGCCATAACTTATC (35)</td>
</tr>
<tr>
<td>bsh1-R</td>
<td>CCGCTCGAGTTAGTAACTGCATGTTATGGTG (32)</td>
</tr>
<tr>
<td>bsh2-F</td>
<td>GGAATTCATATAGTGACACTGCTAACTTATACAAA (39)</td>
</tr>
<tr>
<td>bsh2-R</td>
<td>CCGCTCGAGTTAGTGGGCGGTGAGGAA (27)</td>
</tr>
<tr>
<td>bsh3-F</td>
<td>GGAATTCATATGAGTGACTATGCTAACTTACAAA (36)</td>
</tr>
<tr>
<td>bsh3-R</td>
<td>CGGCTCGAGTTAGTGGGCGGTGAGGAA (28)</td>
</tr>
<tr>
<td>bsh4-F</td>
<td>GGAATTCATATGAGTGACTTAACTTACAAA (38)</td>
</tr>
<tr>
<td>bsh4-R</td>
<td>CGGCTCGAGTTAGTGGGCGGTGAGGAG (29)</td>
</tr>
</tbody>
</table>

Note: the number of nucleotides in parenthesis
RESULTS AND DISCUSSION

Screening BSH-positive lactic acid bacteria

In recent years, the interest in lactic acid bacteria that can deconjugate bile salts to prevent hypercholesterolaemia in obese people, or can lower serum cholesterol levels in hypercholesterolaemic people, has increased. BSH activity in the intestinal microflora has been screened in a number of studies and for specific groups of bacteria like Lactobacillus, Bifidobacterium, and Enterococcus (Begley et al., 2006). To understand the distribution of BSH activity in lactic acid bacteria, we have screened BSH and bile salt tolerance of Lactobacillus isolated from Vietnamese adult origins.

Oxgall powder from Sigma-Aldrich was chosen because this mixture was closer to human bile in terms of its glycine-conjugated bile acids to taurine-conjugated bile acids ratio. In previous studies, 0.3% bile has been considered to be the crucial concentration at which to evaluate bile-tolerant probiotic Lactobacillus. In this regard, 11 strains of Lactobacillus showed good resistance to bile (data not shown). These findings corresponded with those of studies where L. plantarum strains from cheeses displayed good resistance to bile salts (Georgieva et al., 2008; Pisano et al., 2008; Zago et al., 2011).

Acid tolerance

The ability of probiotic organisms to survive in acidic environments is an important characteristic of probiotics. In this study, Lactobacillus strains were incubated under conditions of pH 2 and pH 3 to evaluate their tolerance to acidic pH. As shown in Table 2, of the five tested Lactobacillus strains, Lac.VFE-14 had the highest acidic tolerance ability while this activity of Lac.VFE-09 was lowest as expressed by cell survival percentage.

At pH 3, the viability of Lac.VFE-14 was 98.54% ± 6.68, followed by Lac.VFE-04, Lac.VFE-08, Lac.VFE-11, and Lac.VFE-09. At pH 2, the cell viability was reduced in all of them. The survival of Lac.VFE-14 was 88.22 ± 5.14, where as in Lac.VFE-09, this percentage was 69.06 ± 3.89 (Table 2). Similar results were reported by Mirelahi, where the L. plantarum A7, L. rhamnosus GG, and L. acidophilus strains showed tolerance to pH 3 (Pisano et al., 2008). Another study was conducted by Deshpande demonstrated that L. casei can survive at pH 2, 3, and 4 for at least 90 min (Mirelahi et al., 2009, Deshpande et al., 2014, Zago et al., 2011). However, Lactobacillus strains isolated from Koozeh cheese decreased significantly after incubation at pH 3 and at pH ≤ 2, and there were no viable bacterial cells detected after the first hour of incubation (Hassanzadazar et al., 2012).

Bile salt tolerance

Bile tolerance is considered as another important characteristic of Lactobacillus strains, which enables them to survive, grow, and exert their action during gastrointestinal transit. Five selected Lactobacillus strains isolated from Vietnamese healthy adult feces were screened for their bile salt tolerance by exposure to oxgall up to 1%.

Table 2. Survival of Lactobacillus isolates in the presence of Oxgall and low pH.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Growth (%) in the presence of oxgall</th>
<th>Growth (%) at low pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.3%</td>
<td>0.5%</td>
</tr>
<tr>
<td>Lac.VFE-04</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Lac.VFE-08</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Lac.VFE-09</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Lac.VFE-11</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Lac.VFE-14</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>
As described in Table 2, all five of the Lactobacillus strains could grow well at 0.3% and 0.5% oxgall. At 1% oxgall, except Lac.VFE-09, all remaining strains were able to grow and survive. In some reports, Lactobacillus spp. does not grow at levels greater than 0.3% of oxgall (Zheng et al., 2013). In one study, researchers showed that survival rates of L. plantarum CGMCC 8198 was 48%, while L. casei 1.539 had a survival rate of only 8% when cultured in MRS broth containing 0.3% bile salts for 4 h (Gu et al., 2014). McAuliffe et al., 2005, indicated that L. acidophilus NCFM did not grow in MRS broth, nor on MRS plates supplemented with 0.3% and 0.5% oxgall (McAuliffe et al., 2005). In line with the findings of our investigation, Nawaz et al., 2017, revealed that among the 25 tested lactic acid bacterial strains, there were 10 strains that could grow well in an MRS medium supplemented with bile salt up to 1% oxgall (Nawaz et al., 2017). As such, isolated Lactobacillus strains from Vietnamese healthy people are able to low pH tolerance, especially these Lactobacillus strains showed the highly tolerance to oxgall.

The fact that some Lactobacillus strains were able to grow in the presence of conjugated bile salts, while they were not able to deconjugate them, means that the capacity to express bile salt hydrolase is not related to the capacity to resist the toxicity of conjugated bile salts (Belicová et al., 2013). That explains why all Lactobacillus strains that were able to survive in the presence of oxgall were selected to examine for bile salt deconjugation.

**Bile salt deconjugation**

In our study, bile salt deconjugating activity of all Lactobacillus strains was tested with 5 mM of GC, 5 mM of GDC, 5 mM of TC, and 5 mM of TDC. Five strains (Lac.VFE-04, Lac.VFE-08, Lac.VFE-09, Lac.VFE-11, and Lac.VFE-14) were concentrated and spotted on the same medium for the direct plate assay. The BSH-positive strains could be identified by halos of precipitation and/or clearing of the medium around active colonies (Fig. 1). The obtained results indicated that the Lac.VFE-04, Lac.VFE-08, and Lac.VFE-14 were able to deconjugate GDC.

*Figure 1. BSH plate activity by Lactobacillus on solid MRS medium. Plates were incubated anaerobically for 48 h at 37ºC. (a) MRS; (b) MRS containing 5mM GC; (c) 5 mM GDC; (1) Lac.VFE-04; (2) Lac.VFE-08; (3) Lac.VFE-09; (4) Lac.VFE-11; (5) Lac.VFE-14.*

Figure 1 shows the results of positive and negative hydrolysis on solid plates of MRS medium supplemented with bile salts by five strains of Lactobacillus. As we can see on the Table 3, three strains Lac.VFE-04, Lac.VFE-08, and Lac.VFE-14 were able to hydrolyze GDC, whereas this activity of Lac.VFE-09 and Lac.VFE-11 was not stable.

Lim et al., observed that only 12.8% of Lactobacillus strains isolated from faeces of healthy humans had BSH activity (Lim et al., 2004). Similar to our findings, in a study
conducted by Rani et al., 2017, the researchers tested the ability of BSH from \textit{L. gasseri} FR4 to GC, GDC, TC, and TDC. BSH of \textit{L. gasseri} FR4 showed greater hydrolysis capability towards glycine-conjugated bile salts (GC and GDC) than to taurine-conjugated bile salts (TC and TDC) (Rani, Anandharaj et al., 2017). The highest BSH activity was observed with the GDC substrate.

In contrast with our findings, the investigation conducted by Belicová in 2013 indicated that 11 \textit{Lactobacillus} strains that were isolated from Slovak Bryndza cheese could grow in the presence of TC, TDC, GC, and GDC, but not all of them were able to deconjugate bile salts. Among them, \textit{L. plantarum} CK06, B01, K21, and ZS15 strains showed deconjugation activity on GC, TC, and TDC, but there were no strains that demonstrated deconjugation activity on GDC (Hassanzadazar et al., 2012).

In another study, González-Vázquez et al. investigated the hydrolysis of GC, GDC, TC, and TDC by \textit{L. casei} J57 isolated from Pulque and \textit{L. casei} Shirotai. They reported that \textit{L. casei} J57 showed bile salt hydrolase activity with all bile salts at different concentrations expressed as unit/mg of protein. With GC, the value was 44.91, whereas with GDC, TC, and TDC, the values were 45.27, 671.72, and 61.57, respectively. However, \textit{L. casei} Shirotai did not show any activity in glycine conjugates, while its activity in the TC was 1046.15 and TDC was 264.69 unit/mg of protein (Zhang et al., 2009).

Table 3. The bile salt hydrolase activity on different substrates of selected \textit{Lactobacillus} strains.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Bile salt deconjugating assays</th>
<th>GC (5mM)</th>
<th>GDC (5mM)</th>
<th>TC (5mM)</th>
<th>TDC (5mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lac.VFE-04</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lac.VFE-08</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lac.VFE-09</td>
<td>-</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lac.VFE-11</td>
<td>-</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lac.VFE-14</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Hydrolase activity and glycine

Deconjugation is catalyzed by bile salt hydrolases, which hydrolyze the amide bond and liberate the glycine/taurine moiety from the steroid core of the bile salts. The resulting free bile acids or deconjugated bile acids are excreted via the feces.

Table 4. The bile salt hydrolase activity and glycine.

<table>
<thead>
<tr>
<th>Strain</th>
<th>GDC (5 mM)</th>
<th>Glycine (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lac.VFE-04</td>
<td>+</td>
<td>1.1</td>
</tr>
<tr>
<td>Lac.VFE-08</td>
<td>+</td>
<td>0.6</td>
</tr>
<tr>
<td>Lac.VFE-14</td>
<td>+</td>
<td>0.9</td>
</tr>
</tbody>
</table>

In MRS broth, BSH catalyzes the hydrolysis of glycine-conjugated bile acids into the glycine amino acid residue and the free bile acid. BSH activity in a cell-free extract of each strain Lac.VFE-04, Lac.VFE-08, and Lac.VFE-14 was determined by measuring the amount of amino acids resulting from the hydrolysis of the amide bond of bile salts. Using HPLC assay, most results were consistent with those of the plate assay (Table 4). The plate assay showed the BSH-positive activity in the presence of 5 mM GDC, and as the culture spotted on the plates was more concentrated, the precipitated halo zones were observed. Moreover, BSH activity in the supernatant could be further investigated.

Bile salt hydrolase genes

The presence and genetic organization of \textit{bsh} genes in \textit{Lactobacillus} are highly variable. In some species, only a single \textit{bsh} gene has been reported (Zhang et al., 2009; Kumar et al., 2013),
while in *L. plantarum* WCFS1, *L. acidophilus* NCFM, *L. johnsonii* NCC533, and *L. gasseri* ATCC 33323, multiple *bsh* genes have been recorded and characterized (Lambert et al., 2008; Zhang et al., 2009, Kumar et al., 2013). In our study, four predicted bile salt hydrolase genes (*bsh1*, *bsh2*, *bsh3*, and *bsh4*) were found in Lac.VFE-04, Lac.VFE-08, and Lac.VFE-14 but they could only deconjugate GDC (Fig. 2).

In some *Lactobacillus*, function of each *bsh1*, *bsh2*, *bsh3*, and *bsh4* in deconjugation activity have been investigated by overexpression. For example, Ren *et al.* conducted a study in 2011 researching 4 *bsh* genes in *L. plantarum* ST-III. Their results showed that the proteins expressed from the *bsh1*, *bsh2*, *bsh3*, and *bsh4* genes all reacted with the substrates tested, which included TC, GC, TDC, GDC, taurochenodeoxycholic acid (TCDC), and glycochenodeoxycholic acid (GCDC). Specifically, they found that BSH1 showed much greater hydrolysis activity than the other three proteins on GDC (Ren *et al.*, 2011). The author group suggests that all 4 bile salt hydrolases may be responsible for the bile salt hydrolysis activity in *L. plantarum* ST-III (Lambert *et al.*, 2008).

In *L. plantarum* WCFS1, the functionality of four predicted *bsh1*, *bsh2*, *bsh3*, and *bsh4* genes was explored by overexpression. The obtained results indicated that, BSH1 was shown to be responsible for the majority of BSH activity of *L. plantarum* WCFS1. In addition, *bsh1* of *L. plantarum* WCFS1 was shown to be involved in conferring tolerance specifically GDC, but not with TDC (Lambert *et al.*, 2008).

In another study, genes coding for bile salt hydrolase of *L. plantarum* CGMCC 8198 were identified, analyzed and cloned. Using RT-PCR analysis, only *bsh2*, *bsh3* and *bsh4* genes could be cloned from *L. plantarum* CGMCC 8198, and they all had high similarity with *L. plantarum* WCFS1 and ST-III. The three recombinant BSHs had similar activities for the hydrolysis of TDCA, while the hydrolyzing activity of BSH2 against GDC was 2.3-fold and 5.5-fold greater than those of BSH3 and BSH4, respectively. Therefore, BSH2 might play a major role in the bile salt hydrolysis activity in *L. plantarum* CGMCC 8198 (Gu *et al.*, 2014).

![Figure 2](image.png)

Each of the *bsh1*-deficient derivatives displayed GDC-mediated growth inhibition at concentrations as low as 0.1% (wt/vol) GDC. In contrast, strains containing an intact *bsh1* gene...
were capable of sustaining normal growth characteristics at 0.5% to 0.7% (wt/vol) GDC. Analogous with the limited level of hydrolytic activity relating to bile salts, bsh2, bsh3, and bsh4 did not appear to contribute significantly to tolerance of GDC (Zhang et al., 2009; Kumar et al., 2013).

*L. plantarum* appeared to be remarkably more sensitive to GDC, with obvious differences visible between the strains. The results clearly established that the presence of all bsh1, bsh2, bsh3, and bsh4 in *L. plantarum* VFE-04 and *L. plantarum* VFE-14 enhances GDC bile salt deconjugate. Moreover, in our study, besides these *L. plantarum*, *L. rhamnosus* VFE-08 was also able to deconjugate GDC.

In next studies, overexpression of BSH1, BSH2, BSH3, and BSH4 of *L. plantarum* VFE-04, *L. rhamnosus* VFE-08 and *L. plantarum* VFE-14 will be carried out to verify which *bsh* homologs are responsible for GDC deconjugation of these bacteria.

**CONCLUSION**

Intestinal microbes in the small intestine are capable of deconjugation of glycine or taurine from bile salts using BSH. The obtained characteristics of *L. plantarum* VFE-04, *L. rhamnosus* VFE-08, and *L. plantarum* VFE-14 indicated that these bacteria might be excellent candidates to improve the health of patients suffering from hypercholesterolemia.

**Conflicts of Interest:** The authors declare that they have no conflicts of interest.

**Acknowledgments:** This work was funded by the Vietnam Academy of Science and Technology (Grant no. CT0000.03/18-19)

**REFERENCES**


Xuan Thach Tran et al.

534
Investigation of tolerance of Lactobacillus casei to the presence of acids, bile salts and deconjugation of bile salts. Int J Curr Microbiol 7, 600-612.


Thu HT, Thach TX, Hoa NT, Hien VT, Duy ND, Quyen DV, Nhung NTT (2020) H$_2$O$_2$ production in Lactobacillus strains isolated from the intestinal microbiome of healthy people. *Academia J Biol* 42(1).


