Heliyon 8 (2022) e12272

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Distribution, cholesterol-lowering and immunomodulation effects of lactic acid bacteria from fermented mussel (*Hoi-dong*)

Engkarat Kingkaew [a], Hiroshi Konno [b], Yoshihito Hosaka [b], Wongsakorn Phongsopitanun [a], Somboon Tanasupawat [a,*]

[a] *Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand*
[b] *Akita Konno CO., LTD., 248 Aza Kariwano, Daisen-shi, Akita 019-2112, Japan*

HIGHLIGHTS

- Distribution of lactic acid bacterial species in Thai fermented mussels was investigated.
- Seven *Lactiplantibacillus plantarum* subsp. *plantarum* isolates exhibited bile salt hydrolase activity.
- *Lacticaseibacillus rhamnosus* LM1-1 and *Enterococcus thailandicus* LM4-1 were shown to have high levels of cholesterol assimilation.
- Heat-killed LAB cells of isolates exhibited the immunomodulation effect to levels of IL-12, IFN-γ, hBD-2, and NO production.
- Thai traditional fermented mussel is an attractive source of potential probiotics.

ARTICLE INFO

Keywords:
Lactic acid bacteria (LAB)
Thai fermented mussel
Cholesterol-lowering effects
Immunomodulation
Probiotics

ABSTRACT

Forty-eight lactic acid bacteria (LAB) isolated from fermented mussels in Thailand were evaluated for their probiotic properties, bile salt hydrolase (BSH), cholesterol assimilation and immunomodulatory effects. They were identified as *Companilactobacillus formosensis* (Group I, 10 isolates), *Lentilactobacillus buchneri* (Group II, 8 isolates), *Lactiplantibacillus plantarum* subsp. *plantarum* (Group III, 16 isolates), *Lacticaseibacillus rhamnosus* (Group IV, 1 isolate), *Pediococcus pentosaceus* (Group V, 5 isolates) and *P. acidilactici* (Group V, 1 isolate), *Enterococcus thailandicus* (Group VI, 2 isolates), *En. hirae* (Group VII, 1 isolate), *En. durans* (Group VI, 1 isolate), *Lactococcus lactis* subsp. *lactis* (Group VII, 1 isolate), *Lc. lactis* subsp. *hordinae* (Group VII, 1 isolate), and *Leuconostoc lactis* (Group VIII, 1 isolate), based on their phenotypic and genetic characteristics. Seven isolates, *L. plantarum* subsp. *plantarum* LM6-1, LM6-2, LM7-2-2B, LM12-1, LM14-1, LM15-1P and LM15-2 expressed bile salt hydrolase activity. All isolates assimilated cholesterol ranging from 20.73 to 79.40%. BSH-producing isolates were tolerant to acidic and bile conditions and showed the adhesion ability to Caco-2 cells. The BSH-producing and selected isolates showed the immunomodulatory effects to stimulate interleukin-12 (IL-12), interferon-gamma (IFN-γ), human beta defensin-2 (hBD-2) and nitric oxide (NO) production at various levels. Therefore, these results indicated that the isolates meet the standard probiotic criteria and beneficial effects.

1. Introduction

Hoi-dong is a traditional low-salt fermented green mussel meat produced organically from *Perna viridis* (*Hoi-ma-laeng-poo*). It has a dark orange semi-solid appearance with a sour and salty flavor (Figure 1) (Phithakpol et al., 1995; Tanasupawat and Komagata, 1995). *L. pentosus*, *L. plantarum*, and *Tetragenococcus halophilus* isolates were found in the products (Tanasupawat and Daengsubha, 1983; Tanasupawat and Komagata, 1995). Lactic acid bacteria (LAB) play an essential role in fermentation, resulting in improved taste, aroma, and texture. In addition, they could be used in food preservation. They are also used as probiotics in several Asian fermented foods (Ngasotter et al., 2020). LAB are classified as normally regarded as safe (GRAS) (FAO/WHO, 2002). Presently, various investigations support the beneficial significance of probiotics as a functional food with cholesterol-lowering, and immunomodulatory effects (Albano et al., 2018; Domingos-Lopes et al., 2020; Hameed et al., 2022).

* Corresponding author.
E-mail address: Somboon.T@Chula.ac.th (S. Tanasupawat).

https://doi.org/10.1016/j.heliyon.2022.e12272
Received 18 April 2022; Received in revised form 22 June 2022; Accepted 2 December 2022

Figure 1. Fermented mussel (*Hoi-dong*).

Probiotics have various health effects, such as the ingestion of LAB alleviating certain risk factors for coronary disease (CAD) (De Vries et al., 2006). According to Albano et al. (2018), even at 1% decrease in blood cholesterol can reduce the CAD risk. Furthermore, LAB are presently still interesting since they modulate immunity and immune-boosting effect and are used in special disorders such as immunodeficiency and autoimmune diseases (Thamacharoensuk et al., 2017; Iwabuchi et al., 2012). Interleukin-12 (IL-12) is a pro-inflammatory cytokine associated in limiting of infection, cancer, as well as the induction of IFN-γ production (Thamacharoensuk et al., 2017). IFN-γ has a role in the prevention of intracellular pathogen infection. There have been numerous investigations of LAB-stimulating IL-12 and IFN-γ secretion (Chen et al., 2013; Moon et al., 2019; Nakai et al., 2019; Thamacharoensuk et al., 2017). Moreover, human beta defensin-2 (hBD-2) is a human antimicrobial peptide that serves vital functions in host defense and is induced by inflammation or infection. Several LAB isolates have been shown to stimulate BD expression, hence enhancing BD expression could prevent infections (Kobatake and Kabuki, 2019). In addition, nitric oxide (NO) plays an essential function in infection defense and immunomodulatory effects (Wang et al., 2009) and there have been numerous investigations into LAB-induced NO production (Kmonickova et al., 2012; Surayot et al., 2014).

Fermented food products might be a source of novel LAB isolates with probiotic potential. Studies on the bioactive properties of LAB isolated from fermented mussels are scarce to none. Currently, the study of Nanasombat et al. (2012) and Boonprab (2022) reported the biological activity and the using of LAB starter. The purpose of this study is to determine the distribution of LAB from Thai fermented mussel (*Hoi-dong*) and screen their bile salt hydrolase activity, cholesterol assimilation capacity, immunomodulatory effects as well as related probiotic properties, *in vitro*.

2. Materials and methods

2.1. Raw material and isolation of LAB

Eighteen fermented mussel (*Hoi-dong*) samples were gathered from Samut Prakarn (13°35′37.2″N 100°35′46.6″E), Bangkok (13°44′35.1″N 100°30′15.3″E), Rayong (12°37′59.5″N 101°28′40.5″E), Samut Songkhram (13°25′30.9″N 99°57′17.8″E), Samut Sakhon (13°30′52.6″N 100°23′07.5″E), Nakhon Pathom (13°49′02.7″N 100°03′28.6″E) and Chonburi provinces (13°20′19.0″N 100°55′20.2″E) (Table 1). For each sample, 10 g was homogenized to 90 mL MRS broth (Difco) as well as incubated at 30 °C for 72 h (De Man et al., 1960). After incubation period, one loopful was streaked on MRS agar with 0.3% (w/v) $CaCO_3$ and incubated under the same conditions. The colonies with clear zone were picked up for purification. Pure cultures were strored at −20 °C in 40% (v/v) glycerol and lyophilized with 10% (w/v) skim milk.

2.2. Identification methods

2.2.1. Phenotypic characterization

Colony appearance, cell shape, cell arrangement, and Gram staining were determined after cultivation on MRS agar plate incubated at 30 °C for 48 h. Physiological and biochemical characteristics including propagation at increasing NaCl concentration (4%, 6%, and 8%), temperature (15 °C, 30 °C, and 45 °C), and pH (3.0, 6.0 and 9.0), catalase activity, nitrate reduction, gas generation, hydrolysis of aesculin and arginine, and acid formation from carbohydrates were determined as described by Tanasupawat et al. (1998). Hierarchical cluster analysis based on the phenotypic characteristics was performed using SPSS v22.

Table 1. Isolate number, group, nearest relatives, 16S rRNA gene sequence similarity (%) of the representative isolates.

Isolate no.	Group: Nearest relatives	Similarity (%)	Length (bp)	Accession no.	Cholesterol assimilation ability (%)	BSH activity
LM15-2A	I: *Companilactobacillus formosensis* S215^T	99.86	1,354	LC547212	62.07 ± 3.06	–
LM16-2		99.71	1,358	LC547232	30.73 ± 4.16	–
LM10-2M		–	–	–	60.73 ± 4.62	–
LM15-2B		99.85	1,354	LC546812	49.40 ± 3.46	–
LM18-3		99.85	1,367	LC547231	57.40 ± 7.21	–
LM7-1SP		99.93	1,352	LC546814	59.40 ± 7.21	–
LM7-2S		99.79	1,411	LC702436	64.07 ± 6.43	–
LM10-3M		100	1,367	LC546813	32.07 ± 3.06	–
LM10-1M		99.64	1,375	LC702435	74.07 ± 5.03	–
LM15-3		99.56	1,378	LC547229	64.73 ± 3.06	–
LM17-6	II: *Lentilactobacillus buchneri* JCM 1115^T	99.93	1,382	LC702433	52.07 ± 2.31	–
LM17-7		–	–	–	51.40 ± 6.00	–
LM17-2		–	–	–	42.07 ± 1.15	–
LM17-5		–	–	–	45.40 ± 5.29	–
LM17-4		99.93	1,375	LC547234	36.73 ± 2.31	–
LMK9-3		99.81	1,394	LC547223	28.07 ± 6.11	–
LM7-3		–	–	–	38.73 ± 11.37	–
LM18-4		99.93	1,359	LC702434	50.07 ± 6.11	–
LM16-1	III: *Lactiplantibacillus plantarum* subsp. *plantarum* ATCC 14917^T	100	1,384	LC546818	68.73 ± 2.31	–
LM6-1		100	1,375	LC546819	67.40 ± 3.46	+
LM7-2-2B		99.78	1,337	LC546820	67.40 ± 2.00	+
LM15-1P		99.93	1,370	LC546815	67.40 ± 8.72	+
LM6-2		99.93	1,389	LC547211	54.07 ± 11.37	+
LM14-1		99.85	1,345	LC547215	46.73 ± 4.16	+
LM15-2		100	1,387	LC546816	67.40 ± 6.93	+
LM12-1		100	1,384	LC547230	40.07 ± 8.08	+
LM18-2		100	1,384	LC547227	49.40 ± 5.29	–
LMK11-2		100	1,335	LC547222	55.40 ± 6.00	–
LM12-2		–	–	–	38.07 ± 2.31	–
LM2-3		–	–	–	38.07 ± 6.43	–
LM3-2		–	–	–	45.40 ± 8.00	–
LM3-1		100	1,379	LC702437	72.73 ± 4.62	–
LM16-3		–	–	–	34.07 ± 3.06	–
LMK11-3		–	–	–	37.40 ± 9.17	–
LM1-1	IV: *Lacticaseibacillus rhamnosus* JCM 1136^T	100	1,342	LC546811	77.40 ± 2.00	–
LMK9-1	V: *P. pentosaceus* DSM 20336^T	100	1,368	LC547220	34.07 ± 10.26	–
LM13-1		99.86	1,404	LC547226	54.73 ± 1.15	–
LM17-3		99.93	1,450	LC547228	52.73 ± 2.31	–
LM13-3		99.93	1,362	LC702898	40.07 ± 9.87	–
LM5-2		–	–	–	36.07 ± 8.33	–
LM5-1	V: *P. acidilactici* DSM 20284^T	99.93	1,355	LC547214	62.07 ± 6.43	–
LM4-1	VI: *En. thailandicus* DSM 21767^T	100	1,326	LC546817	79.40 ± 4.00	–
LM4-2		100	1,369	LC547218	20.73 ± 11.02	–
LM1-2	VI: *En. hirae* ATCC 9790^T	100	1,373	LC547213	68.07 ± 1.15	–
LM2-1	VI: *En. durans* NBRC 100479^T	99.63	1,335	LC547217	48.07 ± 4.16	–
LM2-2	VII: *Lc. lactis* subsp. *lactis* JCM 5805^T	100	1,385	LC547219	42.73 ± 10.26	–
LM8-2	VII: *Lc. lactis* subsp. *hordniae* NBRC 100931^T	99.93	1,378	LC547224	68.73 ± 8.08	–
LMK9-2L	VIII: *Leuconostoc lactis* JCM 6123^T	99.71	1,357	LC547233	26.73 ± 4.16	–

Sample LM1 and LM2 are collected from Samut Prakarn; LM3, LM4, LM16, LM17 and LM18 from Bangkok; LM5 and LM6 from Rayong; LM7, LM8 and LM9 from Samut Songkhram; LM10, LMK11 are collected from Samut Sakhon; LM12 and LM13 from Nakhon Pathom; and LM14 and LM15 are from Chonburi.

2.2.2. Genotypic characterization

The 16S rRNA gene sequences of isolates were PCR amplified (Phuengjayaem et al., 2017) and analyzed using a DNA sequencer (at Microgen, Inc.) with universal primers (Lane 1991). On the EzBiocloud system, the sequence similarity values between the isolates and associated reference isolates were computed (Yoon et al., 2017). A phylogenetic tree based on the neighbor-joining (NJ) method (Saitou and Nei, 1987) was constructed using MEGA 7 (Kumar et al., 2016). The confidence values of each branch in the phylogenetic tree were computed using a bootstrap analysis with 1000 replications (Felsenstein, 1985). The identified sequences were submitted into DDBJ (DNA Data Bank of Japan).

2.3. Bile salt hydrolases (BSH) activity

The BSH activity was determined as informed by Shehata et al. (2016). A portion (20 μL) of the overnight culture broth was spotted on MRS agar containing 0.5% (w/v) taurodeoxycholic acid (TDCA) (sodium salt hydrate) as well as 0.037% (w/v) calcium chloride ($CaCl_2$). Plates were incubated at 37 °C for 72 h under anaerobic condition. Precipitated zone around colonies or white opaque colonies indicated bile salt hydrolase activity. As the negative control, the MRS was used. The BSH-producing LAB isolate was selected for evaluation of probiotic properties.

2.4. Cholesterol assimilation

MRS broth containing cholesterol-polyethylene glycol (PEG) 600 (Sigma, India) (final concentration 100 μg/ml) was used to determined cholesterol assimilation capability. Each loopful (1%, v/v) was seeded into MRS containing cholesterol-PEG 600 and incubated anaerobically at 37 °C for 24 h. The cholesterol was isolated following the method of Tomaro-Duchesneau et al. (2014). The residual quantity of cholesterol was determined using modified procedure of Rudel and Morris (1973). A standard curve was generated using the following cholesterol concentrations: 0, 3.125, 6.25, 12.5, 25, 50, 75, 100, as well as 125 μg/ml in MRS. The amount of cholesterol was read off a standard curve. The capability was reported as the cholesterol assimilated (%). The percentage of cholesterol assimilated was quantify using the following Eq. (1):

$$\text{Cholesterol assimilated } (\mu g/\text{ml}) = [\text{Cholesterol } (\mu g/\text{ml})]_{0\ \text{h}} - [\text{Cholesterol } (\mu g/\text{ml})]_{24\ \text{h}}$$

$$\%\ \text{Cholesterol assimilated} = \left[\frac{\text{Cholesterol assimilated } (\mu g/\text{ml})}{\text{Cholesterol } (\mu g/\text{ml})_{0\ \text{h}}}\right] \times 100 \quad (1)$$

2.5. Evaluation of probiotic properties

2.5.1. LAB cell suspension

According to observation of Pithva et al. (2014), cell suspension was prepared. The selected isolates were propagated in MRS broth at 30 °C for 24 h. After incubation period, the cells were collected by centrifugation at 14,000 rpm for 10 min at 4 °C, washed twice with phosphate-buffered saline (PBS; 0.1 M, pH 7.2, containing 0.85% (w/v) NaCl), and solubilized in phosphate buffer (0.1 M, pH 7) to obtain bacterial suspension of $A_{600} = 1$ and 10^9 CFU/ml.

2.5.2. Acid and bile tolerance

The acid and bile tolerance were observed by the modified observation of Thamacharoensuk et al. (2017). In brief, the cell suspension was inoculated into MRS broth (pH 2 and pH 3) or MRS broth containing 0.3% and 0.8% (w/v) bile salt and incubated at 37 °C for 3 h. The viable cells were enumerated by a 10-fold serial dilution, spot plate technique as well as incubated at 37 °C for 24 h. The viable cells were reported as log CFU/ml.

2.5.3. Adhesion assay

The adhesion capacity was determined using Caco-2 cells following the investigation of Han et al. (2017) with modification. Caco-2 cells were provided by Professor Shinichi Yokota, Sapporo Medical University School of Medicine. Caco-2 cells were routinely proliferated in Dulbecco modified Eagle Minimum Essential Medium (DMEM) containing 10% (v/v) fetal bovine serum (FBS), and 1% (v/v) penicillin-streptomycin (PS) at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. The Caco-2 cells (5×10^5 cell/ml) was inoculated and incubated at 37 °C in 5% CO_2. PBS was used to wash Caco-2 cells and the cell suspension was centrifuged at 14,000 rpm for 5 min at 4 °C and solubilized again in DMEM containing no antibiotics. Each LAB cell suspension was added as well as incubated for 90 min at 37 °C in 5% CO_2 atmosphere. Following incubation, Caco-2 cells were cleansed by PBS. 0.05% of Triton-X100 solution was used to lyse the cells. The adherence cells were counted by spot-plate technique on MRS agar as well as incubated at 37 °C for 48 h. As control, the *Lacticaseibacillus rhamnosus* GG was used. The adhesion capability of selected isolates was evaluated using the following Eq. (2):

$$\text{Adhesion percentage } (\%) = \frac{N_t}{N_0} \times 100 \quad (2)$$

where; N_t = the quantity of adherent LAB cells to the Caco-2 cells, N_0 = the sum of LAB cell inoculated.

2.5.4. The immunomodulatory effects

The selected isolates were prepared and evaluated for immunomodulatory effects following the method of Hosaka et al. (2021).

2.5.4.1. Preparation of sterilized lactic acid bacteria powder. Each isolate was propagated in MRS broth medium (Difco) as well as incubated with shaking (120 rpm) at 30 °C for 24 h. The LAB pellet was centrifuged at 1,000 rpm for 10 min. Cells were rinsed with distilled water and then lyophilized to obtain LAB powder. Test sample was solubilized in PBS at 200 μg/ml.

2.5.4.2. Cell culture. RAW264.7 cells were proliferated in DMEM (Sigma) containing 5% FBS (Biological Industries) as well as 0.2% PS (Gibco) in a 5% CO_2 incubator at 37 °C. Caco-2 cells were provided by Professor Shinichi Yokota, Sapporo Medical University School of Medicine. Cultures were propagated in DMEM (Sigma) containing 5% FBS as well as 0.25% PS in a 5% CO_2 incubator at 37 °C. THP-1 cells were cultivated in RPMI 1640 medium (Nacalai Tesque Inc., Japan) containing 10% FBS as well as 0.2% PS in a 5% CO_2 incubator at 37 °C.

Caco-2 cells (1.5×10^5 cells) were innoculated on cell culture inserts (Falcon, 24-Well Hanging Inserts 0.4 μm) and cultured for 3 days. The media supplemented with 5 mM sodium butyrate was substituted as well as incubated for 4 days to trigger differentiation. Transepithelial electrical resistance (TEER) using Millicell-ERS (Merk) was applied to evaluate differentiated cells, and differentiated cells (>400 Ωxcm^2) were used. THP-1 cells were innoculated on a multi-well plate (24 well, Falcon) as well as incubated for 3 days in media containing cholecalciferol (Vitamin D_3; 100 ng/ml) and phorbol12-myristate13-acetate (PMA; 10 nM) to differentiate into macrophage-like cells. After differentiation, Caco-2 and THP-1 cells were co-cultured in Transwell.

2.5.4.3. Production of Nitric oxide (NO). NO production was determined as reported by Yang et al. (2018). RAW264.7 cells were solubilized in DMEM medium (5% FBS + 0.2% PS) at a concentration of 3×10^5 cells/ml, innoulcated in each 24-well multi-well plate and incubated in a 5% CO_2 incubator at 37 °C for 24 h. The test sample was added to stimulate the cells (20 μg/ml; final conc.). The negative control was PBS, while the positive control was lipopolysaccharide (LPS) (10 g/ml) (Fujifilm Wako). Following activation, the medium was harvested, centrifuged at 12,000 rpm for 20 min and evaluated by Griess reaction, as reported by Baek et al. (2015). A portion of each Griess reagent, medium supernatant sample, and 3.125–125 μg/ml sodium nitrite ($NaNO_3$) standard solution was supplemented and incubated for 20 min. The absorbance at 550 nm was used as well as the nitrite concentration was quantify by standard curve.

2.5.4.4. Intestinal immunity model. Co-culture cell culture inserts (apical side) and multi-well plates (basal side) were used to simulate an intestinal immune model. Test sample dissolved in RPMI 1640 medium was seeded to the apical side (final concentration 20 μg/ml), as well as the cells were triggered in a 5% CO_2 incubator at 37 °C for 48 h. Following

incubation, the basal side of the medium was collected, and centrifugated at 12,000 rpm for 20 min, the supernatant was harvested to remove foreign substances. For IL-12 and IFN-γ, proteins were precipitated by applying a 25% volume of 100% trichloroacetic acid (TCA) to the supernatant sample. After a 2-minute heat treatment at 100 °C, the precipitates were cleaned with acetone to remove TCA and solubilized in 1× sample buffer for enrichment.

SDS-PAGE was used to isolate the protein following the procedure of Laemmli (1970). According to Towbin et al. (1979), the target proteins were observed via Western blot. Standard curves were constructed using

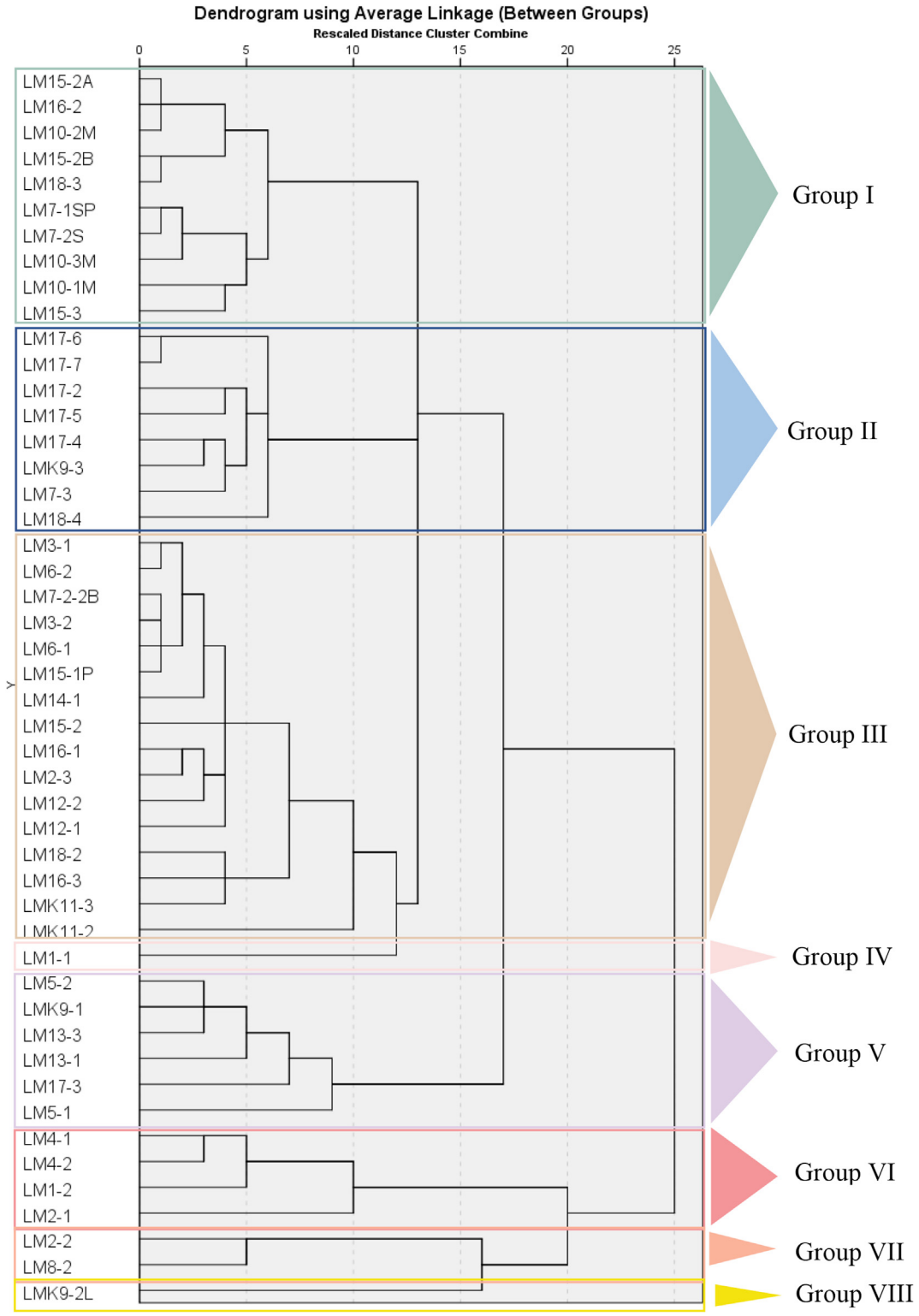

Figure 2. Dendrogram of the hierarchical cluster based on phenotypic characteristics.

IFN-γ (Gibco) as well as IL-12 (Gibco) standard to quantify the IFN-γ and IL-12 production. As an endogenous control, production was adjusted by measuring β-actin. For hBD-2, unenriched supernatant was quantified by the Dot blot, as well as the hBD-2 production was adjusted from the total protein by CBB staining. The values were determined relative to PBS (non-stimulation).

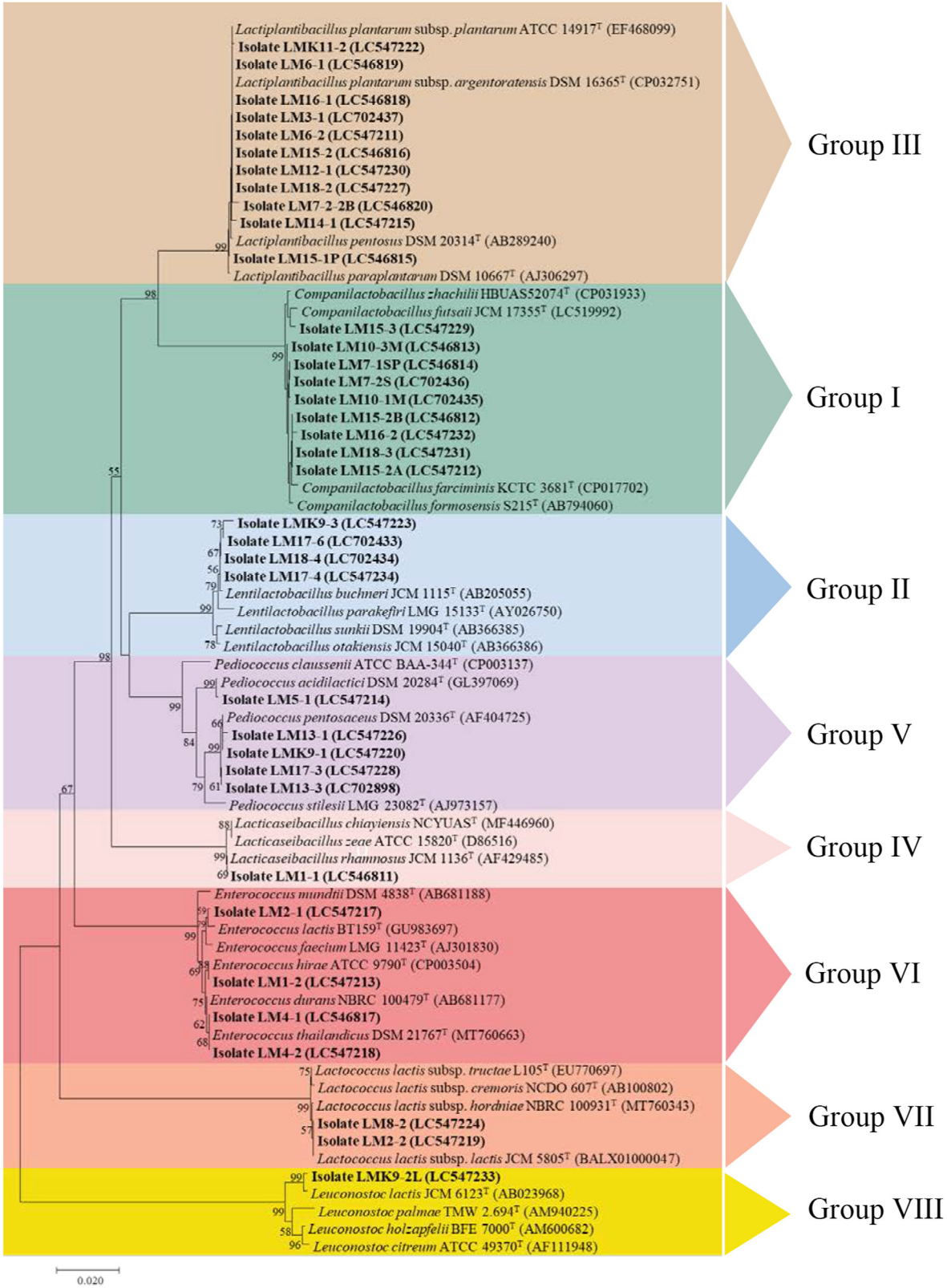

Figure 3. Neighbor-joining tree based on 16S rRNA gene of the representative isolates from each group.

2.6. Statistical analysis

All tests were carried out in triplicate. Observations were reported as the mean ± standard deviation (SD). The acid and bile tolerance results and adhesion results were analyzed by one-way analysis of variance (ANOVA) using SPSS v 22.0 software. For comparison, Duncan's Multiple Range Test (DMRT) was used for mean values at a significant level of $p < 0.05$. The immunomodulatory effect was analyzed by Welch's t-test at a significant level of $p < 0.05$.

3. Results and discussion

3.1. Identification of isolates

Forty-eight LAB isolates were isolated from Thai fermented mussel (*Hoi-dong*) samples from various provinces (Table 1). All isolates were Gram-positive, catalase-negative, and facultatively anaerobic belonging to the members of genera *Companilactobacillus*, *Lentilactobacillus*, *Lactiplantibacillus*, *Lacticaseibacillus*, *Pediococcus*, *Enterococcus*, *Lactococcus* and *Leuconostoc*; they did not reduce nitrate. They were divided into 8 Groups when the hierarchical cluster was analyzed based on their phenotypic characteristics, and the 16S rRNA gene sequence similarity of the representative isolates was determined (Figures 2 and 3 and Table 1).

Group I included ten rod-shaped isolates (LM15-2A, LM16-2, LM10-2M, LM15-2B, LM18-3, LM7-1SP, LM7-2S, LM10-3M, LM10-1M and LM15-3). They produced no gas from glucose. They proliferated at pH 3, in 8% NaCl, at 15 °C and 45 °C but they could not proliferate at pH 9.0. They hydrolyzed arginine, produced D-lactic acid and contained *meso*-DAP in the cell wall. However, they synthesized no acid from arabinose, cellobiose, lactose, mannitol, melibiose, raffinose, rhamnose as well as sorbitol. The representative isolates in this group showed 99.56%–100% 16S rRNA gene sequence similarity (Table 1) to *Companilactobacillus formosensis* S215^T (Figure 2). Therefore, they were identified as *Companilactobacillus formosensis* (Zheng et al., 2020). Their variable phenotypic characteristics are presented in Table 2.

Group II included eight rod-shaped isolates (LM17-6, LM17-7, LM17-2, LM17-5, LM17-4, LMK9-3, LM7-3, and LM18-4). They synthesized gas from glucose. They propagated at pH 3 and 9, 15 °C as well as 45 °C and in 8% NaCl. The isolates had no *meso*-DAP in the cell wall. DL-lactic acid was produced. All isolates produced no acid from lactose, mannose, rhamnose and salicin. They were able to hydrolyze arginine. The representative isolates in this cluster showed 99.81%–99.93% 16S rRNA gene sequence similarity (Table 1) to *Lentilactobacillus buchneri* JCM 1115^T (Figure 2). Therefore, they were identified as *Lentilactobacillus buchneri* (Zheng et al., 2020). Their variable phenotypic characteristics are illustrated in Table 2.

Group III consisted of sixteen rod-shaped isolates (LM16-1, LM6-1, LM7-2-2B, LM15-1P, LM6-2, LM14-1, LM15-2, LM12-1, LM18-2, LMK11-2, LM12-2, LM2-3, LM3-2, LM3-1, LM16-3, LMK11-3). They did not produce no gas from glucose. They proliferated at pH 3 and in 8% NaCl. The isolates contained *meso*-DAP in the cell wall. DL-lactic acid was synthesized. The representative isolates in this cluster presented

Table 2. Phenotypic characteristics of isolates.

Characteristics	I	II	III	IV	V	VI	VII	VIII
No. of isolate	10	8	16	1	6	4	2	1
Cell shape	Rods	Rods	Rods	Rods	Tetracocci	Cocci in chains	Cocci in chains	Cocci in chains
Gas from glucose	−	+	−	−	−	−	−	+
Growth in 6% NaCl	+	+	+	+	+	+	+	+
Growth in 8% NaCl	+	+	+	+	+	+	−	−
Growth at pH 3	+	+	+	+	+	+	+	−
pH 9	−	+	+ (−5)	−	−	+	+	+
Growth at 15 °C	+	+	+	+	+	+	+	+
45 °C	+	+	+ (−1)	+	− (+1)	+	−	+
Arginine hydrolysis	+	+	+ (−4)	+	− (+1)	+	+	−
Acid from:								
L-Arabinose	−	+	+	+	+	−	+	+
D-Cellobiose	−	− (+1)	+	+	+	+	w1	−
Fructose	+	+	+	+	+	+	+	+
D-Galactose	+	+	+	+	+	+ (−1)	+	+
D-Glucose	+	+	+	+	+	+	+	+
Lactose	−	−	+	+	+ (−1)	+	+	+
D-Mannose	+	−	+	+	+	+	+	+
D-Maltose	w5	+	+	+	+ (−1)	+	+	+
D-Mannitol	−	− (+1)	+ (−1)	+	− (+1)	+ (−1)	+	−
D-Melibiose	−	+	+ (−1)	+	w3	+ (−1)	w1	+
D-Raffinose	−	w4	+ (−1)	+	w3	+ (−1)	−	+
L-Rhamnose	−	−	+ (−1)	+	− (+2)	w2	−	−
D-Ribose	+	+	+	+	+	+	+	+
Salicin	+	−	+	+	+	+ (−1)	+	+
D-Sorbitol	−	− (+1)	+ (−2)	+	− (+1)	+ (−1)	−	−
D-Sucrose	+	+ (−3)	+	+	+ (−1)	+ (−1)	w1	+
D-Trehalose	+ (−4)	+	+	+	+	+ (−1)	+	−
D-Xylose	+ (−2)	+	+	+	+	+ (−1)	+	+
Aesculin	+	+	+ (−2)	−	+	+ (−1)	−	−
meso-DAP	+	−	+	−	−	−	−	−
Isomer of lactic acid	D	DL	DL	L	DL	L	L	D

+, positive reaction; w, weak reaction; −, negative reaction. Numbers in parentheses indicate the number of isolates showing the reaction.

99.78%–100% 16S rRNA gene sequence similarity (Table 1) to *Lactiplantibacillus plantarum* subsp. *plantarum* ATCC 14917^T (Figure 2). Hence, they were identified as *Lactiplantibacillus plantarum* subsp. *plantarum* (Zheng et al., 2020). Their variable phenotypic characteristics are illustrated in Table 2.

Group IV contained one rod-shaped isolates (LM1-1). It produced no gas from glucose. It propagated at pH 3, 15 °C and 45 °C and in 6% and 8% NaCl but did not propagate at pH 9. The isolate contained no *meso*-DAP in the cell wall. L-lactic acid was generated. It did not synthesized acid from aesculin. It hydrolyzed arginine. The representative isolate in this cluster displayed 100% 16S rRNA gene sequence similarity (Table 1) to *Lacticaseibacillus rhamnosus* JCM 1136^T (Figure 2). Consequently, it was identified as *Lacticaseibacillus rhamnosus* (Zheng et al., 2020).

Group V was comprised of six tetracoccal isolates (LMK9-1, LM13-1, LM17-3, LM13-3, LM5-2, and LM5-1). They produced no gas from glucose. They proliferated at pH 3 and, 15 °C, and in 8% NaCl but did not proliferate at pH 9. DL-lactic acid was produced. The representative isolates in this group included LMK9-1, LM13-1, LM17-3, and LM13-3, which exhibited 99.86%–100% 16S rRNA gene sequence similarity (Table 1) to *Pediococcus pentosaceus* DSM 20336^T (Figure 2), and isolate LM5-1 revealed 99.93% 16S rRNA gene sequence similarity (Table 1) to *Pediococcus acidilactici* DSM 20284^T (Figure 2). Their variable phenotypic characteristics are presented in Table 2.

Group VI contained four coccal isolates (LM4-1, LM4-2, LM1-2 and LM2-1). They could not synthesize gas from glucose. They developed at pH 3 and 9, 15 °C and 45 °C, and in 6% and 8% NaCl. The isolates had no *meso*-DAP in the cell wall. L-lactic acid was produced. All isolates produced no acid from arabinose. Acid production was variably observed in galactose, mannitol, melibiose, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose, xylose and aesculin. They hydrolyzed arginine. The representative isolate LM4-1 and LM4-2 revealed 100% 16S rRNA gene sequence similarity (Table 1) to *Enterococcus thailandicus* DSM 21767^T (Figure 2), isolate LM1-2 exhibited 100% 16S rRNA gene sequence similarity (Table 1) to *Enterococcus hirae* ATCC 9790^T (Figure 2), and LM2-1 exhibited 99.63% 16S rRNA gene sequence similarity (Table 1) to *Enterococcus durans* NBRC 100479^T (Figure 2).

Group VII consisted of two coccal isolates (LM2-2 and LM8-2). They produced no gas from glucose. They proliferated at pH 3 and 9, 15 °C, and in 6% but did not proliferate at 45 °C, pH 9 and in 8% NaCl. L-lactic acid was synthesized. All isolates could not synthesize acid from raffinose, rhamnose, sorbitol and aesculin. They hydrolyzed arginine. The representative isolate LM2-2 expressed 100% 16S rRNA gene sequence similarity (Table 1) to *Lc. lactis* subsp. *lactis* JCM 5805^T (Figure 2), and isolate LM8-2 expressed 99.93% 16S rRNA gene sequence similarity (Table 1) to *Lc. lactis* subsp. *hordniae* NBRC 100931^T (Figure 2) and they were identified as *Lc. lactis*.

Group VIII included one coccal isolates (LMK9-2L). It generated gas from glucose. It propagated at pH 9, 15 °C and 45 °C, and in 6% but did not propagate at pH 3 and in 8% NaCl. The isolate contained no *meso*-DAP in the cell wall. It synthesized D-lactic acid. It generated no acid from cellobiose, mannitol, rhamnose, sorbitol, trehalose and aesculin. It could not hydrolyze arginine. The representative isolate LMK9-2L revealed 99.71% 16S rRNA gene sequence similarity (Table 1) to *Leuconostoc lactis* JCM 6123^T (Figure 2) and was identified as *Leuconostoc lactis*.

3.2. Bile salt hydrolase activity

BSH activity has been regarded as a factor related to the cholesterol-lowering activity, and BSH activity is now often referred as an essential feature for choosing probiotics (Miremadi et al., 2014). BSH activity promotes bacterial growth and colonization in gut by deconjugating bile salts (Begley et al., 2006). Out of 48 isolates, only 7 isolates, LM15-1P, LM15-2, LM6-1, LM7-2-2B, LM6-2, LM14-1, and LM12-1 expressed BSH activity by the development of opaque white colonies (Table 1). These BSH-positive isolates were recognized as *L. plantarum* subsp. *plantarum* (99–100% similarity). Based on the screening, this study is consistent with several earlier publications (Abushelaibi et al., 2017; Liu et al., 2017). The presence of BSH activity help to diminish the cholesterol quantity and make BSH-producing strain endure to bile condition (Noriega et al., 2006). The *in vivo* study of Costabile et al. (2017) found that the ingestion of *L. plantarum* ECGC 13110402 (great BSH-producing strain) twice daily could significantly reduce the cholesterol, and it could also improve the quantity of high-density lipoprotein. Besides, the application of *L. rhamnosus* BFE5264 resulted in a consequential lowering of the serum cholesterol amount in murine model (Park et al., 2018). Furthermore, this work might demonstrate the presence of BSH-producing isolates in non-human isolation sources.

3.3. Cholesterol assimilation

Dyslipidaemia is a modifiable risk factor for cardiovascular disease (CVD), which is a leading cause of mortality (Labarthe and Dunbar, 2012). Hence, the reducing of cholesterol level is vital for prevention. In this study, all isolates revealed that the cholesterol assimilation ranged from 20.73% to 79.40% (Table 1). Only two isolates showed the percentage of cholesterol assimilation to be greater than 75%. *En. thailandicus* LM4-1 and *L. rhamnosus* LM1-1 potentially assimilated cholesterol at 79.40% and 77.40%, respectively. Moreover, it could be concluded that the amount of assimilated cholesterol revealed a wide variation among isolates. The cholesterol assimilation ability result in this study is in agreement with the findings of various earlier observations (Miremadi et al., 2014; Shehata et al., 2016; Tomaro-Duchesneau et al., 2014). Furthermore, probiotic species (i.e., *L. bulgaricus*, *L. sporogenes* and *L. reuteri*) could decrease cholesterol in human study (Khare and Gaur, 2020). Besides, *L. paracasei* DTA81 revealed a great cholesterol assimilation ability and lowered the total cholesterol in mice model (Tarrah et al., 2021). Remarkably, BSH activity and cholesterol assimilation are the cholesterol-lowering mechanisms as well as desirable probiotic properties (Ishimwe et al., 2015). LAB can utilize cholesterol for their physiological functions; therefore, luminal cholesterol quantity accessible for absorption are decreased (Bordoni et al., 2013).

3.4. Acid and bile tolerance

Acid and bile tolerance are the fundamental characteristics, as it dictates their capacity to endure in the acidic gastric environment as well as small intestine, and as a result, their ability to perform their functional role as a probiotic (Ruiz et al., 2013; Tannock, 2004). Based on BSH-positive activity, all BSH-positive isolates were selected to investigate. The impacts of an acidic and bile environment on selected isolates are illustrated in Table 3. In the acidic conditions, the findings revealed that none of the isolates could survive at pH 2. However, all isolates tolerated at pH 3 and revealed a statistical difference in cell viability compared to the MRS control (18.19–35.91% reduction). This observation is consistent with earlier research (Hassanzadazar et al., 2012). The endurance at pH 3 was established as a criterion for probiotics (Liong and Shah, 2005).

With various degrees of bacterial availability, all isolates were capable of remaining alive in the content of different percentage of bile salts (Table 3). Statistically, the vitality of isolates significantly altered compared to the MRS control. In the case of isolate LM15-1P, this isolate was tolerated only in the presence of 0.3% bile salt. However, the vitality of *L. plantarum* subsp. *plantarum* LM6-1, LM6-2, LM7-2-2B, LM12-1, LM14-1, LM14-2, and LM15-2 was enhanced (+0.11–11.85 % reduction) in the level of 0.3–0.8% bile salts with statistical differences compared to the MRS control. This observation is in accordance with the earlier observation (Thamacharoensuk et al., 2017). Consequently, selected isolates could endure and propagate under the bile environment, and bile salts might enhance the vitality.

Their high endurance to low-pH conditions and the occurrence of bile salts, these isolates might endurance in the stomach and intestine or even

Table 3. Survival of selected isolates after incubation for 3 h at various pH and bile concentrations.

Isolate no.	Viable cells (log CFU/ml)					% Reduction[b]			
	MRS[a]	pH 2	pH 3	0.3% Bile	0.8% Bile	pH 2	pH 3	0.3% Bile	0.8% Bile
LM6-1	8.07 ± 0.16	0.00 ± 0.00*	6.11 ± 0.18*	8.95 ± 0.14*	8.75 ± 0.26*	100.00	24.29	+10.90	+8.43
LM6-2	9.03 ± 0.23	0.00 ± 0.00*	4.85 ± 0.33*	9.81 ± 0.22*	9.74 ± 0.23*	100.00	46.29	+8.64	+7.86
LM7-2-2B	8.69 ± 0.21	0.00 ± 0.00*	6.34 ± 0.09*	9.72 ± 0.12*	9.21 ± 0.19*	100.00	27.04	+11.85	+5.98
LM12-1	8.94 ± 0.19	0.00 ± 0.00*	5.73 ± 0.34*	9.85 ± 0.20*	9.67 ± 0.19*	100.00	35.91	+10.18	+8.17
LM14-1	8.79 ± 0.20	0.00 ± 0.00*	5.87 ± 0.38*	9.62 ± 0.15*	8.80 ± 0.30	100.00	33.22	+9.44	+0.11
LM15-1P	9.24 ± 0.06	0.00 ± 0.00*	7.07 ± 0.26*	9.62 ± 0.15*	9.01 ± 0.09*	100.00	23.48	+4.11	2.49
LM15-2	8.80 ± 0.04	0.00 ± 0.00*	5.98 ± 0.07*	9.19 ± 0.08*	8.93 ± 0.20	100.00	32.05	+4.43	+1.48

Data expressed as mean ± SD.
*$p < 0.05$, compared to negative control.
[a] MRS used as a negative control.
[b] Percentage reduction of bacterial number as compare to negative control; +, indicated enhance of bacterial viability.

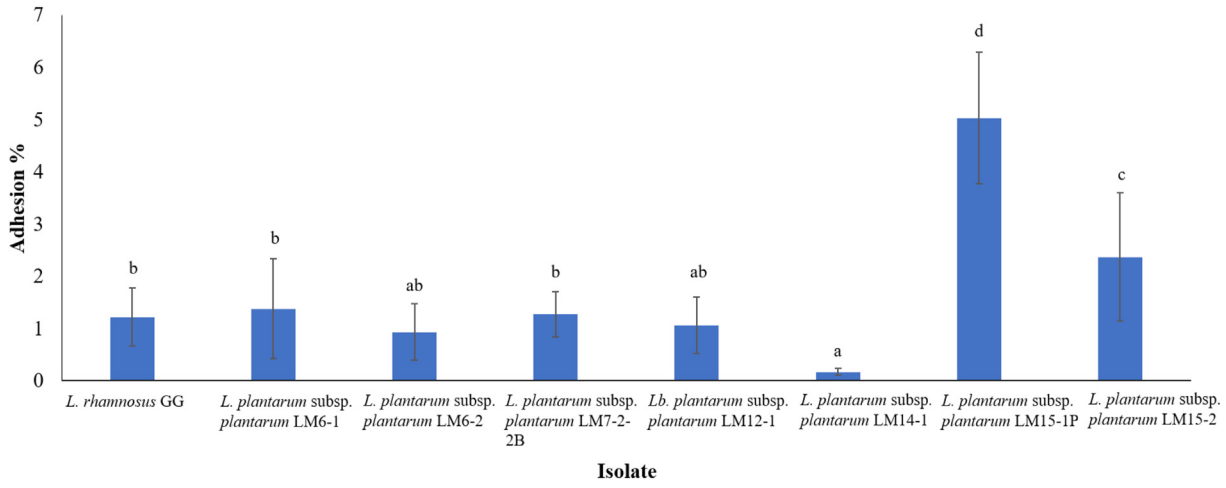

Figure 4. Percentage of selected isolates adhesion to Caco-2 cell lines. Selected isolates were enumerated by bacterial culture and interpreted as the percentage adherence compared with the control. All experiments are done in triplicate and the results were reported as the mean ± standard deviation (SD). The different alphabets mean significant difference ($p < 0.05$).

compete with other bacterial groups in this condition, suggesting a promising probiotic potential.

3.5. Adhesion properties

Based on BSH-positive activity and acid and bile endurance, seven isolates, including *L. plantarum* subsp. *plantarum* LM6-1, LM6-2, LM7-2-2B, LM12-1, LM14-1, LM15-1P, and LM15-2 were chosen to determine. The adhesion capability is illustrated in Figure 4. *L. plantarum* subsp. *plantarum* LM14-1 showed the lowest adhesion ability at 0.17 ± 0.06%. While the adhesion ability of *L. plantarum* subsp. *plantarum* LM6-1 (1.38 ± 0.95%), LM7-2-2B (1.27 ± 0.43%), LM12-1 (1.06 ± 0.54%), and LM6-2 (0.93 ± 0.54%) did not revealed statistical difference when compared with *L. rhamnosus* GG (1.22 ± 0.55%; positive control). Furthermore, *L. plantarum* subsp. *plantarum* LM15-1P (5.03 ± 1.26%) and LM15-2 (2.37 ± 1.23%) showed greater adhesion capability with a statistical difference compared to *L. rhamnosus* GG. Adhesion ability of LAB in this work is compatible with published research findings (Duary et al., 2011; García-Cayuela et al., 2014; Thamacharoensuk et al., 2017). From this study, it could be indicated that the adhesion ability of selected isolates to Caco-2 was isolate-specific and varied within the same species (Duary et al., 2011). In conclusion, most of the *L. plantarum* subsp. *plantarum* isolated from the fermented mussel samples showed similar and/or better able to adhere epithelial cells under *in vitro* investigation as compared to the *L. rhamnosus* GG. These fermented food isolates show remarkable potential and might be potential candidate probiotics for further intensive *in vivo* investigations to evaluate their additional well-being effects due to better gut colonization.

3.6. Immunomodulatory effects of LAB

The observation illustrated that the immunomodulatory effects of the chosen and representative isolates varied with and without statistically significant differences from the control (Table 4).

For IL-12 induction, the ability to stimulate IL-12 production was best in *Lc. lactis subsp. lactis* LM2-2 (53.98 ± 7.66 ng/ml), whereas *L. plantarum* subsp. *plantarum* LM6-1 had the lowest (7.15 ± 1.22 ng/ml). The IL-12 induction ability of LAB in this finding is consistent with past publication (Chen et al., 2013; Iwabuchi et al., 2012; Thamacharoensuk et al., 2017).

For IFN-γ induction, *L. buchneri* LM17-6 had the highest ability to stimulate IFN-γ production (60.43 ± 20.35 ng/ml), while *L. plantarum* subsp. *plantarum* LM6-1 had the lowest (21.84 ± 6.64 ng/mL). The IFN-γ induction in this work is in accordance with earlier findings (Ou et al., 2011; Yamane et al., 2018).

For hBD-2 production, *En. thailandicus* LM4-1 and *En. durans* LM2-1 increased hBD-2 production, but *En. hirae* LM1-2 and *L. plantarum* subsp. *plantarum* LM7-2-2B decreased it. According to the *in vitro* results, *En. thailandicus* LM4-1 and *En. durans* LM2-1 had a stimulatory effect on hBD-2 expression. The result of hBD-2 stimulation in this research is in accordance with the published investigation (Kobatake and Kabuki, 2019; Schlee et al., 2008). As a result, this study demonstrates that

Table 4. Immunomodulatory effects of the selected and representative isolates.

Species/isolate no.	IL-12 (ng/ml)	IFN-γ (ng/ml)	hBD-2 (relative value)	NO (μM)
C. formosensis LM10-1M	29.90 ± 5.15	26.88 ± 8.52	1.29 ± 0.19	14.15 ± 0.07**
L. buchneri LM17-6	10.31 ± 2.74*	60.43 ± 20.35	1.43 ± 0.18	10.38 ± 0.04**
L. plantarum subsp. *plantarum* LM6-1	7.15 ± 1.22*	21.84 ± 6.64	2.26 ± 0.20*	17.89 ± 0.05**
L. plantarum subsp. *plantarum* LM6-2	20.62 ± 4.82	49.25 ± 18.21	1.91 ± 0.23*	13.52 ± 0.28**
L. plantarum subsp. *plantarum* LM7-2-2B	9.97 ± 3.92*	35.42 ± 11.44	0.98 ± 0.11	16.65 ± 0.08**
L. plantarum subsp. *plantarum* LM12-1	53.12 ± 6.43*	59.93 ± 16.02	1.67 ± 0.25	16.64 ± 0.05**
L. plantarum subsp. *plantarum* LM14-1	9.21 ± 3.15*	31.01 ± 8.57	1.50 ± 0.10*	17.76 ± 0.17**
L. plantarum subsp. *plantarum* LM15-1P	51.78 ± 4.72*	27.40 ± 4.63	1.58 ± 0.04*	15.75 ± 0.14**
L. plantarum subsp. *plantarum* LM15-2	24.77 ± 3.42	35.91 ± 8.79	1.61 ± 0.06*	16.03 ± 0.39**
L. rhamnosus LM1-1	33.74 ± 8.43	25.96 ± 9.17	1.18 ± 0.05*	17.44 ± 0.24**
P. pentosaceus LM13-1	22.31 ± 6.72	47.79 ± 19.05	1.50 ± 0.10*	18.19 ± 0.36**
P. acidilactici LM5-1	23.15 ± 4.38	43.03 ± 14.72	1.43 ± 0.11*	19.59 ± 0.17**
Lc. lactis subsp. *lactis* LM2-2	53.98 ± 7.66*	53.55 ± 21.27	2.04 ± 0.06*	17.20 ± 0.33**
Lc. lactis subsp. *hordinae* LM8-2	20.32 ± 10.85	45.68 ± 14.93	1.48 ± 0.06*	15.89 ± 0.17**
En. thailandicus LM4-1	16.03 ± 5.76	33.89 ± 11.99	3.03 ± 0.23*	14.68 ± 0.23**
En. hirae LM1-2	10.43 ± 5.27*	27.05 ± 7.66	0.85 ± 0.07	12.13 ± 0.15**
En. durans LM2-1	18.38 ± 7.41	33.64 ± 11.14	3.01 ± 0.25*	19.15 ± 0.18**
Len. lactis LMK9-2L	10.60 ± 6.35*	27.29 ± 6.92	1.54 ± 0.26	6.78 ± 0.11**
PBS (no stimulation)	29.52 ± 5.87	43.23 ± 12.72	1.00 ± 0.00	Not detected
LPS (positive control)	Not determined			32.47 ± 0.14

Data expressed as mean ± SD.
*$p < 0.05$, compared to PBS (no stimulation) within each column.
**$p < 0.05$, compared to LPS (positive control).

beneficial LAB promote innate immunity through defensin induction. Also, LAB stimulation is an attractive, innovative therapy technique for enhancing innate immunity (Schlee et al., 2008).

For nitric oxide (NO) production, NO production is physiologically advantageous to the host's immune response. From the outcomes of NO assay, all representative isolates stimulate NO production at a wide range of rates with statistically significant differences from the control (Table 4). The highest NO production was found in *P. acidilactici* LM5-1 (19.59 ± 0.17 μM), followed by *En. durans* LM2-1 (19.15 ± 0.18 μM), *P. pentosaceus* LM13-1 (18.19 ± 0.36 μM), and *L. plantarum* subsp. *plantarum* LM6-1 (17.89 ± 0.05 μM) and LM14-1 (17.76 ± 0.17 μM). The NO-induced production of LAB in this examination is similar to earlier publications (Kmonickova et al., 2012; Korhonen et al., 2001; Surayot et al., 2014).

As a consequence, these isolates have the potential to be effective against invading pathogens via stimulation immunity (Kang et al., 2021a, b; Kato et al., 1999). Surprisingly, the heat-killed cells in this study still had immunomodulation activities; hence, the benefits of inactive cells include a lower risk of antibiotic resistance and sepsis and an extension of life span since there is no requirement to retain the viability (Shripada et al., 2020; Zendeboodi et al., 2020). Furthermore, this obeservation revealed that bacterial isolates, even though they belonged to the same species, might have various functional properties (Kang et al., 2021a,b).

4. Conclusions

This observation demonstrated the distribution of LAB in Thai fermented mussel (Hoi-dong) which includes the genera *Companilactobacillus*, *Enterococcus*, *Lentilactobacillus*, *Lactiplantibacillus*, *Lactococcus, Leuconostoc,* and *Pediococcus*. This is the first observation on the LAB distribution these food origins. Seven *L. plantarum* subsp. *plantarum* isolates expressed BSH activity by the development of an opaque white colony as well as could tolerate and propagate in acidic (pH 3) and bile salt (0.3 and 0.8%) environments. Besides, they also had a great adhesion capability to Caco-2 cells. Additionally, the BSH-producing isolates as well as representative isolates showed immunostimulatory effects. *Lc. lactis* subsp. *lactis* LM2-2 induced the most IL-12 production, while *L. buchneri* LM17-6 induced the most IFN-γ production, *En. thailandicus* LM4-1 induced the most hBD-2 secretion, and *P. acidilactici* LM5-1 potentially stimulated NO production. The function of LAB in hypercholesterolemia management and immunomodulatory is increasingly receiving attention. Consequently, these isolates may be regarded as good probiotics since they have cholesterol-removing effects, immunomodulatory ability, adhesion ability, and tolerance of acid and bile, all of which are beneficial probiotic characteristics. Additional research, such as clinical trials, is required.

Declarations

Author contribution statement

Engkarat Kingkaew: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Hiroshi Konno: Performed the experiments; Analyzed and interpreted the data.

Yoshihito Hosaka: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Wongsakorn Phongsopitanun: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Somboon Tanasupawat: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Funding statement

This study was supported by the Thailand Research Fund and the National Research Council of Thailand for the 2017 Royal Golden Jubilee Ph.D. Program as a scholarship to E. K. (PHD/0226/2560) and the Grant for International Research Integration: Research Pyramid, Ratchadaphiseksomphot Endowment Fund (CUGRP-61-01-33-01), Chulalongkorn University.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

The authors thank the Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University for providing research facilities.

References

Abushelaibi, A., Al-Mahadin, S., El-Tarabily, K., Shah, N.P., Ayyash, M., 2017. Characterization of potential probiotic lactic acid bacteria isolated from camel milk. LWT–Food Sci. Technol. 79, 316–325.

Albano, C., Morandi, S., Silvetti, T., Casiraghi, M.C., Manini, F., Brasca, M., 2018. Lactic acid bacteria with cholesterol-lowering properties for dairy applications: *In vitro* and *in situ* activity. J. Dairy Sci. 101 (12), 10807–10818.

Baek, K.-S., Hong, Y.D., Kim, Y., Sung, N.Y., Yang, S., Lee, K.M., Park, J.Y., Park, J.S., Rho, H.S., Shin, S.S., 2015. Anti-inflammatory activity of AP-SF, a ginsenoside-enriched fraction, from Korean ginseng. J. Ginseng Res. 39 (2), 155–161.

Begley, M., Hill, C., Gahan, C.G., 2006. Bile salt hydrolase activity in probiotics. Appl. Environ. Microbiol. 72 (3), 1729–1738.

Boonprab, K., 2022. Rice flour powder carrying mixed starter culture of *Lactiplantibacillus plantarum* KU-LM173 and *Pediococcus acidilactici* KU-LM145 for fermented mussel, *Perna viridis* Linnaeus 1758. J. Appl. Microbiol. 132 (2), 1197–1209.

Bordoni, A., Amaretti, A., Leonardi, A., Boschetti, E., Danesi, F., Matteuzzi, D., Roncaglia, L., Raimondi, S., Rossi, M., 2013. Cholesterol-lowering probiotics: *in vitro* selection and *in vivo* testing of bifidobacteria. Appl. Microbiol. Biotechnol. 97 (18), 8273–8281.

Costabile, A., Buttarazzi, I., Kolida, S., Quercia, S., Baldini, J., Swann, J.R., Brigidi, P., Gibson, G.R., 2017. An *in vivo* assessment of the cholesterol-lowering efficacy of *Lactobacillus plantarum* ECGC 13110402 in normal to mildly hypercholesterolaemic adults. PLoS One 12 (12), e0187964.

Chen, C.Y., Tsen, H.Y., Lin, C.L., Lin, C.K., Chuang, L.T., Chen, C.S., Chiang, Y.C., 2013. Enhancement of the immune response against *Salmonella* infection of mice by heat-killed multispecies combinations of lactic acid bacteria. J. Med. Microbiol. 62, 1657–1664.

De Man, J., Rogosa, d., Sharpe, M.E., 1960. A medium for the cultivation of lactobacilli. J. Appl. Bacteriol. 23 (1), 130–135.

De Vries, M.C., Vaughan, E.E., Kleerebezem, M., de Vos, W.M., 2006. *Lactobacillus plantarum*—survival, functional and potential probiotic properties in the human intestinal tract. Int. Dairy J. 16 (9), 1018–1028.

Domingos-Lopes, M.F.P., Stanton, C., Ross, R.P., Silva, C.C.G., 2020. Histamine and cholesterol lowering abilities of lactic acid bacteria isolated from artisanal Pico cheese. J. Appl. Microbiol. 129 (6), 1428–1440.

Duary, R.K., Rajput, Y.S., Batish, V.K., Grover, S., 2011. Assessing the adhesion of putative indigenous probiotic lactobacilli to human colonic epithelial cells. Indian J. Med. Res. 134 (5), 664.

FAO/WHO working group, 2002. Guidelines for the Evaluation of Probiotics in Food. FAO/WHO Working Group, pp. 1–11.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39 (4), 783–791.

García-Cayuela, T., Korany, A.M., Bustos, I., de Cadiñanos, L.P.G., Requena, T., Peláez, C., Martínez-Cuesta, M.C., 2014. Adhesion abilities of dairy *Lactobacillus plantarum* strains showing an aggregation phenotype. Food Res. Int. 57, 44–50.

Hameed, A., Condò, C., Tauseef, I., Idrees, M., Ghazanfar, S., Farid, A., Muzammal, M., Al Mohaini, M., Alsalman, A.J., Al Hawaj, M.A., 2022. Isolation and characterization of a cholesterol-lowering bacteria from Bubalus bubalis raw milk. Fermentation 8 (4), 163.

Han, Q., Kong, B.H., Chen, Q., Sun, F.D., Zhang, H., 2017. *In vitro* comparison of probiotic properties of lactic acid bacteria isolated from Harbin dry sausages and selected probiotics. J. Funct.Foods 32, 391–400.

Hassanzadazar, H., Ehsani, A., Mardani, K., Hesari, J., 2012. Investigation of antibacterial, acid and bile tolerance properties of lactobacilli isolated from Koozeh cheese. Vet. Res. Forum 3 (3), 181. Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Hosaka, Y., Itoh, K., Matsutani, S., Kawate, S., Miura, A., Mizoura, Y., Yamada, S., Konno, H., Grave, E., Nagata, K., 2021. Fermented food Tempeh induces interleukin 12 and enhances macrophage phagocytosis. J. Food Biochem. 45 (11), e13958.

Ishimwe, N., Daliri, E.B., Lee, B.H., Fang, F., Du, G., 2015. The perspective on cholesterol-lowering mechanisms of probiotics. Mol. Nutr. Food Res. 59 (1), 94–105.

Iwabuchi, N., Yonezawa, S., Odamaki, T., Yaeshima, T., Iwatsuki, K., Xiao, J.-Z., 2012. Immunomodulating and anti-infective effects of a novel strain of *Lactobacillus paracasei* that strongly induces interleukin-12. FEMS Immunol. Med. Microbiol. 66 (2), 230–239.

Kang, C.-H., Kim, J.-S., Kim, H., Park, H.M., Paek, N.-S., 2021a. Heat-killed lactic acid bacteria inhibit nitric oxide production via inducible nitric oxide Synthase and cyclooxygenase-2 in RAW 264.7 cells. Probiotics Antimicrob. Proteins 13 (6), 1530–1538.

Kang, C.-H., Kim, J.-S., Park, H.M., Kim, S., Paek, N.-S., 2021b. Antioxidant activity and short-chain fatty acid production of lactic acid bacteria isolated from Korean individuals and fermented foods. 3 Biotech 11 (5), 217–217.

Kato, I., Tanaka, K., Yokokura, T., 1999. Lactic acid bacterium potently induces the production of interleukin-12 and interferon-γ by mouse splenocytes. Int. J. Immunopharm. 21 (2), 121–131.

Khare, A., Gaur, S., 2020. Cholesterol-lowering effects of *Lactobacillus* species. Curr. Microbiol. 77 (4), 638–644.

Kmonickova, E., Kverka, M., Tlaskalová-Hogenová, H., Kostecka, P., Zídek, Z., 2012. Stimulation of nitric oxide, cytokine and prostaglandin production by low-molecular weight fractions of probiotic *Lactobacillus casei* lysate. Neuroendocrinol. Lett. 33 (3), 166–172.

Kobatake, E., Kabuki, T., 2019. S-layer protein of *Lactobacillus helveticus* SBT2171 promotes human β-defensin 2 expression via TLR2–JNK signaling. Front. Microbiol. 2414.

Korhonen, R., Korpela, R., Saxelin, M., Mäki, M., Kankaanranta, H., Moilanen, E., 2001. Induction of nitric oxide synthesis by probiotic *Lactobacillus rhamnosus* GG in J774 macrophages and human T84 intestinal epithelial cells. Inflammation 25 (4), 223–232.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33 (7), 1870–1874.

Labarthe, D.R., Dunbar, S.B., 2012. Global cardiovascular health promotion and disease prevention 2011 and beyond. Circulation 125 (21), 2667–2676.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227 (5259), 680–685.

Lane, D.J., 1991. 16S/23S rRNA sequencing. In: Nucleic Acid Techniques in Bacterial Systematics, pp. 115–175.

Liong, M.T., Shah, N.P., 2005. Acid and bile tolerance and cholesterol removal ability of lactobacilli strains. J. Dairy Sci. 88 (1), 55–66.

Liu, Y.F., Zhao, F.C., Liu, J.Y., Wang, H.M., Han, X., Zhang, Y.X., Yang, Z.Y., 2017. Selection of cholesterol-lowering lactic acid bacteria and its effects on rats fed with high-cholesterol diet. Curr. Microbiol. 74 (5), 623–631.

Miremadi, F., Ayyash, M., Sherkat, F., Stojanovska, L., 2014. Cholesterol reduction mechanisms and fatty acid composition of cellular membranes of probiotic Lactobacilli and Bifidobacteria. J. Funct.Foods 9, 295–305.

Moon, P.-D., Lee, J.S., Kim, H.-Y., Han, N.-R., Kang, I., Kim, H.-M., Jeong, H.-J., 2019. Heat-treated *Lactobacillus plantarum* increases the immune responses through activation of natural killer cells and macrophages on *in vivo* and *in vitro* models. J. Med. Microbiol. 68 (3), 467–474.

Nakai, H., Hirose, Y., Murosaki, S., Yoshikai, Y., 2019. *Lactobacillus plantarum* L-137 upregulates hyaluronic acid production in epidermal cells and fibroblasts in mice. Microbiol. Immunol. 63 (9), 367–378.

Nanasombat, S., Phunpruch, S., Jaichalad, T., 2012. Screening and identification of lactic acid bacteria from raw seafoods and Thai fermented seafood products for their potential use as starter cultures. Songklanakarin J. Sci. Technol. 34 (3).

Ngasotter, S., Waikhom, D., Mukherjee, S., Devi, M.S., Singh, A.S., 2020. Diversity of lactic acid bacteria (LAB) in fermented fish products: a review. Int. J. Curr. Microbiol. Appl. Sci. 9 (5), 2238–2249.

Noriega, L., Cuevas, I., Margolles, A., Los Reyes-Gavilan, C.G.D., 2006. Deconjugation and bile salts hydrolase activity by *Bifidobacterium* strains with acquired resistance to bile. Int. Dairy J. 16 (8), 850–855.

Ou, C.C., Lin, S.L., Tsai, J.J., Lin, M.Y., 2011. Heat-killed lactic acid bacteria enhance immunomodulatory potential by skewing the immune response toward Th1 polarization. J. Food Sci. 76 (5), M260–M267.

Park, S., Kang, J., Choi, S., Park, H., Hwang, E., Kang, Y., Kim, A., Holzapfel, W., Ji, Y., 2018. Cholesterol-lowering effect of *Lactobacillus rhamnosus* BFE5264 and its influence on the gut microbiome and propionate level in a murine model. PLoS One 13 (8), e0203150.

Phithakpol, B., Varanyanond, W., Reungmaneepaitoon, S., Wood, H., 1995. The Traditional Fermented Foods of Thailand ASEAN Food Handling Bureau. Kuala Lumpur, p. 157.

Phuengjayaem, S., Phinkian, N., Tanasupawat, S., Teeradakorn, S., 2017. Diversity and succinic acid production of lactic acid bacteria isolated from animals, soils and tree barks. Res. J. Microbiol. 12, 177–186.

Pithva, S., Shekh, S., Dave, J., Vyas, B.R., 2014. Probiotic attributes of autochthonous *Lactobacillus rhamnosus* strains of human origin. Appl. Biochem. Biotechnol. 173 (1), 259–277.

Rudel, L.L., Morris, M., 1973. Determination of cholesterol using *o*-phthalaldehyde. J. Lipid Res. 14 (3), 364–366.

Ruiz, L., Margolles, A., Sánchez, B., 2013. Bile resistance mechanisms in *Lactobacillus* and *Bifidobacterium*. Front. Microbiol. 4, 396.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4 (4), 406–425.

Schlee, M., Harder, J., Köten, B., Stange, E.F., Wehkamp, J., Fellermann, K., 2008. Probiotic lactobacilli and VSL# 3 induce enterocyte β-defensin 2. Clin. Exp. Immunol. 151 (3), 528–535.

Shehata, M., El Sohaimy, S., El-Sahn, M.A., Youssef, M., 2016. Screening of isolated potential probiotic lactic acid bacteria for cholesterol lowering property and bile salt hydrolase activity. Ann. Agric. Sci. 61 (1), 65–75.

Shripada, R., Gayatri, A.-J., Sanjay, P., 2020. Chapter 5 – paraprobiotics. In: Faintuch, J., Faintuch, S. (Eds.), Precision Medicine for Investigators, Practitioners and Providers. Acad. Pr, pp. 39–49.

Surayot, U., Wang, J., Seesuriyachan, P., Kuntiya, A., Tabarsa, M., Lee, Y., Kim, J.-K., Park, W., You, S., 2014. Exopolysaccharides from lactic acid bacteria: structural analysis, molecular weight effect on immunomodulation. Int. J. Biol. Macromol. 68, 233–240.

Tanasupawat, S., Daengsubha, W., 1983. *Pediococcus* species and related bacteria found in fermented foods and related materials in Thailand. J. Gen. Appl. Microbiol. 29 (6), 487–506.

Tanasupawat, S., Komagata, K., 1995. Lactic acid bacteria in fermented foods in Thailand. World J. Microbiol. Biotechnol. 11 (3), 253–256.

Tanasupawat, S., Okada, S., Komagata, K., 1998. Lactic acid bacteria found in fermented fish in Thailand. J. Gen. Appl. Microbiol. 44 (3), 193–200.
Tannock, G.W., 2004. A special fondness for lactobacilli. Appl. Environ. Microbiol. 70 (6), 3189–3194.
Tarrah, A., dos Santos Cruz, B.C., Sousa Dias, R., da Silva Duarte, V., Pakroo, S., Licursi de Oliveira, L., Gouveia Peluzio, M.C., Corich, V., Giacomini, A., Oliveira de Paula, S., 2021. *Lactobacillus paracasei* DTA81, a cholesterol-lowering strain having immunomodulatory activity, reveals gut microbiota regulation capability in BALB/c mice receiving high-fat diet. J. Appl. Microbiol. 131 (4), 1942–1957.
Thamacharoensuk, T., Taweechotipatr, M., Kajikawa, A., Okada, S., Tanasupawat, S., 2017. Induction of cellular immunity interleukin-12, antiproliferative effect, and related probiotic properties of lactic acid bacteria isolated in Thailand. Ann. Microbiol. 67 (8), 511–518.
Tomaro-Duchesneau, C., Jones, M.L., Shah, D., Jain, P., Saha, S., Prakash, S., 2014. Cholesterol assimilation by *Lactobacillus* probiotic bacteria: an *in vitro* investigation. BioMed Res. Int. 2014, 1–9.
Towbin, H., Staehelin, T., Gordon, J., 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA 76 (9), 4350–4354.
Wang, J., Mendelsohn, R., Dinar, A., Huang, J., Rozelle, S., Zhang, L., 2009. The impact of climate change on China's agriculture. Agric. Econ. 40 (3), 323–337.
Yamane, T., Sakamoto, T., Nakagaki, T., Nakano, Y., 2018. Lactic acid bacteria from kefir increase cytotoxicity of natural killer cells to tumor cells. Foods 7 (4), 48.
Yang, Y., Xing, R., Liu, S., Qin, Y., Li, K., Yu, H., Li, P., 2018. Immunostimulatory effects of sulfated chitosans on RAW 264.7 mouse macrophages via the activation of PI3 K/Akt signaling pathway. Int. J. Biol. Macromol. 108, 1310–1321.
Yoon, S.-H., Ha, S.-M., Kwon, S., Lim, J., Kim, Y., Seo, H., Chun, J., 2017. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int. J. Syst. Evol. Microbiol. 67 (5), 1613.
Zendeboodi, F., Khorshidian, N., Mortazavian, A.M., da Cruz, A.G., 2020. Probiotic: conceptualization from a new approach. Curr. Opin. Food Sci. 32, 103–123.
Zheng, J., Wittouck, S., Salvetti, E., Franz, C., Harris, H., Mattarelli, P., O'Toole, P.W., Pot, B., Vandamme, P., Walter, J., Watanabe, K., Wuyts, S., Felis, G.E., Gänzle, M.G., Lebeer, S., 2020. A taxonomic note on the genus *Lactobacillus*: description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. Int. J. Syst. Evol. Microbiol. 70 (4), 2782–2858.