© 2023 Journal of Pharmacy & Pharmacognosy Research, 11 (6), 1044-1055, 2023
ISSN 0719-4250
https://jppres.com

DOI: https://doi.org/10.56499/jppres23.1713_11.6.1044

Original Article

Bioactive compound and chemical characterization of lactic acid bacteria from fermented food as bio-preservative agents to control food-borne pathogens

[Compuestos bioactivos y caracterización química de bacterias lácticas de alimentos fermentados como agentes bioconservantes para el control de patógenos alimentarios]

Surachai Techaoei[1], Khemjira Jarmkom[1], Thisakorn Dumrongphuttidecha[1], Warachate Khobjai[2*]

[1]Faculty of Integrative Medicine, Rajamangala University of Technology Thanyaburi, Pathum Thani, 12130, Thailand.
[2]Faculty of Medicine, Siam University, 38 Petchkasem road, Bang Wa, Pasi Charoen, Bangkok, 10160, Thailand.
*E-mail: warachate.kho@siam.edu

Abstract

Context: Lactic acid bacteria (LAB), which are found in many fermented foods, are known to produce antimicrobial compounds that play a vital role in food bio-preservation.

Aims: To screen, identify, characterize, and determine the secondary metabolites of LAB isolated from Thai fermented foods that are beneficial against *Escherichia coli* and *Staphylococcus aureus*.

Methods: The antimicrobial activity of cell free supernatant (CFS) was evaluated by agar well diffusion assay, and determining the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC). Bacterial strains were identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), and chemical compound was conducted by gas chromatography and mass spectrometry (GC-MS). Additionally, microbial dynamic and bile salt tolerance were assessed.

Results: Seven of the 90 lactic acid isolates from Thai fermented foods, showed antibacterial activity against *E. coli* and *S. aureus* in the 12.00–16.00 mm inhibitory zone. It was identified that the bacteria were *Lactobacillus pentosus*, *Lactobacillus farciminis*, *Lactobacillus brevis*, and *Lactobacillus plantarum*. The best antibacterial activity was represented by LBST1861 strain, which also provided bile salt resistance at 0.3% for 24 hours and had MIC and MBC values of 12.5 mg/mL and 50.0 mg/mL against *S. aureus* and *E. coli*, respectively. Furthermore, the GC-MS discovered a total of 16 chemical compounds that may be used to limit microbial growth and has a potential to be employed as a bio-preservative.

Conclusions: The most potent strain of LBST1861 strain against *S. aureus* and *E. coli* as *L. plantarum*, isolated from fermented foods in Thailand, generated significant bioactive chemicals that can be applied to promote food products.

Keywords: antimicrobial activity; bioactive compounds; fermented foods; lactic acid bacteria; probiotics.

Resumen

Contexto: Se sabe que las bacterias lácticas (BAL), que se encuentran en muchos alimentos fermentados, producen compuestos antimicrobianos que desempeñan un papel vital en la bioconservación de los alimentos.

Objetivos: Examinar, identificar, caracterizar y determinar los metabolitos secundarios de las BAL aisladas de alimentos fermentados tailandeses que son beneficiosos contra *Escherichia coli* y *Staphylococcus aureus*.

Métodos: La actividad antimicrobiana del sobrenadante libre de células (SFC) se evaluó mediante un ensayo de difusión en pocillos de agar y determinando la concentración inhibitoria mínima (CIM) y la concentración bactericida mínima (CBM). Las cepas bacterianas se identificaron mediante espectrometría de masas por ionización de desorción láser asistida por matriz en tiempo de vuelo (MALDI-TOF MS), y el compuesto químico se realizó mediante cromatografía de gases y espectrometría de masas (GC-MS). Además, se evaluaron la dinámica microbiana y la tolerancia a las sales biliares.

Resultados: Siete de los 90 aislados de ácido láctico procedentes de alimentos fermentados tailandeses mostraron actividad antibacteriana contra *E. coli* y *S. aureus* en la zona inhibitoria de 12,00-16,00 mm. Se identificó que las bacterias eran *Lactobacillus pentosus*, *Lactobacillus farciminis*, *Lactobacillus brevis* y *Lactobacillus plantarum*. La mejor actividad antibacteriana estuvo representada por la cepa LBST1861, que también proporcionó una resistencia a las sales biliares del 0,3% durante 24 horas y tuvo unos valores MIC y MBC de 12,5 mg/mL y 50,0 mg/mL contra *S. aureus* y *E. coli*, respectivamente. Además, la GC-MS descubrió un total de 16 compuestos químicos que pueden utilizarse para limitar el crecimiento microbiano y tiene potencial para emplearse como bioconservante.

Conclusiones: La cepa LBST1861 más potente contra *S. aureus* y *E. coli* como *L. plantarum*, aislada de alimentos fermentados en Tailandia generó importantes sustancias químicas bioactivas que pueden aplicarse a la promoción de productos alimentarios.

Palabras Clave: actividad antimicrobiana; alimentos fermentados; bacterias lácticas; compuestos bioactivos; probióticos.

ARTICLE INFO
Received: June 11, 2023.
Accepted: September 26, 2023.
Available Online: October 3, 2023.

AUTHOR INFO
ORCID:
0000-0003-4321-4060 (ST)
0009-0007-5109-7024 (TD)
0009-0006-4639-8079 (KJ)
0000-0002-2710-6601 (WK)

INTRODUCTION

Probiotics are confirmed to be a mixture of living, advantageous microbes, such as bacteria, fungus, and/or yeast that naturally live in the host (Pinto et al., 2020). Lactic acid bacteria (LAB) are the main grouped of probiotic bacteria consist mostly of genus of *Lactobacillus*, *Bifidobacterium* and *Streptococcus*. Normally, the highlight of LAB has been used in treating gastrointestinal disease because they really maintenance of intestinal homeostasis and the regulation of adapted immune responds to environmental antigens (de Lima Barros et al., 2021) In generally, Probiotics act in three ways: by inhibiting microbes, improving barrier performance, and modulating immune response (Ng et al., 2009).

Among the LAB, the several strains have been potentially probiotic properties and commercially importantly bioactive molecules such as bacteriocin, vitamins, exopolysaccharides, gamma-aminobutyric acid, flavor substances, and antioxidant substances (Wang et al., 2021). The LAB has been used in the production of dairy products (Arqués et al., 2015; Swain et al., 2014), fermented meat and vegetable products (Bartkiene et al., 2019), and marine products (Zorriehzahra et al., 2016). Therefore, the search for new probiotic LAB strains could produce bioactive metabolites against foodborne pathogens for food safety and food spoilage prevention.

Therefore, the aim of this research was to discover the probiotic LAB that produced the extracellular metabolites of Thai fermented food from agricultural products in Thailand evidence of anti-foodborne bacteria.

MATERIAL AND METHODS

Collection of fermented foods

The twenty Thai fermented foods were also collected from Talad-Thai market (14.08259°N, 100.63239°E) where is the largest Thailand's of international wholesale and retail market for agricultural products which located at Pathum Thani, Thailand during October-November 2021.

Screening and isolation of LAB

All samples of a Thai fermented food were serially diluted, and dilutions of 10^5, 10^6, and 10^7 dilutions in sterilizing 0.85% normal saline solution were put to MRS agar plates along with an indicator called bromocresol green. The plates were incubated anaerobically for 48 h at 37°C. Gram reaction, catalase test, formation of acid from glucose, and growth at various temperatures were utilized as biochemical assays. Colonies that approximated the yellow zone were picked, and they were then sub-inoculated into MRS media. The isolated isolates were put into 10 mL of MRS medium, where they were given two days at 37°C to grow anaerobically (Suwannaphan, 2021).

Antimicrobial screening

All LAB strains were grown in MRS for two days at 37°C in an aerobic environment to reach the late exponential phases, and the appropriate cell-free supernatants (CFS) were obtained by filtering them through a 0.45 µm filter before centrifuging at 7,000 g for five minutes. The antibacterial efficacy of CFS against the pathogenic pathogens *S. aureus* and *E. coli* was tested using the agar well diffusion method. Briefly, a test tube with 5 mL of nutrient broth (NB) was filled with the tested bacterium, and after 24 h of aeration (180 rpm shaking) at 37°C, active cultures were obtained. The following stage was creating a suspension of various bacterial strains containing 10^7 colony forming units (CFU)/mL. A sterile glass spreader was used to cover the whole surface of each nutrient agar plate, or sterile cotton swabs were used to swab the plate with the tested microorganisms. A sterile glass Pasteur pipette was used to create eight 6 mm-diameter wells. After then, 30 µL of CFS was transferred to the wells and kept in an anaerobic environment for 24 h at 37°C. Then, a zone of inhibition (ZOI) enclosing the well was looked for after incubation by visually looking at the plates. The calipers were used to calculate the diameter of the inhibitory zone in millimeters. Ampicillin (50 µg/mL) served as the positive control, and MRS broth as the negative. Further characteristics were given for the LAB isolates having the largest zones of inhibition against indicator bacteria. For subsequent screening and characterization, those isolates were sub-cultured on MRS agar and preserved in MRS-glycerol solution (30%). All the assays were performed in triplicate (Goa et al., 2022; Manguntungi et al., 2021; Surco-Laos et al., 2022).

Growth dynamics of LAB

The highest antimicrobial activity, LABST1861 was injected into 1 L of MRS medium and statically cultured for 72 h at 37°C. Every four hours, 10 mL of the cell culture was removed to measure the cell density using a UV spectrometer at 600 nm (Zhang et al., 2017).

Bile salts of LAB resistance

The resistance to bile salts of a selected LBST1861 strain was measured using the methodology of Guan et al. (2017) with a few modifications. It was investi-

gated whether a particular lactic acid bacteria could endure in the presence of varying bile salt concentrations. In brief, MRS broth containing 1% seed cultures of chosen LAB was treated with bile salts in a range of concentrations (0.1% to 0.5%, w/v) and MRS broth devoid of bile salts acting as the control. The culture was cultivated for 24 h at 37°C before being measured for optical density (OD) at 600 nm using a UV-Vis Spectrophotometer. Three different investigations were conducted.

Production of selected lactic acid bacteria

Fermentation of the LABST1861 was performed anaerobically for 48 h at 37°C after it was introduced to 1 L of MRS broth containing 1% seed culture. After fermentation, a centrifuge operating at 7,000 rpm for 15 min was used to separate the antibacterial metabolite and then stored until it was needed at 4°C (Ibrahim et al., 2021).

Extraction of bioactive compounds

The method reported by Arasu et al. (2013) and Zhang et al. (2017) was adjusted for the extraction of bioactive compounds. Following the ethyl acetate extraction (ethyl acetate: the CFS = 1:1, v/v), the CFS was concentrated by evaporation. Then, the antibacterial potential of the crude metabolites was evaluated.

MIC and MBC of bioactive compound of LAB

The bioactive of LBST1861 was initially dissolved into 5% DMSO at a concentration of 12 mg/mL, and it was thereafter serially diluted twice to achieve final concentrations ranging from 6 to 0.375 mg/mL. 96-well plates containing the tested bacterial suspension (10^6 CFU/mL) and an equivalent volume of the diluted sample were combined, incubated at 37°C for 24 h, and then subjected to UV spectrophotometer detection at OD 600 nm. The lowest concentration at which no indication had formed was known as the MIC. The MBC value was then calculated by transferring the MIC value into a fresh agar palate and incubating it there for 24 h at 37°C. The absence of bacterial growth was used to define the minimum bactericidal concentration (MBC) value (Xinran et al., 2018).

Bacterial identification

Each potential lactic acid bacterium was identified by Nacef et al. (2017) utilizing morphology, biochemistry, and MALDI-TOF MS (Matrix Supported Laser Desorption/Ionization Flight Time Mass Spectrometry, Biotypes 0.6, Bruker, Germany).

Chemical analysis of bioactive compounds

The analysis was carried out using a GC-MS system (7890A-5975C, Agilent Technologies Inc., Santa Rosa, CA, USA) and an HP-5 MS capillary column (30 m, 0.25 mm, 0.25 µm, Agilent Technologies Inc., Santa Rosa, CA, USA) in the analytical laboratory of the Faculty of Integrative Medicine at Rajamangala University of Technology Thanyaburi, Thailand. A volume of 1 µL was used to inject the sample. As the carrier gas, 1 mL/min of helium (99.999%) was utilized. The column temperature program consisted of 40°C for 5 min, followed by increases of 5°C/min to 150°C and 10°C/min to 210°C. The injection port temperature was 280°C. The symptoms of MS Capillary column, 5975B Inert XL MS system, quadrupole mass spectrometer, and electron ionization at 70 eV. The MS source and Quadrupole were maintained at 230°C and 150°C, respectively (Ibranhim et al., 2021).

Statistical analysis

Three replicates were used in the trials. Using the statistics tool in GraphPad Prism, the data were expressed using the mean and standard deviation (SD). The significant values were considered at 95 % confidence, $p<0.05$.

RESULTS AND DISCUSSION

Isolation of lactic acid bacteria

A total of 7 samples of fermented fish; (Pickled fish (pla-som: PS), fermented fish (pla-kradii: PK), fermented fish (pla-chon: PC), fermented fish (pla-nil: PN), fermented fish (pla-tapian: PT), fermented fish (pla-soi: PSI), and fermented fish (pla-chalad: PCL), 2 samples of fermented shrimp (Kapi: KP) and Pickled shrimp (kung-jom: KJ), 4 samples of fermented vegetable; (pickled onions: PO, fermented cabbage (FC), fermented bamboo (FB), and fermented bamboo shoot (FBS), 1 samples of fermented crab (FCB), and 1 sample of fermented sour pork (name: NM) were included (Figs. 1-2). All lactic acid bacteria were Gram positive cocci (11.11%), coccobacilli (7.78%), and bacilli (81.11%) with acid producing and catalase negative. According to Dopazo et al. (2023), Asia has traditionally employed lactic acid bacteria as a traditional preservative. In addition, people in Latin American nations have a long tradition of creating fermented beverages and culinary concoctions out of regionally specific ingredients (Carboni et al., 2023).

Bio-metabolites screening of LAB

Table 1 displays how *S. aureus* and *E. coli* are affected by 90 isolated LAB strains' inhibitory activities. As can be observed, 32 strains tested against *S. aureus*

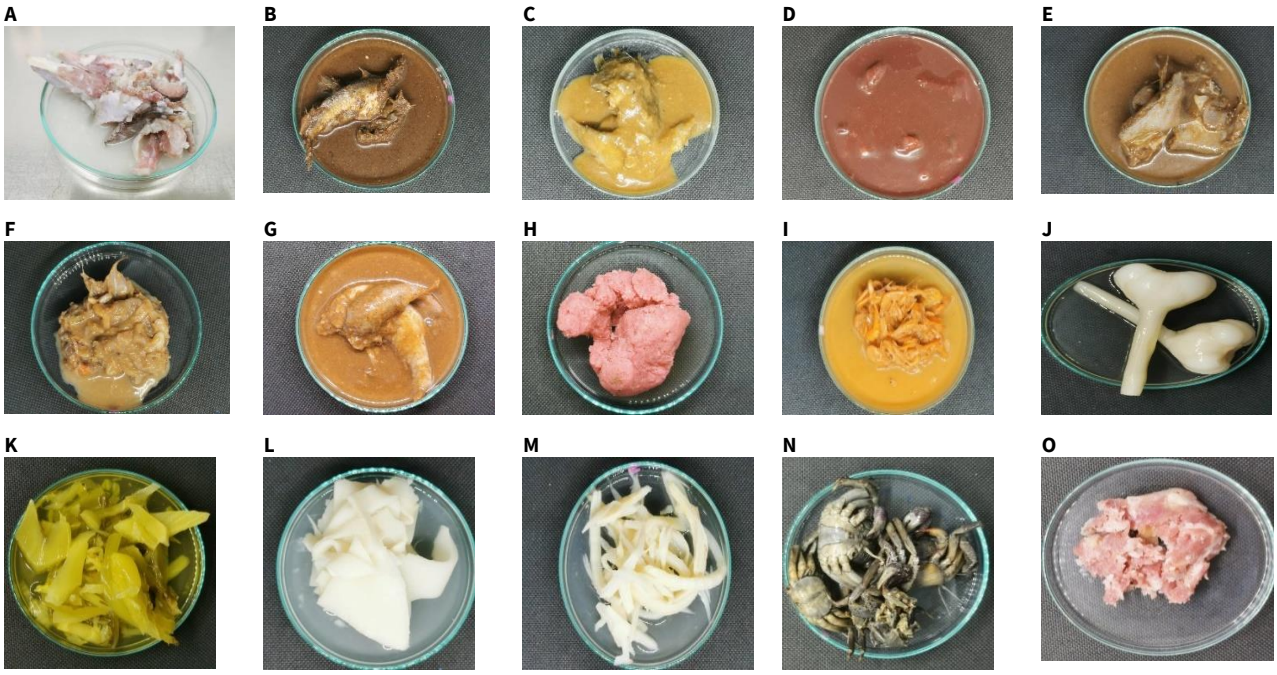


Figure 1. Thai traditional fermented foods collected at Talad Thai, Pathum Thani, Thailand.

(A-G) fermented fish, **(H-I)** fermented pork, **(J-M)** fermented vegetable, **(N)** fermented crab, and **(O)** fermented shrimp. The sample was kept in sterile sample bag under ice container and transferred to laboratory room until 3 h.

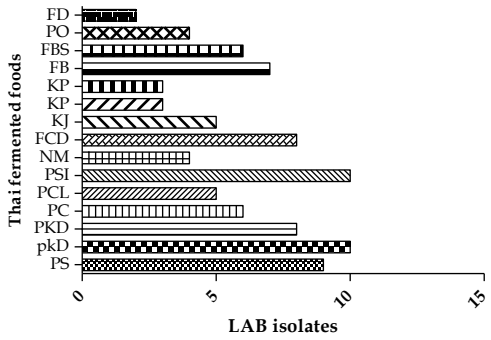


Figure 2. The total number of isolated lactic acid bacteria from Thai fermented foods.

Table 1. Antimicrobial properties of LAB isolates against *S. aureus* and *E. coli*

Isolates	anti-*S. aureus*	anti-*E. coli*
LBST15	++	++
LBST67	++	++
LBST195	++	++
LBST196	++	++
LBST2011	++	++
LBST1811	++	++
LBST1861	+++	+++

++, diameter of inhibition zone 12.00-14.00 mm; +++, 14.00-16.00 mm; ++++, more than 16.00 mm. The diameter of the inhibitory zone was 6 mm, which is the same as a hole punch. The experiment was conducted three times

and *E. coli* showed antibacterial activity with an inhibition zone of at least 10 mm. Among these stains, LBST15, LBST67, LBST195, LBST196, LBST2011, LBST1811, and LBST1861 having an inhibition zone of more than 12.00 mm, were further assessed for their

physiological and biochemical features. In the present study, the LBST1861 strain displayed the best antibacterial activity against *S. aureus* and *E. coli*, with inhibition zones of 14.33 ± 0.58 ($p<0.001$) and 16.67 ± 0.58 ($p<0.002$), respectively. The antibacterial activity of the positive control (amphicilin, 50 g/mL) was seen against *S. aureus* and *E. coli*, with inhibition zones of 18.83 ± 0.29 and 19.5 ± 0.50 mm, whereas the negative control (MRS broth) showed no antibacterial action against *E. coli* or *S. aureus.*

In various studies, LAB from home-made fermented foods has been shown in numerous studies to have the ability to suppress *S. aureus* as well as to have an impact on *Salmonella*, *E. coli*, and *Bacillus* (Ren et al., 2018). The findings indicated that LAB is capable of producing an antibiotic material to block several types of pathogens (Yi et al., 2020). Similar findings have been made in previous research, which found that the CFS of lactic acid bacteria may stop the growth of Gram-positive bacteria (*S. aureus a*nd *Listeria monocytogenes*) and Gram-negative bacteria (*E. coli*, *Shigella sonnei, Pseudomonas fluorescens,* and *Salmonella typhimurium*) (Arrioja-Bretón et al., 2020).

Identification of LAB

The MALDI-TOF mass spectra that each of three colonies of different LAB strains showed allowed for the identification of the bacteria. When the resulting MALDI-TOF MS profiles were compared to the reference spectra created to demonstrate how similar the two sets of spectra were in the BioTyper database. Among the 32 representative isolates, 7 possible LAB strains were discovered down to the species level (log score 2.0). As a results, LAB strains (LBST15, LBST67, LBST195, LBST196, LBST2011, LBST1811, and LBST1861) were identified as *L. brevis, L. plantarum, L. farciminis,* and *L. pentosus* belonging to Lactobacillus genera (Table 2). However, the morphological and biochemical criteria must be employed in categorization, such as Gram stain, bacterial shape, catalase activity, temperature growth, bile salt resistance, and colony color (Rao et al., 2015).

Numerous studies have shown that the basic components and the conditions of the fermentation process affect the bacterial community of fermented food (Jung et al., 2014; Zabat et al., 2018). According to research, the major bacteria included in conventionally fermented vegetables include *Lactobacillus*, *Serratia*, *Leuconostoc*, *Pediococcus*, and *Weissella* (He et al., 2020; Lee et al., 2018). Typically, methods based on physiological and biochemical parameters, as well as phenotypic traits, are employed to distinguish LAB from fermented foods. These challenging and time-consuming processes, however, may undervalue the diversity of microorganisms that survive in a food environment (Liu et al., 2014; Kanak and Yilmaz, 2018). On the other hand, nucleic acid-based molecular approaches are known to be efficient for recognizing the variety of microbes in food samples (O'Sullivan et al., 2013). As a result, the MALDI-TOF MS identification method is a rapid, accessible, trustworthy, and reliable method (Huppertsberg and Knepper, 2018). It is therefore seen as a desirable alternative to biological processes, especially molecular biological techniques (Ahmad et al., 2012). According to Duková et al. (2012), the MALDI-TOF MS methodology has a success rate of 93% when detecting *Lactobacillus* sp. at the species level in contrast to the PCR approaches, which have a success rate of 77%.

Table 2. Genera, species, and identification scores were obtained using morphology, biochemical, and MALDI-TOF MS for each strain identified throughout this investigation.

Test	Isolates						
	LBST15	**LBST67**	**LBST195**	**LBST196**	**LBST2011**	**LBST1811**	**LBST1861**
Morphology	rod	rod	rod	rod	rod	rod	rod
Gram staining	+	+	+	+	+	+	+
Catalase test	-	-	-	-	-	-	-
Growth at temperature (37°C)	+	+	+	+	+	+	+
Bile salt (0.3%)	+	+	+	+	+	+	+
Color of colony	Creamy	Creamy	Creamy	Creamy	Creamy	Creamy	Creamy
MALDI-TOF matching species	*L. pentosus*	*L. farciminis*	*L. brevis*	*L. brevis*	*L. brevis*	*L. plantarum*	*L. plantarum*
Score value	2.24 ± 0.06	2.14 ± 0.05	2.21 ± 0.02	2.18 ± 0.09	2.22 ± 0.10	2.08 ± 0.08	2.39 ± 0.06

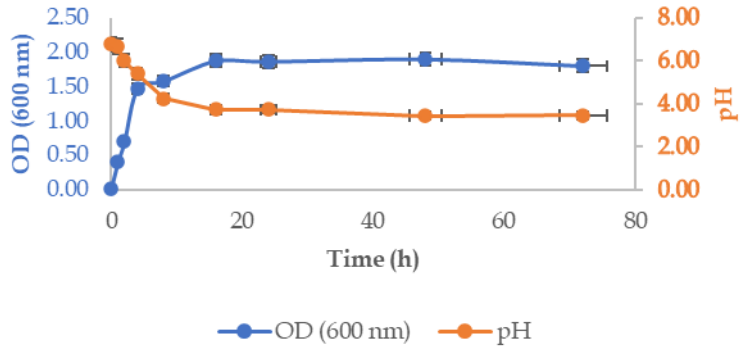


Figure 3. Dynamic growth curve of the lactic acid bacteria strain LBST1861 on MRS broth for 72 h at 37 °C.

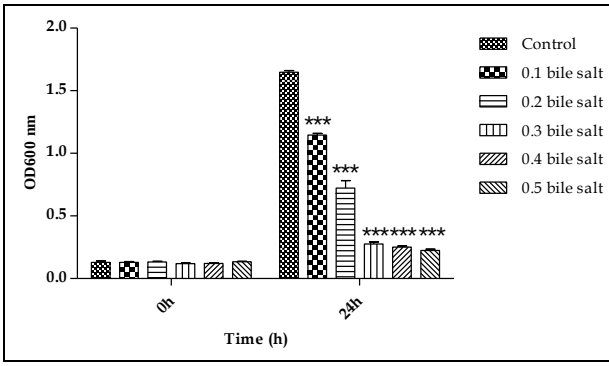


Figure 4. The survival of lactic acid bacteria strain LBST1861 in MRS broth supplemented with 0.1-0.5% bile salts after 24 h at 37°C. *p<0.05, ** p<0.01, ***p<0.001

Growth dynamic of LAB strain LBST1861

The results of the dynamic growth for the lactic acid bacteria, LBST1861, are shown in Fig 3. The graph displays the growth rate (optical density at 600 nm) of bacterial cells as well as the pH level during the fermentation of the isolates. After barely two hours of incubation, the chosen bacteria started growing rapidly, and the exponential phase immediately started after that. The stationary phase replaces the log phase after 16 h of incubation. The results showed that bacteria can produce more lactic acid when they reach late log-phases, as seen by the pH lowering to 3.73 on day 16 and remained constant during stationary phase. In a previous study, Zhang et al. (2021) found that lower OD 600 nm values at lower pH were indicative of greater inhibition of the increase in lactic acid or drop in pH. However, the intrinsic and extrinsic motivation of bacterial strains play a role in the degree to which cell growth and metabolites increase (Connell, et al., 2022) such as the fermentation process, microbial strains, pH, temperature, mineral content, carbon supply, nitrogen source, and other factors (Abbasiliasi, et al., 2017).

Bile salt tolerance

Bacterial probiotics must be able to endure passage through both the small and large intestines because they are frequently taken externally. In light of this, one of the important probiotic selection criteria is resistance to bile salts found in the small intestine tract and stomach acid (Ruiz et al., 2013). According to the outcome of the current investigation, LBST1861 was able to tolerate bile salt concentrations at 24 h that were 0.1%, 1.4 times, and 0.2% lower than those of the control group (Fig. 4). Additionally, in order for bacteria to colonize and get involved in metabolic activity in the host's small intestine, resistance to bile salts is necessary (Foley et al., 2021; Shehata et al., 2016).

MIC and MBC of CFS of lactic acid bacteria

The MIC and MBC results of the LBST1861 extract are shown in Fig. 5. The MIC and MBC of LAB extract against *S. aureus* and *E. coli* were found to be 12.5 mg/mL and 50.0 mg/mL in our investigation. As previously noted, a number of foodborne pathogens may be susceptible to the antibacterial effects of different LAB (Bajpai et al., 2016). According to Zhang et al. (2017) and Ren et al. (2018), a considerable amount of the cell-free supernatant from lactic acid-producing bacteria was soluble in organic solvents including ethanol, methanol, and ethyl acetate (Girma and Aemiro, 2021). Ren et al. (2018) also showed the solubility of the bacteriocin compounds in organic solvent.

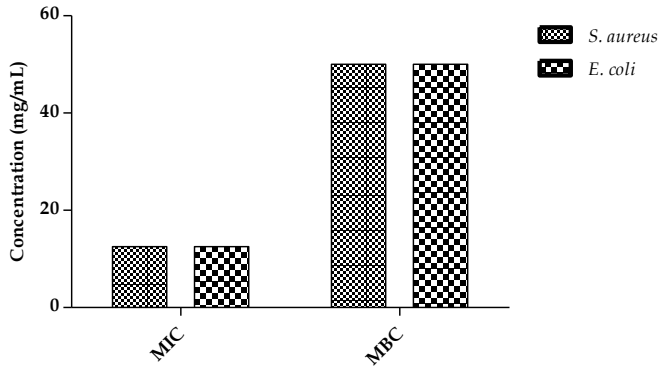


Figure 5. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of LBST1861 extract.

Table 3. Biologically active substances of the extract of lactic acid bacteria LBST1861.

No.	Components	RT	Area (%)	Function	References
1	2-Pyrrolidinone	19.37	1.00	F	Nomiya et al., 2000
2	Methyl salicylate	21.30	0.11	B, F	Vlachojannis et al., 2015
3	1-Tetradecene	26.71	1.15	B, F	Lammers et al., 2021
4	Tetradecane	26.91	0.77	F	Khan and Javaid, 2019
5	Pheno l,2,4-bis(1,1-dimethylethyl)	30.50	0.68	F	Teresa et al., 2014; Devi et al., 2021
6	Dichloroacetic acid,4-hexadecyl ester	33.49	0.90	F	Jaradat et al., 2021
7	Hexadecane	33.72	0.72	B	Tiji et al., 2021
8	Naphthalene,1,2-3-trimethyl-4-propenyl	35.65	0.15	B	Yan et al., 2017
9	Pyrrolo [1,2-a]pyrazine-1,4-dione, hexahydro	37.53	1.55	B	Kiran et al., 2018
10	E-15-Heptadecenal	37.77	1.13	B	Supardy et al., 2012
11	Octadecane	37.87	0.82	B	Rouis-Soussi et al., 2014
12	Isopropyl myristate	38.24	0.82	N	
13	Bis(2-ethylhexyl) phthalate	39.42	18.16	B, L	Javed et al., 2022
14	Pyrrolo [1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)	40.30	2.46	B	Ser al., 2015
15	Dibutyl Phthalate	40.57	1.67	B	Lalitha et al., 2021
16	3-Eicosene, (E)-	41.30	0.40	B	Lulamba et al., 2021

RT: retention time; F: antifungal activity; B: antibacterial activity; L: Larvicidal activity, N: no function.

Chemical characterization of LAB

Out of the 16 chemicals produced in the ethyl acetate extract of lactic acid bacteria strains LBST1861, only 15 compounds, including 2-pyrrolidinone, methyl salicylate, 1-tetradecene, tetradecane, phenol, 2,4-bis(1,1-dimethylethyl), dichloroacetic acid, 4-hexadecyl ester, hexadecane, naphthalene, 1,2-3-trimethyl-4-propenyl, hexahydro-3-(2-methylpropyl), dibutyl phthalate, 3-eicosene, (E)-, isopropyl myristate, bis(2-ethylhexyl) phthalate, pyrrolo [1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl), dibutyl phthalate, 3-eicosene, (E) (Table 3). These substances demonstrated biological activity that was resistant to antibacterial, antifungal, anthelmintic, and larvicidal effects. As demonstrated by earlier research, lactic acid bacterial secondary metabolites are highly powerful against bacterial pathogens (Javed et al., 2022; Kiran et al., 2018; Lalitha et al., 2021; Lammers et al., 2021; Lulamba et al., 2021; Mgomi et al., 2023; Rouis-Soussi et al., 2014; Ser et al., 2015; Tiji et al., 2021; Vlachojannis et al., 2015; Yan et al., 2017), and antifungal activity (Devi et al., 2021; Jaradat et al., 2021; Lammers et al., 2021; Mgomi et al., 2023; Nomiya et al., 2000; Teresa et al., 2014; Vlachojannis et al., 2015). According to research compounds, the CFS extract has excellent potential for compounds that can fight bacterial infections.

CONCLUSION

In the current study, 90 LAB strains collected from various fermented sources in Thailand, however only seven isolates were chosen based on their potential for antibacterial activity. The only strain, LBST1861, was identified as *Lactobacillus plantarum* by MALDITOF-MS, morphology, and biochemical characterization. It showed the best activity against *E. coli* and *S. aureus*. Bioactive chemicals demonstrate that 16 chemical compounds are effective against foodborne infections. To ascertain the mechanism(s) and substance stability of the bacterial isolate, additional *in vitro* investigations as well as an *in vivo* study are needed.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

This study was funded by the Science, Research and Innovation Promotion Funding (TSRI) (Grant No. FRB650070/0168). Under the direction of Rajamangala University of Technology Thanyaburi (FRB65E1211), these research block grants were administered.

REFERENCES

Abbasiliasi S, Tan JS, Ibrahim TAT, Bashokouh F, Ramakrishnan NR, Mustafa S, Ariff AB (2017) Fermentation factors influencing the production of bacteriocins by lactic acid bacteria: A review. RSC Adv 7(47): 29395–29420. https://doi.org/10.1039/C6RA24579J

Ahmad F, Babalola OO, Tak HI (2012) Potential of MALDI-TOF mass spectrometry as a rapid detection technique in plant pathology: Identification of plant-associated microorganisms. Anal Bioanal Chem 404: 1247–1255. https://doi.org/10.1007/s00216-012-6091-7

Arasu MV, Jung MW, Ilavenil S, Jane M, Kim DH, Lee KD, Park HS, Hur TY, Choi GJ, Lim YC, Al-Dhabi NA, Choi KC (2013) Isolation and characterization of antifungal compound from *Lactobacillus plantarum* KCC-10 from forage silage with potential beneficial properties. J Appl Microbiol 115: 1172–1185. https://doi.org/10.1111/jam.12319

Arqués JL, Rodríguez E, Langa S, Landete JM, Medina M (2015) Antimicrobial activity of lactic acid bacteria in dairy products and gut: effect on pathogens. Biomed Res Int 2015: 584183. https://doi.org/10.1155/2015/584183

Arrioja-Bretón D, Mani-López E, Palou E, López-Malo A (2020) Antimicrobial activity and storage stability of cell-free supernatants from lactic acid bacteria and their applications with fresh beef. Food Control 115: 107286. https://doi.org/10.1016/j.foodcont.2020.107286

Bajpai VK, Han JH, Rather IA, Park C, Lim J, Paek WK, Lee JS, Yoon, JI, Park YH (2016) Characterization and antibacterial potential of lactic acid bacterium *Pediococcus pentosaceus* 4I1 isolated from freshwater fish *Zacco koreanus*. Front Microbiol 7: 2037. https://doi.org/10.3389/fmicb.2016.02037

Bartkiene E, Bartkevics V, Mozuriene E, Lele V, Zadeike D, Juodeikiene G (2019) The safety, technological, nutritional, and sensory challenges associated with lacto-fermentation of meat and meat products by using pure lactic acid bacteria strains and plant-lactic acid bacteria bioproducts. Front Microbiol 10: 1036 https://doi.org/10.3389/fmicb.2019.01036

Carboni AD, Martins GN, Gómez-Zavaglia A, Castilho PC (2023) Lactic acid bacteria in the production of traditional fermented foods and beverages of Latin America. Fermentation 9(4): 315. https://doi.org/10.3390/fermentation9040315

Connell E, Le Gall G, Pontifex MG, Sami S, Cryan JF, Clarke G, Müller M, Vauzour D (2022) Microbial-derived metabolites as a risk factor of age-related cognitive decline and dementia. Mol Neurodegeneration 17(1): 43. https://doi.org/10.1186/s13024-022-00548-6

de Lima Barros SÉ, dos Santos Rocha C, de Moura MSB, Barcelos MP, da Silva Hage-Melim LI (2021) Potential beneficial effects of kefir and its postbiotic, kefiran, on child food allergy. Food Funct 12(9): 3770–3786. https://doi.org/10.1039/D0FO03182H

Devi TS, Vijay K, Vidhyavathi RM, Kumar P, Govarthanan M, Kavitha T (2021) Antifungal activity and molecular docking of phenol, 2,4-bis (1,1-dimethylethyl) produced by plant growth-promoting actinobacterium *Kutzneria* sp. strain TSII from mangrove sediments. Arch Microbiol 203: 4051–4064. https://doi.org/10.1007/s00203-021-02397-1

Dopazo V, Illueca F, Luz C, Musto L, Moreno A, Calpe J, Meca G (2023) Evaluation of shelf life and technological properties of bread elaborated with lactic acid bacteria fermented whey as a bio-preservation ingredient. LWT 174: 114427. https://doi.org/10.1016/j.lwt.2023.114427

Dušková M, Šedo O, Kšicová K, Zdráhal Z, Karpíšková R (2012) Identification of lactobacilli isolated from food by genotypic methods and MALDI-TOF MS. Int J Food Microbiol 159(2): 107–114. https://doi.org/10.1016/j.ijfoodmicro.2012.07.029

Foley MH, O'Flaherty S, Allen G, Rivera AJ, Stewart AK, Barrangou R, Theriot CM (2021) Lactobacillus bile salt hydrolase substrate specificity governs bacterial fitness and host colonization. Proc Natl Acad Sci USA 118(6): e2017709118. https://doi.org/10.1073/pnas.2017709118

Girma A, Aemiro A (2021) Antibacterial activity of lactic acid bacteria isolated from fermented Ethiopian traditional dairy products against food spoilage and pathogenic bacterial strains. J Food Qual 2021: 1–10. https://doi.org/10.1155/2021/9978561

Goa T, Beyene G, Mekonnen M, Gorems K (2022) Isolation and characterization of lactic acid bacteria from fermented milk produced in Jimma Town, Southwest Ethiopia, and evaluation of their antimicrobial activity against selected pathogenic bacteria. Int J Food Sci 2022: 2076021. https://doi.org/10.1155/2022/2076021

Guan X, Xu, Q, Zheng, Y, Qian L, Lin B (2017) Screening and characterization of lactic acid bacteria strains that produce fermented milk and reduce cholesterol levels. Braz J Microbiol 48: 730–739. https://doi.org/10.1016/j.bjm.2017.02.011

He Z, Chen H, Wang X, Lin X, Ji C, Li S, Liang H (2020) Effects of different temperatures on bacterial diversity and volatile flavor compounds during the fermentation of suancai, a traditional fermented vegetable food from northeastern China. LWT 118: 108773. https://doi.org/10.1016/j.lwt.2019.108773

Huppertsberg S, Knepper TP (2018) Instrumental analysis of microplastics—benefits and challenges. Anal Bioanal Chem 410: 6343–6352. https://doi.org/10.1007/s00216-018-1210-8

Ibrahim GA, Sharaf OM, Al-Gamal MS, Youssef AM, Dabiza NM, El-Ssayad MF (2021) Extraction, evaluation and structure elucidation of bioactive metabolites of *Lactobacillus helveticus* CNRZ32. Biointerface Res Appl Chem 11(1): 7667–7688. https://doi.org/10.33263/BRIAC111.76777688

Jaradat N, Ghanim M, Abualhasan MN, Rajab A, Kojok B, Abed R, Mousa A, Arar M (2021) Chemical compositions, antibacterial, antifungal and cytotoxic effects of *Alhagi mannifera* five

extracts. J Complement Integr Med 19(4): 869–877. https://doi.org/10.1515/jcim-2021-020

Javed MR, Salman M, Tariq A, Tawab A, Zahoor MK, Naheed S, Shahid M Ijaz A, Ali H (2022) The antibacterial and larvicidal potential of bis-(2-ethylhexyl) phthalate *from Lactiplantibacillus plantarum*. Molecules 27(21): 7220. https://doi.org/10.3390/molecules27217220

Jung JY, Lee SH, Jeon CO (2014) Kimchi microflora: history, current status, and perspectives for industrial kimchi production. Appl Microbiol Biotechnol 98: 2385–2393. https://doi.org/10.1007/s00253-014-5513-1

Kanak EK, Yilmaz SÖ (2018) Maldi-tof mass spectrometry for the identification and detection of antimicrobial activity of lactic acid bacteria isolated from local cheeses. Food Sci Technol 39: 462–469. https://doi.org/10.1590/fst.19418

Khan IH, Javaid A (2019) Antifungal, antibacterial and antioxidant components of ethyl acetate extract of quinoa stem. Plant Prot 3(3): 125–130. https://doi.org/10.33804/pp.003.03.0150

Kiran GS, Priyadharsini S, Sajayan A, Ravindran A, Selvin J (2018) An antibiotic agent pyrrolo [1, 2-a] pyrazine-1, 4-dione, hexahydro isolated from a marine bacteria *Bacillus tequilensis* MSI45 effectively controls multi-drug resistant *Staphylococcus aureus*. RSC Adv 8(32): 17837–17846. https://doi.org/10.1039%2Fc8ra00820e

Lalitha P, Parthiban A, Sachithanandam V, Purvaja R, Ramesh R (2021) Antibacterial and antioxidant potential of GC-MS analysis of crude ethyl acetate extract from the tropical mangrove plant *Avicennia officinalis* L. S Afr J Bot 142: 149–155. https://doi.org/10.1016/j.sajb.2021.06.023

Lammers A, Zweers H, Sandfeld T, Bilde T, Garbeva P, Schramm A, Lalk M (2021) Antimicrobial compounds in the volatilome of social spider communities. Front Microbiol 12: 700693. https://doi.org/10.3389/fmicb.2021.700693

Lee Y, Cho Y, Kim E, Kim HJ, Kim HY (2018) Identification of lactic acid bacteria in galchi-and myeolchi-jeotgalby 16S rRNA sequencing, MALDI TOF mass spectrometry, and PCR-DGGE. J Microbiol Biotechnol 28(7): 1112–1121. https://doi.org/10.4014/jmb.1803.03034

Liu W, Pang H, Zhang H, Cai Y (2014) Biodiversity of lactic acid bacteria. Lactic acid bacteria: Fundamentals and practice. In: Zhang H, Cai Y (eds). Lactic Acid Bacteria. Dordrecht: Springer, pp. 103–203. https://doi.org/10.1007/978-94-017-8841-0_2

Lulamba TE, Green E, Serepa-Dlamini MH (2021) *Photorhabdus* sp. ETL antimicrobial properties and characterization of its secondary metabolites by gas chromatography–mass spectrometry. Life 11(8): 787. https://doi.org/10.3390/life11080787

Manguntungi B, Mustopa AZ, Meilina L, Nurfatwa M, Vanggy LR, Irawan S, Tamzil MS, Aprilian T, Yulianti (2021) The profile analysis of lactic acid bacteria (LAB) from Sumbawa white honey and its potential producing antibacterial compounds. Walailak J Sci Tech 18(15): 22204–22212. https://doi.org/10.48048/wjst.2021.22204

Mgomi FC, Yang YR, Cheng G, Yang ZQ (2023) Lactic acid bacteria biofilms and their antimicrobial potential against pathogenic microorganisms. Biofilm 5: 100118. https://doi.org/10.1016/j.bioflm.2023.100118

Nacef M, Chevalier M, Chollet S, Drider D, Flahaut C (2017) MALDI-TOF mass spectrometry for the identification of lactic acid bacteria isolated from a French cheese: The Maroilles. Int Food Microbiol 17: 247: 2–8. https://doi.org/10.1016/j.ijfoodmicro.2016.07.005

Ng SC, Hart AL, Kamm MA, Stagg AJ, Knight SC (2009) Mechanisms of action of probiotics: Recent advances. Inflamm Bowel Dis 15(2): 301-310. https://doi.org/10.1002/ibd.20602

Nomiya K, Takahashi S, Noguchi R, Nemoto S, Takayama T, Oda M (2000) Synthesis and characterization of water-soluble silver(I) complexes with l-histidine (H2his) and (S)-(−)-2-pyrrolidone-5-carboxylic acid (H_2pyrrld) showing a wide spectrum of effective antibacterial and antifungal activities. Crystal structures of chiral helical polymers $[Ag(Hhis)]_n$ and $\{[Ag(Hpyrrld)]_2\}_n$ in the solid state. Inorg Chem 39: 3301–3311. http://dx.doi.org/10.1021/ic990526o

O'Sullivan DJ, Giblin L, McSweeney PL, Sheehan JJ, Cotter PD (2013) Nucleic acid-based approaches to investigate microbial-related cheese quality defects. Front Microbiol 4: 1. https://doi.org/10.3389/fmicb.2013.00001

Pinto A, Barbosa J, Albano H, Isidro J, Teixeira P (2020) Screening of bacteriocinogenic lactic acid bacteria and their characterization as potential probiotics. Microorganims 8(3): 393. https://doi.org/10.3390/microorganisms8030393

Rao KP, Chennappa G, Suraj U, Nagaraja H, Raj HNAPC, Sreenivasa, MY (2015) Probiotic potential of lactobacillus strains isolated from sorghum-based traditional fermented food. Probiotics Antimicrob Proteins 7: 146–156. https://doi.org/10.1007/s12602-015-9186-6

Ren D, Zhu J, Gong S, Liu H, Yu H (2018) Antimicrobial characteristics of lactic acid bacteria isolated from homemade fermented foods. BioMed Res Int 2018: 5416725. https://doi.org/10.1155/2018/5416725

Rouis-Soussi LS, Ayeb-Zakhama AE, Mahjoub A, Flamini G, Jannet HB, Harzallah-Skhiri F (2014) Chemical composition and antibacterial activity of essential oils from Tunisian *Allium nigrum* L. Excli J 13: 526–535. https://doi.org/10.17877/DE290R-170

Ruiz L, Margolles A, Sánchez B (2013) Bile resistance mechanisms in *Lactobacillus* and *Bifidobacterium*. Front Microbiol 4: 396. https://doi.org/10.3389/fmicb.2013.00396

Ser HL, Palanisamy UD, Yin WF, Abd Malek SN, Chan KG, Goh BH, Lee LH (2015) Presence of antioxidative agent, pyrrolo [1,2-a] pyrazine-1,4-dione, hexahydro-in newly isolated *Streptomyces mangrovisoli* sp. nov. Front Microbiol 6: 854. https://doi.org/10.3389/fmicb.2015.00854

Shehata MG, El Sohaimy SA, El-Sahn MA, Youssef MM (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. https://doi.org/10.1016/j.aoas.2016.03.001

Supardy A, Ibrahim D, Sulaiman SF, Zakaria NA (2012) Inhibition of *Klebsiella pneumoniae* ATCC 13883 cells by hexane extract of *Halimeda discoidea* (Decaisne) and the identification of its potential bioactive compounds. J Micrboiol Biotechnol 22(6): 872–881. http://dx.doi.org/10.4014/jmb.1111.11053

Surco-Laos F, Garcia JA, Bendezú MR, Laos-Anchante D, Panay-Centeno JF, Valle-Campos M, Palomino-Jhong JJ, Yarasca-Carlos PE, Loja B, Alvarado AT (2022) *In vitro* antioxidant properties and antimicrobial activity of the ethanolic extract of *Senecio nutans* Sch. Beep. (*Asteraceae*). J Pharm Pharmacogn Res 10(6): 1026–1036. https://doi.org/10.56499/jppres22.1471_10.6.1026

Suwannaphan S (2021) Isolation, identification and potential probiotic characterization of lactic acid bacteria from Thai traditional fermented food. AIMS Microbiol 7(4): 431–446. https://doi.org/10.3934%2Fmicrobiol.2021026

Swain MR, Anandharaj M, Ray RC, Rani RP (2014) Fermented fruits and vegetables of Asia: A potential source of probiotics. Biotechnol Res Int 2014: 250424. http://dx.doi.org/10.1155/2014/250424

Teresa RCM, Rosaura VG, Elda CM, Ernesto GP (2014) The avocado defense compound phenol-2,4-bis (1,1-dimethylethyl) is induced by arachidonic acid and acts via the inhibition of hydrogen peroxide production by pathogens. Physiol Mol

Plant Pathol 8: 32–41. https://doi.org/10.1016/j.pmpp.2014.05.003

Tiji S, Rokni Y, Benayad O, Laaraj N, Asehraou A, Mimouni M (2021) Chemical composition related to antimicrobial activity of Moroccan *Nigella sativa* L. extracts and isolated fractions. Evid Based Complement Alternat Med 2021: 8308050. https://doi.org/10.1155/2021/8308050

Vlachojannis C, Chrubasik-Hausmann S, Hellwig E, Al-Ahmad A (2015) A preliminary investigation on the antimicrobial activity of Listerine®, its components, and of mixtures thereof. Phytother Res 29(10): 1590–1594. https://doi.org/10.1002/ptr.5399

Wang Y, Wu J, Lv, M, Shao Z, Hungwe M, Wang J, Bai X, Xie J, Wang Y, Geng W (2021) Metabolism characteristics of lactic acid bacteria and the expanding application in food industry. Front Bioeng Biotechnol 9: 612285 https://doi.org/10.3389/fbioe.2021.612285

Xinran LV, Miao L, Ma, H, Bai F, Lin Y, Sun M, Li J (2018) Purification, characterization and action mechanism of plantaricin JY22, a novel bacteriocin against *Bacillus cereus* produced by *Lactobacillus plantarum* JY22 from golden carp intestine. Food Sci Biotechnol 27(3): 695–703. https://doi.org/10.1007/s10068-017-0280-2

Yan Z, Zhang Y, Wu H, Yang M, Zhang H, Hao Z, Jiang H (2017) Isolation and characterization of a bacterial strain *Hydrogenophaga* sp. PYR1 for anaerobic pyrene and benzo [a] pyrene biodegradation. RSC Avd 7(74): 46690–46698. https://doi.org/10.1039/C7RA09274A

Yi L, Qi T, Hong Y, Deng L, Zeng K (2020) Screening of bacteriocin-producing lactic acid bacteria in Chinese homemade pickle and dry-cured meat, and bacteriocin identification by genome sequencing. LWT 125: 109177. https://doi.org/10.1016/j.lwt.2020.109177

Zabat MA, Sano WH, Wurster JI, Cabral DJ, Belenky P (2018) Microbial community analysis of sauerkraut fermentation reveals a stable and rapidly established community. Foods 7(5): 77. https://doi.org/10.3390/foods7050077

Zhang L, Wang L, Yi L, Wang X, Zhang Y, Liu J, Guo X, Liu L, Shao C, Lü X (2017) A novel antimicrobial substance produced by *Lactobacillus rhamnous* LS8. Food Control 73: 754–760. https://doi.org/10.1016/j.foodcont.2016.09.028

Zhang M, Yao M, Lai T, Zhao H, Wang Y, Yang Z (2021) Response of *Lactiplantibacillus plantarum* NMGL2 to combinational cold and acid stresses during storage of fermented milk as analyzed by data-independent acquisition proteomics. Foods 10(7): 1514. https://doi.org/10.3390/foods10071514.

Zorriehzahra MJ, Delshad ST, Adel M, Tiwari R, Karthik K, Dhama K, Lazado CC (2016) Probiotics as beneficial microbes in aquaculture: an update on their multiple modes of action: A review. Vet Q 36(4): 228–241. https://doi.org/10.1080/01652176.2016.1172132

AUTHOR CONTRIBUTION:

Contribution	Techaoei S	Jamkom K	Dumrongphuttidecha T	Khobjai W
Concepts or ideas	x	x	x	x
Design	x			x
Definition of intellectual content		x	x	
Literature search	x	x	x	
Experimental studies	x			
Data acquisition	x			x
Data analysis				x
Statistical analysis	x			x
Manuscript preparation	x			
Manuscript editing	x			x
Manuscript review	x	x	x	x

Citation Format: Techaoei S, Jamkom K, Dumrongphuttidecha T, Khobjai W (2023) Bioactive compound and chemical characterization of lactic acid bacteria from fermented food as bio-preservative agents to control food-borne pathogens. J Pharm Pharmacogn Res 11(6): 1044–1055. https://doi.org/10.56499/jppres23.1713_11.6.1044

Supplementary data

Figure 1S. Mass spectrometry detection of metabolites of lactic acid bacteria.

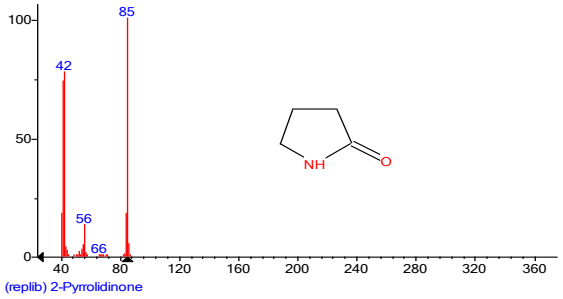
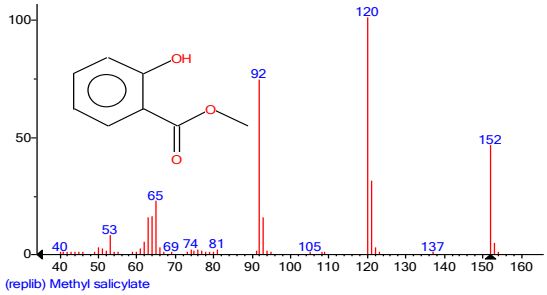
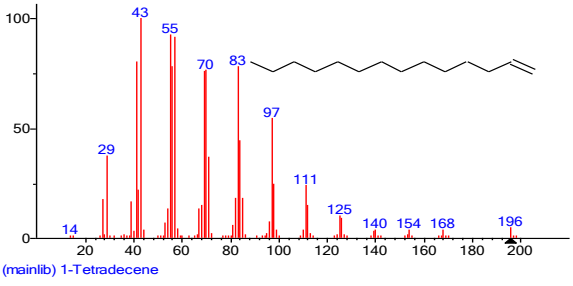
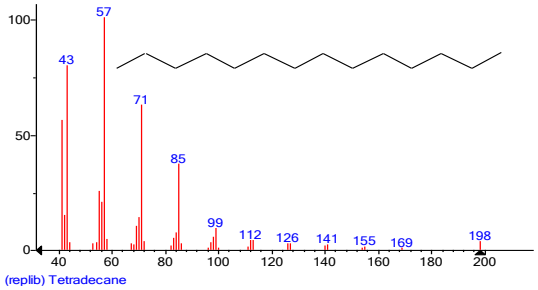
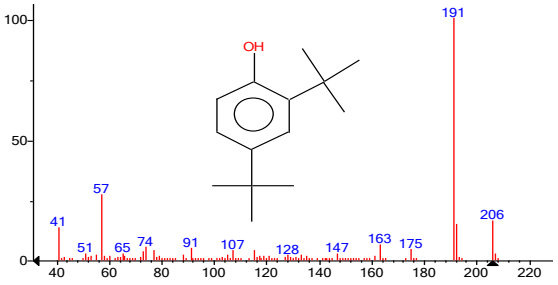
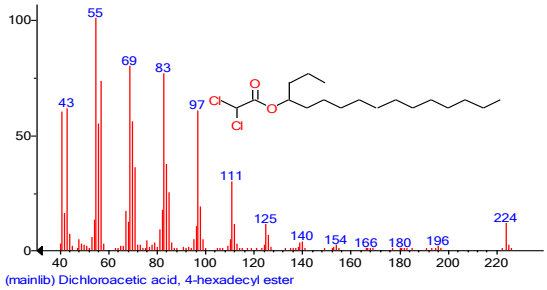
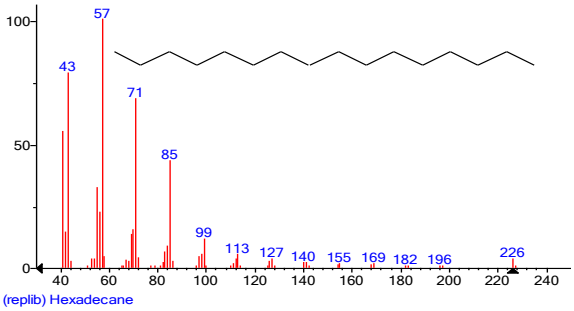
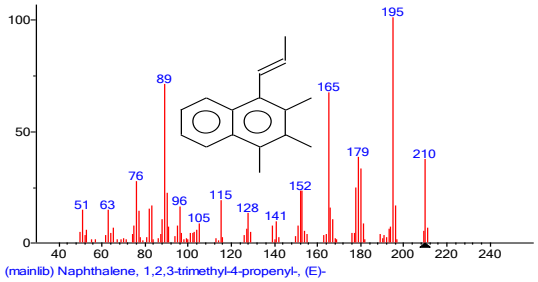
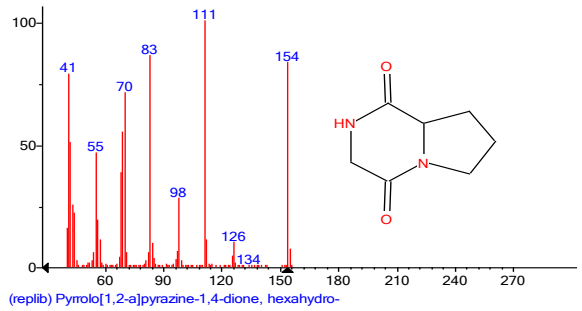
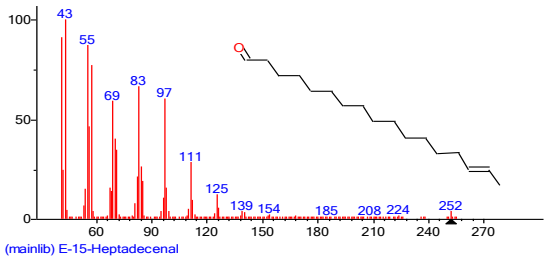
<table>
<tr><td> 
</td><td> 
</td></tr>
<tr><td>2-Pyrrolidinone
(Pyrrolidin-2-one)</td><td>Methyl salicylate
(Methyl 2-hydroxybenzoate)</td></tr>
<tr><td> 
</td><td> 
</td></tr>
<tr><td>1-Tetradecene
(Tetradec-1-ene)</td><td>Tetradecane
(Tetradecane)</td></tr>
<tr><td> 
</td><td> 
</td></tr>
<tr><td>l,2,4-bis(1,1-dimethylethyl)-
(2,4-ditert-butylphenol)</td><td>Dichloroacetic acid,4-hexadecyl ester
(Hexadecan-4-yl 2,2-dichloroacetate)</td></tr>
<tr><td> 
</td><td> 
</td></tr>
<tr><td>Hexadecane
(Hexadecane)</td><td>Naphthalene,1,2-3-trimethyl-4-propenyl,-(E)-
(1,2,3-Trimethyl-4-((E)-prop-1-enyl)naphthalene)</td></tr>
</table>

Figure 1S. Mass spectrometry detection of metabolites of lactic acid bacteria (continued...)



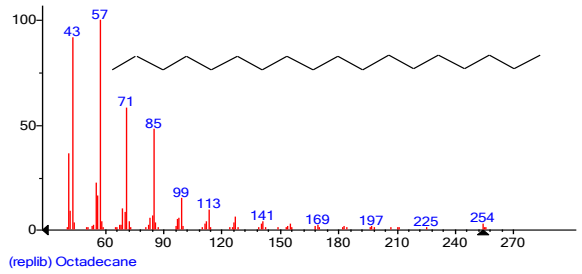
Pyrrolo [1,2-a]pyrazine-1,4-dione,hexahydro

((8aR)-8a-prop-2-enyl-3,6,7,8-tetrahydro-2H-pyrrolo[1,2-a]pyrazine-1,4-dione)



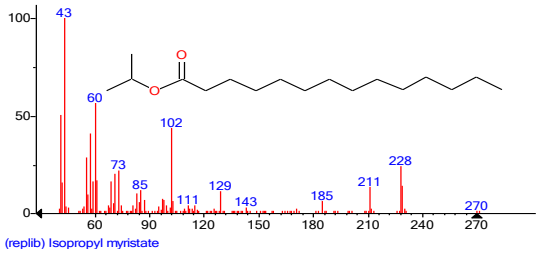
E-15-Heptadecenal

(E)-heptadec-15-enal



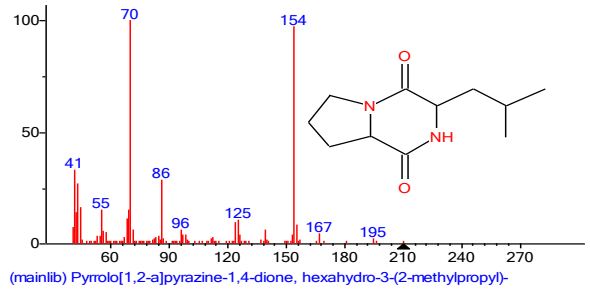
Octadecane

(Octadecane)



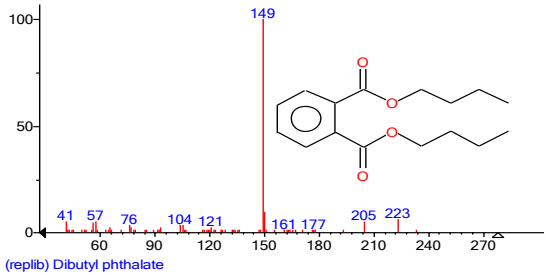
Isopropyl myristate

(Propan-2-yl tetradecanoate)



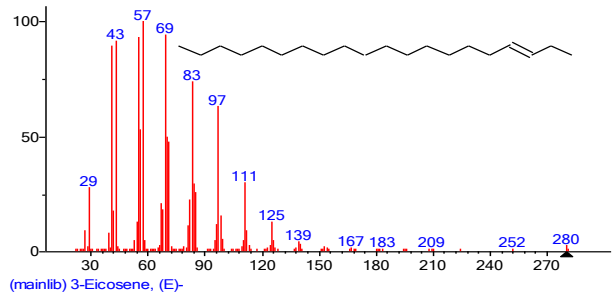
Pyrrolo [1,2-a]pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl)-

(3-(2-methylpropyl)-2,3,6,7,8,8a-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione)



Dibutyl phthalate

(Dibutyl benzene-1,2-dicarboxylate)



3-Eicosene,(E)-

((E)-icos-3-ene)

The compounds names in parenthesis were wrote according to IUPAC (https://iupac.org/).