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Isolation, characterisation, and identification of lactic acid bacteria with high antibacterial activity from commercial fermented papaya-shrimp products

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Abstract

In the present work, the antibacterial potential of lactic acid bacteria (LAB) was investigated from a traditional and region-specific fermented food of southern Vietnam, known as fermented papaya-shrimp (FPS) – a distinctive fermentation matrix combining fruit and seafood ingredients. From 50 FPS samples, 500 single colonies were selected based on their appearance, colour, and structural characteristics, which suggested a resemblance to LAB species on MRS agar. This number was subsequently reduced to 386 suspected LAB isolates after conducting Gram-staining and biochemical tests. These suspected LAB isolates were then categorised into five groups based on their morphological features observed under the microscope. Among the suspected LAB isolates, L3'A, L3'C, and L5'A exhibited the strongest antibacterial activity against all four test organisms: *Escherichia coli* ATCC 25922, *Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC 14028, *Bacillus cereus* ATCC 11778, and *Staphylococcus aureus* ATCC 6538. The largest inhibition zone was recorded for L3'A against *S. aureus* (11.37 ± 0.68 mm), followed by L3'C (10.98 ± 0.52 mm) and L5'A (10.54 ± 1.11 mm), also against this bacterium, indicating that *S. aureus* was the most sensitive to the LAB isolates obtained in the present work. In contrast, *B. cereus* was the most resistant among the test organisms, with inhibition zones of 6.52 ± 0.43 mm for L3'A, 5.23 ± 0.80 mm for L3'C, and 4.54 ± 0.78 mm for L5'A. Overall, L3'A demonstrated the highest antibacterial effectiveness, followed by L3'C and L5'A. Molecular identification *via* 16S rRNA sequencing indicated that L3'A, L3'C, and L5'A were closely related to *Lactiplantibacillus plantarum* (99.90%), *Lactiplantibacillus plantarum* (100%), and *Levilactobacillus brevis* (100%). While these findings highlighted notable antibacterial potential, particularly for *L. plantarum*, the absence of biochemical characterisation of inhibitory compounds limits confirmation of the presence and activity of bacteriocins.

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Introduction

Lactic acid bacteria (LAB) encompass a diverse group of facultatively anaerobic, Gram-positive, non-spore-forming, catalase-negative, and oxidase-negative bacteria that include various genera, both rod-shaped (bacillus) and spherical (coccus) (Rama *et al*., 2024). They are commonly isolated from fermented dairy, vegetable, and meat products, where they contribute to microbial stability and sensory quality through the production of many bioactive compounds (Khaneghah *et al*., 2020; Rama *et al*., 2024). Several LAB species, particularly those from the genera *Lactobacillus*, *Leuconostoc*, and *Pediococcus*, are also classified as probiotics, and have been shown to inhibit foodborne pathogens *via* competitive exclusion and antimicrobial metabolite production (Yousefi *et al*., 2019). The classification of LAB is primarily based on their morphological characteristics, glucose fermentation pathways, optimal growth temperature ranges, salt tolerance, and motility (König and Fröhlich, 2017). Traditionally, the major LAB genera included *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Streptococcus* (Liu *et al*., 2014). However, the formerly large and heterogeneous genus *Lactobacillus*, which contained over 260 species, was reclassified in 2020 into 25 distinct genera, including an emended *Lactobacillus* and 23 newly proposed genera such as *Lactiplantibacillus*,

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Lacticaseibacillus, *Limosilactobacillus*, *Levilactobacillus*, and *Latilactobacillus* (Zheng *et al.*, 2020).

Extensive research has focused on the application of LAB species across various fields, including the development of fermented and functional foods, animal nutrition, and gastrointestinal therapeutics (Thakur *et al.*, 2024; Sarita *et al.*, 2025). Notably, LAB have been shown to effectively inhibit the growth of various bacterial pathogens through multiple mechanisms, including adherence to epithelial cells, modulation of the immune response, and production of antimicrobial substances (Yousefi *et al.*, 2019; Khaneghah *et al.*, 2020). Furthermore, the application of LAB species, along with their antimicrobial products, plays a vital role in inhibiting pathogenic bacteria in both raw and processed food materials, thereby enhancing preservation. LAB synthesise various antimicrobial compounds, including bacteriocins, hydrogen peroxide, and organic acids such as lactic acid (Reis *et al.*, 2012). Among these, bacteriocins are predominantly used in the food industry to mitigate food spoilage, and reduce the risk of foodborne diseases (Özogul and Hamed, 2018; Ahansaz *et al.*, 2023). They possess several notable characteristics, including non-toxicity, susceptibility to inactivation by digestive tract-related proteases, the potential for genetic engineering, and their function as natural food preservatives (Teshome *et al.*, 2022). These theoretical and practical insights highlight the potential of LAB for biopreservation in food and their application as starter cultures in controlled fermentation processes. As a result, there has been a significant increase in interest surrounding the screening, isolation, and characterisation of LAB from diverse sources in recent years (Alonso *et al.*, 2019).

Until now, only a few studies have investigated LAB communities present in complex, mixed-substrate fermentations that incorporate both fruit and seafood components. These combined matrices may represent novel ecological environments, potentially fostering the development of LAB strains with distinctive metabolic and antibacterial profiles. In Vietnam, fermented papaya-shrimp (FPS) is a mixed-substrate fermented product that combines fruit and seafood ingredients, originating from the Mekong Delta region. Although it is currently produced on an industrial scale, it remains scientifically underexplored in terms of its microbial composition. Therefore, the primary objective of the present work was to isolate and identify LAB species exhibiting high antimicrobial activity from the FPS product. Further research will focus on characterising the antimicrobial compounds derived from these isolates.

Materials and methods

FPS sample collection

The present work was conducted at Nong Lam University, Ho Chi Minh City, Vietnam. Fifty samples of the FPS product were collected from various kiosks and households in Thu Duc Ward, Ho Chi Minh City, Vietnam. All samples were collected either immediately or within 24 h after processing, and kept at room temperature to facilitate the observation of product changes and the daily screening of LAB throughout the 7-day fermentation period.

Bacterial indicators

The four bacterial indicators used in the present work, namely *Escherichia coli* ATCC 25922, *Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC 14028, *Bacillus cereus* ATCC 11778, and *Staphylococcus aureus* ATCC 6538, were inoculated in Nutrient Broth (NB; HiMedia, India), and stored at -5°C for short-term storage (up to one month), or at -20°C with added glycerol for long-term storage.

Inoculation and isolation of LABs from FPS products

Firstly, 10 g of FPS was minced and homogenised in 90 mL of sterilised 0.85% NaCl solution within a sterile bag, creating a 10^{-1} solution. Next, 1 mL of this solution was transferred into a sterile test tube containing 9 mL of sterilised 0.85% NaCl solution to achieve a 10^{-2} dilution. The serial dilution process was continued until the appropriate concentrations were successfully prepared. FPS products were then examined for the presence of LAB at dilutions of 10^{-4}, 10^{-5}, and 10^{-6}.

The FPS dilutions were then spread onto de Man, Rogosa and Sharpe agar (MRS; HiMedia, India) by transferring 100 µL of each dilution onto a Petri dish. Once the agar surface had dried completely, the plates were inverted and incubated under anaerobic conditions at 37°C for 48 h. Afterwards, single colonies with distinct morphological characteristics, such as milky white or yellowish-white colonies, were further sub-cultured by streaking onto fresh

MRS agar, and anaerobically incubated at 37°C for 24 to 48 h (Mulaw *et al*., 2019).

Morphological examination

A standard procedure of Gram-staining was applied to observe the morphological features of LAB isolates (Paray *et al*., 2023). As a result, Gram-positive bacilli/cocci were selected for further study (Goa *et al*., 2022).

Catalase test

The aim of this test was to determine the presence of catalase enzymes in suspected LAB isolates. Catalase-negative bacteria were chosen for further test. Hydrogen peroxide is broken into oxygen and water molecules under the catalysis of catalase enzyme ($2H_2O_2 \longrightarrow 2H_2O + O_2$) which results in bubble formation. The catalase test was performed by adding a drop of 3% H_2O_2 solution onto a glass slide on which a microbial colony was spread after 24 h growth in an appropriate condition (Reiner, 2010).

Oxidase test

A 1% solution of N_1N_1-Tetramethyl-phenyldiamine-dihydrochloride (Titan Biotech; India) was used to test the oxidase characteristic of microbial cell. This solution was dropped into a piece of filter paper. Next, a colony of isolated LAB was picked and spread onto that of filter paper. As a result, the appearance of dark purple colour on the filter paper after 5 to 10 sec indicated a positive result, while no colour change was a negative result for the oxidase test (Shields and Cathcart, 2010).

Antimicrobial activity of LAB isolates

The antimicrobial assessment was conducted using the standard agar well diffusion method on Mueller Hinton Agar (MHA; HiMedia, India) plates. Briefly, 100 mL of MHA was inoculated with 100 µL of overnight indicator cultures that had been incubated at 37°C for 24 h in NB (Chaudhary and Saharan, 2019). Wells with a diameter of 8.0 mm were created using sterilised 1 mL pipette tips. Each well was subsequently filled with 100 µL of cell-free supernatant (CFS) obtained from LAB isolates incubated in MRS broth at 37°C for 24 h. The CFS was prepared by centrifuging the cultures at 12,000 rpm for 15 min at 4°C, followed by pH adjustment to 7.0 using 1 N NaOH (Chaudhary and Saharan, 2019). The concentrations of LAB isolates and indicator bacteria were adjusted to approximately 10^7 CFU/mL, as confirmed by the plate-spreading technique. Ampicillin (10 µg/mL; Duchefa, Netherlands) and sterile MRS broth were used as the positive and negative controls, respectively. The diameter of the inhibition zone (in millimetres) was measured after 24 h of incubation at 37°C using a calliper. The final inhibition diameter was calculated by subtracting the 8.0 mm well diameter from the total zone size, and inhibition was considered positive if the clear zone was ≥ 1 mm (Reuben *et al*., 2019). LAB isolates whose CFS exhibited the largest inhibition zones against the indicator strains were selected for further characterisation. These isolates were preserved in MRS broth supplemented with 20% (v/v) glycerol, and stored at -20°C for subsequent studies (Pisano *et al*., 2022).

Molecular identification of LAB isolates

From the stock of high antibacterial isolate, 3 µL of each stock was inoculated into 5 mL MRS broth, and incubated at 37°C for 24 h to prepare for the DNA extraction. Then, these samples were extracted to collect the DNA by using TopPURE® Bacterial Genomic DNA kits (ABT, Vietnam). Bacterial 16S rRNA gene was amplified from the extracted genomic DNA by polymerase chain reaction (PCR) using the following universal primer set: 27F (5'- AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') (James, 2010). The thermal cycles were performed with an initial denaturation step at 95°C for 5 min, followed by 30 cycles of 95°C for 20 sec, 53°C for 30 sec, and 72°C for 90 sec, with a final extension at 72°C for 5 min. Subsequently, gel electrophoresis was performed using 1% agarose and 1X TAE buffer (ABT, Vietnam) at a voltage of 85V for 30 min to identify any bands of 16S rRNA that were successfully extracted and amplified during the PCR process. PCR products were then purified using QIAGEN PCR Purification KIT (QIAGEN, Inc.), and sequenced by First Base Company (Malaysia). The species of LAB isolates were determined by comparing the identified 16S rRNA sequences to the GenBank database of the National Centre for Biotechnology Information (NCBI) using BLAST. The 16S rRNA sequences were analysed using Chromas Software version 2.6.6, and the phylogenetic tree was constructed by Maximum Likelihood method using MEGA 12 program.

Statistical analysis

One-way analysis of variance (ANOVA) and Tukey's *post hoc* test were performed using Minitab Statistical Software version 22 to determine significant differences between the measured properties of different isolates. Significant differences were considered at $p < 0.05$. Mean ± standard deviation (SD) of three replicates was also calculated by Microsoft Excel 2021.

Results

Isolation of LABs from FPS products

From the initial 50 samples of FPS, they were inoculated, incubated, and isolated on MRS agar to collect pure single colonies and examine their morphological characteristics. Based on general feature of LAB on MRS agar, five groups of colonies that were suspected to be LAB were identified and classified as L1, L2, L3, L4, and L5. Table 1 provides a detailed description of the five-group colonies. Most isolated colonies exhibited round shapes with margin, a flat, convex, or raised surface, and in the milky white, opaque white, or off-white colours. The horizontal diameter of colonies L4, and L5 were larger than that of the three groups L1, L2, and L3.

Morphological and biochemical features of LAB isolates

The five groups of LAB isolated colonies on MRS agar, designated as L1, L2, L3, L4, and L5, were subsequently inoculated into MRS broth at 37°C for 24 h, and were then prepared for Gram-staining, catalase, and oxidase tests. Suspected LAB species were Gram-positive, catalase-negative, and oxidase-negative (Goa *et al*., 2022). Based on the observed biochemical results, morphological features, cell shapes, and arrangements under the microscope of 500 isolates, a total of 386 LAB isolates were identified. These isolates were then classified into five new groups - L1', L2', L3', L4', and L5' - corresponding to the original groups L1, L2, L3, L4, and L5 on MRS agar, respectively.

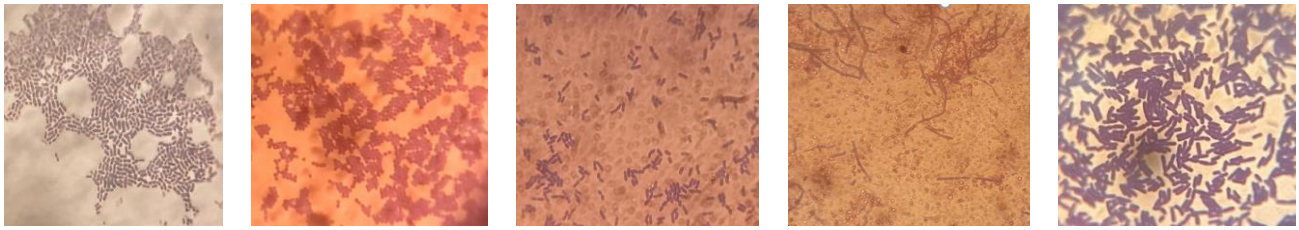
The majority of suspected LAB isolates were rod-shaped, comprising over 90% of the total, which amounted to 349 out of 386 isolates. In contrast, the percentage of spherical LAB isolates L2' was below 10%, totalling 37 out of 386 isolates from 50 FPS samples. Among the rod-shaped isolates, groups L3' and L5' were the most prevalent, with the counts of both isolates being 132 and 96, respectively. The morphological characteristics and number of isolates are described in Figure 1 and Table 2.

Antimicrobial activity of LAB isolates

From each group of isolated LABs, three isolates were randomly selected and labelled as A, B, and C for antimicrobial testing using the agar well diffusion method. The incubated LAB isolates were thoroughly centrifuged as previously described to obtain the CFS for testing. The antibacterial ability of each isolated LAB was assessed by measuring the diameter of the clearing zone surrounding the well.

Table 1. Morphological characteristics of isolated colonies on MRS agar.

Group	Colour	Morphological characteristic
L1	Milky white	Small round shape, entire margin, raised surface
L2	Milky white	Small round shape, entire margin, convex surface
L3	Milky white	Large round shape, entire margin, flat surface
L4	Opaque white	Large round shape, entire margin, convex surface
L5	Off white	Large round shape, entire margin, convex surface



(a) **(b)** **(c)** **(d)** **(e)**

Figure 1. Morphology of suspected LAB isolates under microscope. **(a)** Group L1'; **(b)** Group L2'; **(c)** Group L3'; **(d)** Group L4'; and **(e)** Group L5'.

Table 2. Quantity, morphological, and biochemical characteristics of suspected LAB isolates.

Group	Quantity of isolate	Gram staining	Cell morphology	Catalase	Oxidase
L1’	68	+	Coccobacillus shape	-	-
L2’	37	+	Coccus shape	-	-
L3’	132	+	Short rod shape	-	-
L4’	53	+	Long chain shape	-	-
L5’	96	+	Long rod shape	-	-

(+): Positive; (-): Negative.

It was observed that *S. aureus* ATCC 6538 was the most susceptible to the CFS of LAB isolates, followed by *E. coli* ATCC 25922, *Salmonella* Typhimurium ATCC 14028, and *B. cereus* ATCC 11778, respectively. All of the CFS from the 15 LAB isolates with the highest antibacterial activity demonstrated inhibition against *S. aureus*. Amongst all of the LAB isolates, L3’A, L3’C, and L5’A showed the strongest antibacterial ability. Against *S. aureus,* L3’A exhibited the largest inhibition zone, averaging 11.37 ± 0.68 mm, followed by L3’C at 10.98 ± 0.52 mm, and L5’A at 10.54 ± 1.11 mm.

Against *E. coli*, *Salmonella* Typhimurium, and *B. cereus*, L3’A was also showed the highest inhibition with the average clearing-zone at 7.03 ± 0.72, 4.07 ± 0.54, and 6.52 ± 0.43 mm, respectively. Next, L3’C showed strong inhibition at 6.25 ± 0.86 mm against *E. coli*, 2.89 ± 0.57 mm against *Salmonella* Typhimurium, and 5.23 ± 0.80 mm against *B. cereus*. Finally, L5’A inhibited *E. coli* at 5.05 ± 0.76, *Salmonella* Typhimurium at 3.78 ± 0.57 (larger than that of L3’C), and *B. cereus* at 4.54 ± 0.78 mm.

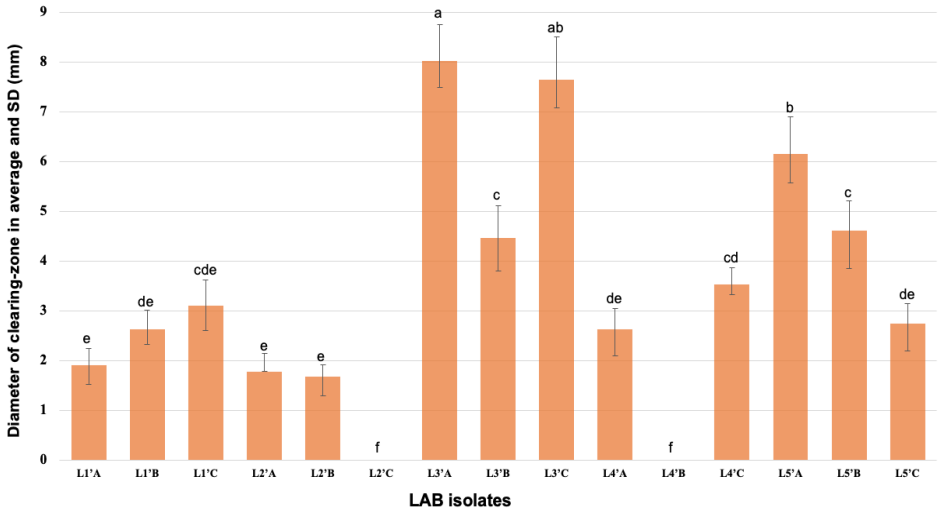
The mean antibacterial activity of each LAB isolate against four different indicator bacteria is displayed in Figure 2. Among the tested LAB isolates, L3’A, L3’C, and L5’A exhibited the strongest antagonistic activity. Notably, L3’A demonstrated significantly larger inhibition zones against *E. coli* (16.03 ± 0.72 mm), *Salmonella* Typhimurium (14.77 ± 0.54 mm), and *B. cereus* (14.63 ± 0.47 mm) compared to L3’C and L5’A ($p < 0.05$). The corresponding inhibition zones for L3’C were 15.65 ± 0.86, 13.59 ± 0.57, and 13.23 ± 0.80 mm, while those for L5’A were 14.15 ± 0.76, 13.25 ± 0.57, and 12.04 ± 0.78 mm, respectively. In contrast, no significant differences were observed among the three isolates against *S. aureus*; however, all three formed a distinct statistical group with significantly higher inhibition compared to the other tested LAB isolates ($p < 0.05$).

Molecular identification of LAB isolates

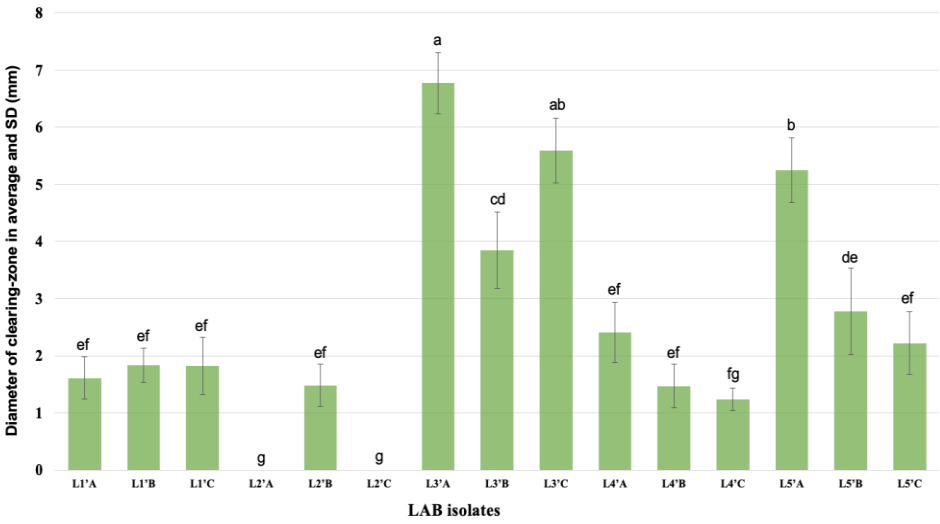
The electrophoresis results of three LAB isolates L3’A, L3’C, and L5’A on the agarose gel are shown in Figure 3. Prominent product bands were observed near the 1,500 bp mark of the standard ladder, corresponding to the expected size of the 16S rRNA gene fragment amplified by the primer pair 27F - 1492R. These findings confirmed the successful amplification of the 16S rRNA gene region from the tested isolates. However, the DNA bands of the three isolates were not perfectly aligned horizontally, which may have resulted from slight variations in the DNA template sequences, or to template fragmentation or degradation during DNA extraction or the PCR process.

The 16S rRNA sequence of three isolates were aligned with their closely related reference bacterial sequences obtained from the GenBank by BLAST. Based on 16S rRNA sequencing and RNA sequence alignment analysis, L3’A and L3’C exhibiting strong antibacterial activity showed > 99% sequence identity with members of the genus *Lactiplantibacillus*, and L5’A was identified as belonging to the genus *Levilactobacillus*. All sequences were submitted to GenBank, and assigned accession numbers: L3’A (PV876510), L3’C (PV876511), and L5’A (PV876512). Specifically, strain L3’A was identified as *Lactiplantibacillus plantarum* (MW857478.1), L3’C as *Lactiplantibacillus plantarum* (MW857478.1), and L5’A as *Levilactobacillus brevis* (PQ396269.1). Additionally, the phylogenetic tree showed that L5’A was closely related to *Levilactobacillus brevis*, while L3’A and L3’C were matched completely with *Lactiplantibacillus plantarum* at 100% (Figure 4).

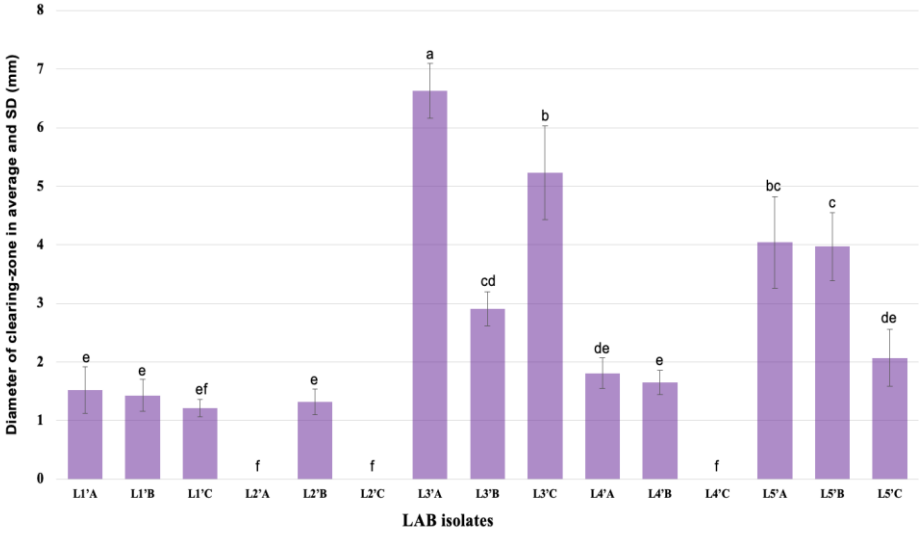
(a)


Diameter of clearing-zone in average and SD (mm)
0 1 2 3 4 5 6 7 8 9
e de cde e e f a c ab de f cd b c de
L1'A L1'B L1'C L2'A L2'B L2'C L3'A L3'B L3'C L4'A L4'B L4'C L5'A L5'B L5'C
LAB isolates

(b)


Diameter of clearing-zone in average and SD (mm)
0 1 2 3 4 5 6 7 8
ef ef ef g ef g a cd ab ef ef fg b de ef
L1'A L1'B L1'C L2'A L2'B L2'C L3'A L3'B L3'C L4'A L4'B L4'C L5'A L5'B L5'C
LAB isolates

(c)


Diameter of clearing-zone in average and SD (mm)
0 1 2 3 4 5 6 7 8
e e ef f e f a cd b de e f bc c de
L1'A L1'B L1'C L2'A L2'B L2'C L3'A L3'B L3'C L4'A L4'B L4'C L5'A L5'B L5'C
LAB isolates

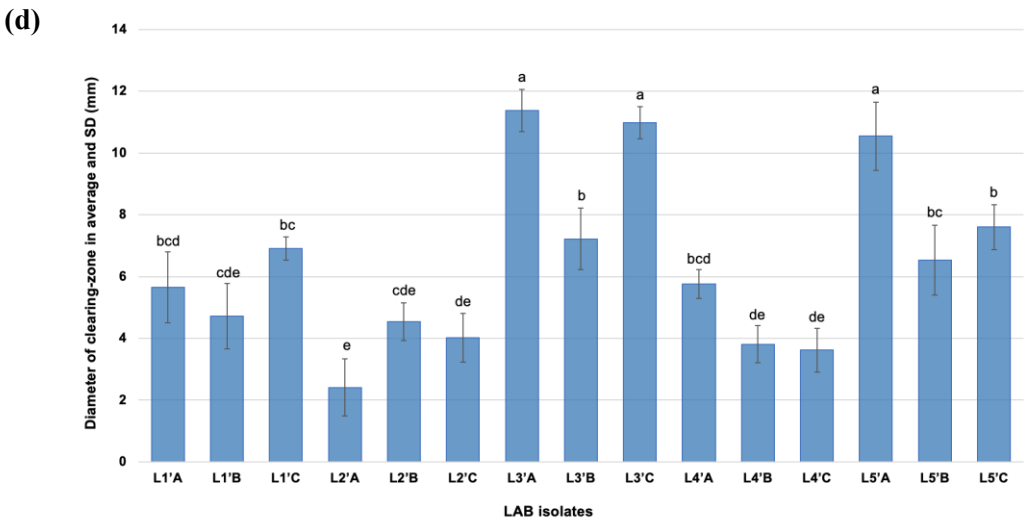


Figure 2. Average clearing-zones diameter (mm) by CFS of LAB isolates against bacterial indicators: **(a)** *Escherichia coli,* **(b)** *Salmonella* Typhimurium, **(c)** *Bacillus cereus,* and **(d)** *Staphylococcus aureus*. Diameter of ZOI is expressed as mean ± SD. Different lowercase letters on columns indicate statistically significant differences at $p < 0.05$.

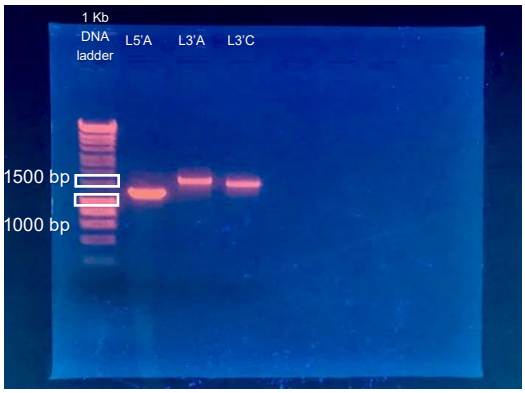


Figure 3. PCR product of three LAB isolates L5'A, L3'A, and L3'C on gel electrophoresis.

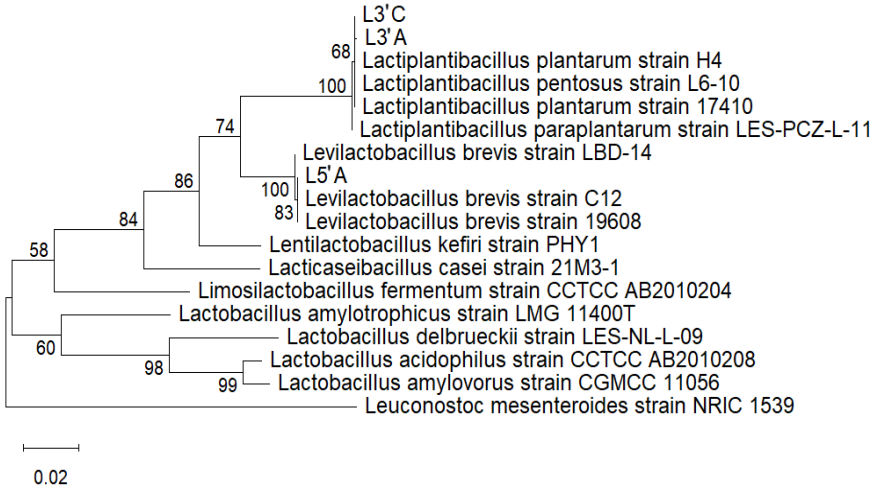


Figure 4. 16S rRNA phylogenetic tree with three 16S rRNA genes of isolated strain (L3'A, L3'C, L5'A) were sequenced and aligned with 14 sequences of 16S rRNA from GenBank (access number: OQ096585.1; OP804298.1; MW866806.1; PP600284.1; ON287034.1; OQ096536.1; MW714719.1; PQ660953.1; MN911358.1; MF045130.1; AM236149.1; PP658340.1; MF045128.1; MF045127.1), and 16S rRNA sequence of *Leuconostoc mensenteroides* (AB023246.1) as outgroup.

Discussion

In the present work, out of 500 isolates obtained from 50 FPS products, only 15 LAB isolates exhibited noticeable inhibition zones against four bacterial indicators. Among them, three LAB isolates: L3'A, L3'C, and L5'A exhibited outstanding antibacterial activities against all four bacterial pathogens, which were then respectively identified as *Lactiplantibacillus plantarum* and *Levilactobacillus brevis* based on the 16S rRNA analysis. Lactobacilli and their beneficial characteristics have been broadly studied and analysed in a variety of sources (Yu *et al.*, 2013; Pot *et al.,* 2014). For example, in the studies by Yang and Chang (2010) and Khemariya *et al*. (2016), *Lactiplantibacillus plantarum* and *Levilactobacillus brevis* were successfully isolated and identified from several plant-based fermented products. Due to recent changes in the taxonomic classification, *Lactobacillus plantarum* was currently renamed as *Lactiplantibacillus plantarum*, and *Lactobacillus brevis* was known as *Levilactobacillus brevis*, respectively (Salvetti *et al*., 2012; Yilmaz *et al*., 2022). The genus *Lactobacillus* historically encompassed 261 species exhibiting extensive diversity at the phenotypic, ecological, and genotypic levels. However, a recent taxonomic revision by Zheng *et al*. (2020) and colleagues proposed the reclassification of the genus into 25 distinct genera including *Acetilactobacillus*, *Agrilactobacillus*, *Amylolactobacillus*, *Apilactobacillus*, *Bombilactobacillus*, *Companilactobacillus*, *Dellaglioa*, *Fructilactobacillus*, *Furfurilactobacillus*, *Holzapfelia*, *Lentilactobacillus*, *Levilactobacillus*, *Lactiplantibacillus*, *Lacticaseibacillus*, *Lactobacillus*, *Lapidilactobacillus*, *Latilactobacillus, Ligilactobacillus, Limosilactobacillus, Liquorilactobacillus, Loigolactobacillus*, *Paucilactobacillus*, *Paralactobacillus*, *Schleiferilactobacillus*, and *Secundilactobacillus*.

In the antagonistic test, the highest sensitivity of *S. aureus* to the CFS of LABs recorded in the present work was similar to the research of Goa *et al*. (2022) and Girma and Aemiro (2021), with the average inhibition zone being in the range of 8 - 14 mm. The study conducted in Jimma Town also reported a strong inhibitory effect of LAB-derived CFS against *E. coli*, with inhibition zones ranging from 2.2 ± 1.9 to 12 ± 1.8 mm (Goa *et al*., 2022). Inhibition zones were also recorded for *Salmonella* Typhimurium and *B. cereus*, though these bacteria showed lower sensitivity to the CFS of LAB. In the present work, the highest inhibition zones were recorded for isolate L3'A, with 6.77 ± 0.54 mm against *Salmonella* Typhimurium and 6.63 ± 0.47 mm against *B. cereus*, which were slightly lower than those reported in previous studies, such as 7.5 mm against *Salmonella* Typhimurium (Sari *et al.*, 2018) and 9.5 ± 0.6 mm against *B. cereus* (Cizeikiene *et al.*, 2013). Another study by Hussein *et al.* (2024) reported that *Lactiplantibacillus plantarum* KR3, isolated from kefir, exhibited significantly larger inhibition zones, including an 8 mm well diameter, against *E. coli* (23 ± 1.64 mm), *Salmonella* Typhimurium (17.1 ± 1.70 mm), and *S. aureus* (20 ± 0.34 mm), compared to L3'A in the present work, which showed inhibition zones of 14.03 ± 0.72, 14.77 ± 0.54, and 16.63 ± 0.47 mm, respectively. Nevertheless, the three LAB isolates tested herein demonstrated robust antimicrobial activity that surpassed many other LAB strains reported in previous studies (Hu *et al.*, 2019; García *et al.*, 2022).

The broad-spectrum antimicrobial activity observed in some *Lactobacillus* species has been widely reported (Jamuna and Jeevaratnam, 2004; Kariyawasam *et al*., 2020; Pisano *et al.*, 2022). The inhibitory effect of LAB isolates is primarily attributed to their production of various antimicrobial metabolites, including organic acids, hydrogen peroxide, diacetyl compounds, bacteriocins, and in some cases, reuterin. Among these, organic acids such as lactic and acetic acids are particularly known for their broad-spectrum activity, as they lower environmental pH, and inhibit the growth of a wide range of microorganisms (Stoyanova *et al.*, 2012). Hydrogen peroxide is produced under aerobic conditions when enzymes like NADH oxidase are active, and it exerts antimicrobial effects especially in the absence of catalase (Reis *et al.*, 2012; Detha and Datta, 2016). Susceptible genera include *Lactococcus* and *Pseudomonas* (Sanam *et al.*, 2022). Diacetyl interferes with the growth and survival of pathogens such as *Aeromonas*, *Bacillus*, *Escherichia*, *Salmonella*, and *Yersinia* (Šušković *et al.*, 2010; Sanam *et al.*, 2022). Bacteriocins, proteinaceous compounds secreted by many LAB strains, disrupt bacterial membranes, leading to growth inhibition or cell death (Parada *et al.*, 2007). Additionally, under anaerobic conditions, certain LAB species like *L. brevis*, *L. reuteri*, and *L. buchneri* can produce

reuterin, which displays potent activity against both bacteria and moulds (Šušković *et al.*, 2010; Lee *et al.*, 2018).

Variations in antibacterial activity among LAB strains may come from differences in bacteriocin-encoding genes or levels of metabolite production (Grossart *et al.*, 2004; Hughes and Andersson, 2017; Wang *et al.*, 2024). Moreover, the origin of isolates – especially those from food sources with low microbial competition – may influence their antimicrobial potential (Łepecka *et al.*, 2021). Experimental factors, such as preparation of the CFS, LAB concentration, and indicator strain density, can also affect the observed inhibitory effects (Sharma and Lee, 2025).

Besides the promising results, the present work had several limitations that should be acknowledged and addressed to guide future research. One major limitation was the lack of in-depth characterisation of bacteriocin production in the potential LAB isolates. Although antibacterial activity was confirmed through pH-neutralised assays, the specific bacteriocins responsible for this activity were neither identified nor analysed. Future research will focus on purifying and characterising these antibacterial peptides using chromatographic and mass spectrometric techniques, such as HPLC and SDS-PAGE, with the aim of comprehensively understanding their structures and antagonistic properties. Another key limitation was on the identification of LAB isolates. While 16S rRNA gene sequencing was performed for molecular identification, it was not sufficient to determine the specific strains of the LAB isolates. Future studies will employ whole-genome sequencing to achieve more precise taxonomic classification.

Conclusion

Lactic acid bacteria (LAB) are originally present in most fermented foods, where they ferment the ingredients, generate various aroma and flavour compounds, and extend the shelf life of food products. In the present work, 386 suspected LAB isolates were successfully obtained from 50 fermented shrimp-papaya (FSP) products collected in Thu Duc Ward, Ho Chi Minh City, Vietnam. These isolates were classified into five groups based on their morphological characteristics. Among these groups, rod-shaped LAB were predominant with the exhibition of four distinctive features.

Subsequently, molecular identification was performed on three isolates that exhibited the strongest antibacterial activity against four indicator bacteria: *Escherichia coli* ATCC 25922, *Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC 14028, *Bacillus cereus* ATCC 11778, and *Staphylococcus aureus* ATCC 6538. As a result, isolates L3'A and L3'C, which produced the largest inhibition zones, were identified as *Lactiplantibacillus plantarum* based on the NCBI database. The third most active isolate, L5'A, was identified as *Levilactobacillus brevis*. In the antibacterial assays, all cell-free supernatants (CFS) were neutralised to eliminate acid effects, thereby indicating that the inhibitory activity was attributable to bioactive metabolites beyond lactic acid. These findings provide a foundation for future characterisation of bacteriocins and other antimicrobial compounds.

Recommendations

Based on the obtained results, further investigation into the antibacterial activity of LAB isolates is necessary to obtain a comprehensive understanding of their influences on both Gram-positive and Gram-negative bacteria. In addition, the two isolates, *Lactiplantibacillus plantarum* and *Levilactobacillus brevis*, identified in the present work should be further examined for probiotic characterisation and considered for potential application as starter cultures in other fermented products or as natural bio-preservatives in various food systems.

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