

# Isolation and probiotic characterization of lactic acid bacteria from “*Nem chua*”, a traditional Vietnamese fermented pork

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**Abstract.** *Truc NT, Thi QVC, Nhung DTK, Hieu TQM, Ut LV, Dang KD. 2026. Isolation and probiotic characterization of lactic acid bacteria from “Nem chua”, a traditional Vietnamese fermented pork. Biodiversitas 27 (2): d270214. <https://doi.org/10.13057/biodiv/d270214>. Nem chua, a renowned traditional Vietnamese fermented pork product, is consumed without heat treatment, thereby posing a potential risk of contamination with pathogenic microorganisms. This investigation concentrated on isolation and characterization Lactic Acid Bacteria (LAB) with potential probiotic properties. In parallel, *Staphylococcus* spp., a pathogenic group commonly associated with foodborne infections in *Nem chua* were also isolated and identified. A total of twenty-one LAB isolates were isolated on MRS (de Man, Rogosa, and Sharpe) medium supplemented with calcium carbonate (CaCO<sub>3</sub>), while six *Staphylococcus* spp. strains were obtained on MacConkey medium from *Nem chua* samples. Probiotic evaluation revealed that all LAB strains produced inhibition zones ranging from 0.63 to 1.56 cm in diameter, as determined by the agar well diffusion method. The acid tolerance of five LAB strains with the strongest antibacterial activity was assessed, with strain L2 exhibiting the highest ΔOD<sub>600</sub> at pH 3.0 after 9 hours of incubation. For bile salt tolerance, three LAB strains were able to grow at 0.30% bile salt, whereas only strain L2 tolerated 1% concentration. Antibiotic susceptibility testing indicated that 42.1% of LAB strains were sensitive to doxycycline, whereas resistance to streptomycin, ciprofloxacin, levofloxacin, and vancomycin were observed in all strains. Furthermore, all LAB strains produced amylase and cellulase, but only three strains (L2, L8, and L16) exhibited extracellular protease activity. Molecular identification based on 16S rRNA sequencing confirmed that strains L2, L8, and L16 corresponded to *Lactobacillus plantarum*, *L. farciminis*, and *Lactiplantibacillus argenteratensis*, respectively. These findings underscore the potential application of isolated LAB isolates for enhancing the safety and quality of traditional *Nem chua*. The identified strains may be associated as utilitarian starter social orders to advance microbial security, material characteristics, and by and expansive quality consistency in customary developed meat era.*

**Keywords:** Lactic acid bacteria, *Lactobacillus plantarum*, *Nem chua*, probiotics, Vietnamese fermented pork

## INTRODUCTION

*Nem chua*, a culturally significant Vietnamese fermented pork product, has a long-standing presence in Vietnamese cuisine and is widely consumed across the country (Nguyen et al. 2010; Pilasombut et al. 2015). The spontaneous fermentation of this product involves a complex microbial community, among which Lactic Acid Bacteria (LAB) play a dominant role (Anh et al. 2015; Nguyen et al. 2015). LAB are essential not only for the development of characteristic flavor of *Nem chua* but also for its preservation, primarily through lactic acid production and competitive inhibition of spoilage and pathogenic microorganisms (Nguyen et al. 2010). However, under traditional small-scale production practices, *Nem chua* may become contaminated with foodborne pathogens like *Salmonella* spp., *Escherichia coli*, and *Staphylococcus aureus*, alongside beneficial microorganisms (Anal et al. 2020; Dang et al. 2024; Quach et al. 2024).

Probiotics are characterized as live microorganisms that bestow a wellbeing advantage to the host when managed in satisfactory sums (FAO/WHO 2002). Among them, LAB are broadly utilized as starter or aid societies in aged meat items due to their capacity to progress microbiological security, tangible quality, and dietary esteem (Hugas et al. 2003; Calo et al. 2018; Drosinos et al. 2021). These microscopic organisms restrain the development of pathogens through fermentation, bacteriocin generation, and competition for supplements, whereas contributing to alluring organoleptic characteristics such as smell and surface (Leroy and De Vuyst 2020). Their metabolic flexibility and security profile have driven to developing intrigued in segregating innate LAB strains with probiotic potential for moving forward conventional matured nourishments.

LAB are widely associated with traditional fermented foods, such as fermented vegetables and fish sauce (Swain et al. 2014; Cai et al. 2024). In Vietnam, several studies have documented the considerable diversity of LAB

associated with *Nem chua* (Nguyen et al. 2015; Phan et al. 2017). A survey of more than 60 *Nem chua* samples collected from different regions, including Ho Chi Minh City, Hue, Thanh Hoa, and Ha Noi, revealed that LAB strains were predominant, comprising approximately 80–100% of the total aerobic bacterial population. The most frequently identified LAB species were *Lactiplantibacillus plantarum* (67.6%), *Pediococcus pentosaceus* (21.6%), *Levilactobacillus brevis* (9.5%), and *Lactobacillus farciminis* (1.3%) (Le et al. 2011). Similarly, a study on *Nem chua* samples collected in Ha Noi reported additional LAB species, including *L. farciminis* (32.8%), *L. plantarum* (26.1%), and *Leuconostoc citreum* (12.6%), as well as other species such as *Lactiplantibacillus pentosus*, *P. pentosaceus*, *Pediococcus acidilactici*, *Leuconostoc fallax*, *Weissella cibaria*, *Weissella paramesenteroides*, *Lactobacillus rossiae*, and *Lactobacillus paracasei* (Doan et al. 2012). Among these, *L. plantarum* strain is particularly valued for its capacity to produce organic acids, enhance sensory attributes, and play a key role in biological preservation.

Numerous prior studies have shown that LAB can produce bacteriocins, proteinaceous compounds capable of inhibiting growth or disrupting the cell structure of foodborne pathogens, such as *S. aureus*, *Listeria monocytogenes*, *E. coli*, and *Salmonella* spp. (Bhattacharya et al. 2022; Darbandi et al. 2022; Verma et al. 2022; Hernandez-Mendoza et al. 2024). In addition, certain LAB strains exhibit antibacterial activity through the biosynthesis of organic acids (e.g., lactic, acetic, and succinic acids) and hydrogen peroxide (Ibrahim et al. 2021). With these attributes, LAB contribute to enhancing food stability during fermentation and may support the maintenance of beneficial intestinal microbiota (Pilasombut et al. 2015; Vo et al. 2021). Although multiple studies have focused on the isolation and characterization of LAB from various food sources across different regions of Vietnam (Phan et al. 2017; Ong et al. 2020), to date, no publication has evaluated the probiotic characteristics of LAB isolated from *Nem chua* in Vinh Long Province. This study is, therefore, the first to investigate the probiotic characteristics of indigenous LAB from *Nem chua* in this region. In the context of strengthening microbiological safety control in fermented foods, isolating and identifying such strains is essential, as traditional fermentation practices may lead to the loss of valuable LAB traits. Hence, the present study aimed to isolate and evaluate LAB strains with probiotic potential from *Nem chua*. In parallel with isolating LAB, the study also aimed to survey the presence of pathogenic microorganisms (used as indicator bacteria) naturally occurring in traditional *Nem chua*. This concurrent isolation was performed to better understand potential microbial hazards associated with the product, thereby supporting the long-term objective of improving its quality and safety.

## MATERIALS AND METHODS

### Sample collection and bacterial isolation

Ten types of *Nem chua* were collected from ten conventional markets across Vinh Long Province, with at least three samples per type. The samples were stored at 4°C and transported to the laboratory within 24 hours for analysis. The isolation of Lactic Acid Bacteria (LAB) followed the method described by Nguyen et al. (2010). Each 10 g sample was homogenized in 90 mL sterile MRS broth and incubated at 37°C for 48 hours. The enrichment culture was serially diluted to 10<sup>-6</sup>, spread on MRS agar containing 1% CaCO<sub>3</sub>, and incubated at 37°C for 24–48 hours. Colonies shaping clear zones were chosen as hypothetical LAB. Similarly, pathogenic bacteria were isolated according to Akilan et al. (2020). A *Nem chua* sample (10 g) was homogenized in 90 mL of sterile TSB (Tryptone Soy Broth) medium and incubated statically at 37°C for 48 hours. The enriched culture was serially diluted with sterile distilled water up to 10<sup>-3</sup> after incubation. 100 µL of each dilution (10<sup>-1</sup>, 10<sup>-2</sup>, and 10<sup>-3</sup>) was spread onto MacConkey agar plates, followed by incubation at 37°C for 24 to 48 hours. Colonies exhibiting distinct morphological characteristics were collected and subcultured on MacConkey agar media.

### Preliminary screening and characterization

Purified colonies were inspected for colony and cell morphology, Gram and spore staining, catalase and oxidase activities (Barrow and Feltham 2004). The isolates were further examined for LAB characteristics (Gram-positive, non-spore-forming, catalase- and oxidase-negative).

### Evaluation of probiotic and enzymatic properties

Antibacterial activity against indicator pathogens (*Salmonella* spp., *E. coli*, and *Staphylococcus* spp.) was evaluated by the agar well method (Mohammed and Çon 2021). All the three bacteria were cultured on MacConkey agar. In this study, these three bacterial species were chosen because they represent major foodborne pathogens commonly associated with fermented meat products and the broader food production chain. In brief, supernatants from 48-hour MRS medium were set in agar wells on TSA plates seeded with pointer strains, and restraint zones were measured after 24 hours at 37°C.

Hemolytic activity was conducted on 5% sheep blood agar (Casarotti et al. 2017). Strains appearing no clear zones were considered non-hemolytic.

Low pH resistance was evaluated in MRS broth balanced to pH 2.0, 3.0, and 4.0 (Kong et al. 2025). Optical density at 600 nm was observed at 0, 3, 6, 9, and 12 hours to assess practicality beneath acidic conditions.

Bile salt resistance was performed on MRS agar containing 0.1–1.0% bile salts (Quach et al. 2025). Development on media with ≥ 0.3% bile demonstrated great resistance.

Antibiotic susceptibility was determined by the disc diffusion method on MHA (Yao et al. 2021) using antibiotic discs of ceftazidime (CEF, 30 µg), ciprofloxacin (CIP, 5 µg), doxycycline (DOX, 30 µg), erythromycin (ERY, 15 µg), gentamicin (GEN, 10 µg), levofloxacin (LEV, 5 µg), neomycin (NEO, 30 µg), streptomycin (STR, 10 µg), sulfamethoxazole (SUL, 25 µg), and vancomycin

(VAN, 30 µg). Strains were classified as resistant, intermediate, or sensitive according to CLSI (2015) and Sharma et al. (2017).

Extracellular enzyme production (protease, amylase, cellulase) was screened to complement probiotic appraisal. Protease activity was determined on casein agar (Rubak et al. 2020), amylase activity on starch agar utilizing Lugol recoloring (Samanta et al. 2011), and cellulase activity on CMC agar with Congo ruddy recoloring (Abouloifa et al. 2020). Clear zones around colonies indicated positive enzyme activity.

### Molecular identification

Genomic DNA was extracated utilizing the TopPURE® Genomic DNA Extraction Kit (ABT, Vietnam) taking after the manufacturer's protocol. PCR intensification of the LABs and *Staphylococcus* bacteria's 16S rRNA gene was performed utilizing preliminaries 27F (5'-AGAGTTTGATCCTGGCTC-3') and 1492R (5'-TACGGTTACCTTGTTACGACT-3'). Thermal cycling was conducted with an initial denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 52°C for 30 s, and 72°C for 1 min, and a final extension at 72°C for 10 min. PCR products (~1,500 bp) were sequenced commercially, and sequences were compared with those in the NCBI database for identification.

### Statistical analysis

All data were analyzed utilizing Microsoft Exceed expectations 2019 and Statgraphics Centurion XV. Differences were assessed by one-way ANOVA and LSD test at  $p < 0.05$ . Arrangements appearing  $\geq 98\%$  likeness to reference strains were adjusted utilizing CLUSTAL W, and a phylogenetic tree was built in MEGA12 computer program by the neighbor-joining strategy with 1,000

bootstrap imitates (Saitou and Nei 1987; Kumar et al. 2024).

## RESULTS AND DISCUSSION

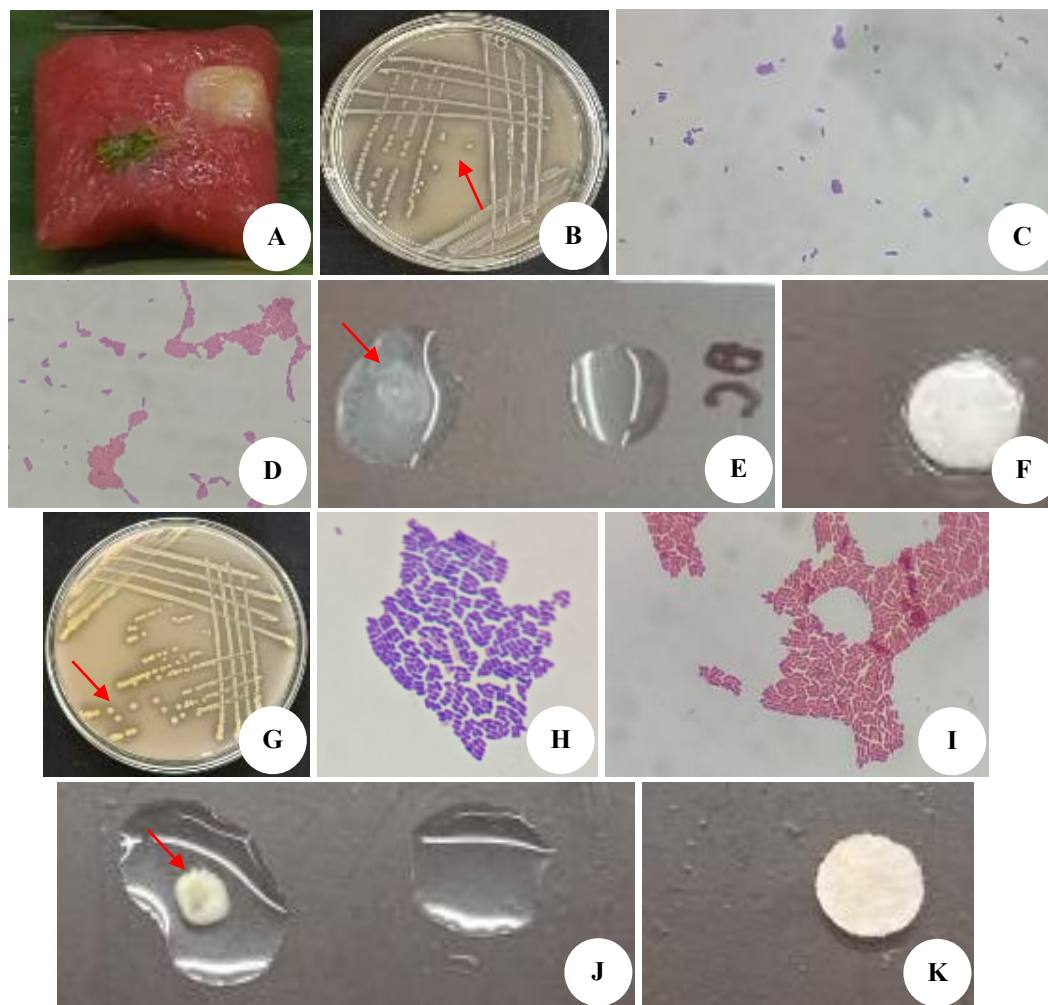
### Isolation of lactic acid bacteria

Isolation and characterization of Lactic Acid Bacteria (LAB) are principal for recognizing strains with probiotic potential and security significance in conventional fermentation. A add up to of 21 LAB strains were isolated from *Nem chua* samples (Figure 1.A) collected from Vinh Long Province. Most confines shown circular, dark, or milky-white colonies with smooth surfaces, extending from level to somewhat arched heights and whole to marginally wrinkled edges (Table 1). All strains were Gram-positive (Figures 1.C and 1.H), non-spore-forming (Figures 1.D and 1.I), and catalase and oxidase negative (Figures 1.E, 1.F, 1.J and 1.K, arrow). Moreover, all isolates produced clear  $\text{CaCO}_3$  degradation zones on MRS agar containing 1%  $\text{CaCO}_3$  (Figures 1.B and 1.G, arrow), confirming their acid-producing nature. The morphological and biochemical characteristics observed in current study are similar to those already detailed for LAB. Lawalata et al. (2023) and Luo et al. (2025) showed similar colony appearances and Gram-positive, catalase-negative highlights in LAB from aged nourishments. Moreover, Jeyagowri et al. (2023) and Zhao et al. (2025) recorded comparable phenotypic characteristics in separates from rice and matured refreshments, separately. Nga et al. (2023) on *Nem chua* moreover detailed circular, milky-white colonies with level or raised surfaces and intaglio edges, encourage approving the consistency of LAB morphology from conventional Vietnamese fermentations.

**Table 1.** Colony morphology and biochemical characteristics of isolated LAB strains from *Nem chua* in Vinh Long Province, Vietnam

| Bacterial isolates | Colony color | Elevation | Margin   | Colony surface | Gram staining | Cell shape | Catalase | Oxidase | Spore |
|--------------------|--------------|-----------|----------|----------------|---------------|------------|----------|---------|-------|
| L1                 | Opaque white | Convex    | Entire   | Smooth         | +             | Rod        | -        | -       | -     |
| L2                 | Milky white  | Convex    | Entire   | Smooth         | +             | Rod        | -        | -       | -     |
| L3                 | Opaque white | Convex    | Entire   | Smooth         | +             | Rod        | -        | -       | -     |
| L4                 | Milky white  | Convex    | Wrinkled | Smooth         | +             | Rod        | -        | -       | -     |
| L5                 | Opaque white | Convex    | Entire   | Smooth         | +             | Rod        | -        | -       | -     |
| L6                 | Opaque white | Convex    | Wrinkled | Smooth         | +             | Rod        | -        | -       | -     |
| L7                 | Opaque white | Convex    | Entire   | Smooth         | +             | Rod        | -        | -       | -     |
| L8                 | Milky white  | Flat      | Serrated | Smooth         | +             | Rod        | -        | -       | -     |
| L9                 | Opaque white | Convex    | Entire   | Smooth         | +             | Rod        | -        | -       | -     |
| L10                | Opaque white | Convex    | Entire   | Smooth         | +             | Rod        | -        | -       | -     |
| L11                | Opaque white | Flat      | Entire   | Smooth         | +             | Rod        | -        | -       | -     |
| L12                | Milky white  | Convex    | Entire   | Smooth         | +             | Rod        | -        | -       | -     |
| L13                | Opaque white | Convex    | Entire   | Smooth         | +             | Rod        | -        | -       | -     |
| L14                | Opaque white | Convex    | Entire   | Smooth         | +             | Rod        | -        | -       | -     |
| L15                | Milky white  | Convex    | Serrated | Smooth         | +             | Rod        | -        | -       | -     |
| L16                | Pale yellow  | Convex    | Entire   | Smooth         | +             | Rod        | -        | -       | -     |
| L17                | Milky white  | Convex    | Entire   | Smooth         | +             | Rod        | -        | -       | -     |
| L18                | Opaque white | Convex    | Entire   | Smooth         | +             | Rod        | -        | -       | -     |
| L19                | Opaque white | Convex    | Entire   | Smooth         | +             | Rod        | -        | -       | -     |
| L20                | Yellow       | Convex    | Entire   | Smooth         | +             | Rod        | -        | -       | -     |
| L21                | Opaque white | Convex    | Entire   | Smooth         | +             | Rod        | -        | -       | -     |

Note: +: Positive reaction, -: Negative reaction



**Figure 1.** Colony morphology and biochemical characteristics of representative LAB strains of L2 and L16 isolated from *Nem chua* in Vinh Long Province, Vietnam. From left to right: A. *Nem chua*, B. Bacterial colony, C. Gram staining, D. Spore staining, E. Catalase reaction, and F. Oxidase reaction of strain L2; G. Bacterial colony, H. Gram staining, I. Spore staining, J. Catalase reaction, and K. Oxidase reaction of strain L16

**Table 2.** Colony morphological and biochemical characteristics of the isolated pathogenic bacteria strains from *Nem chua* in Vinh Long Province, Vietnam

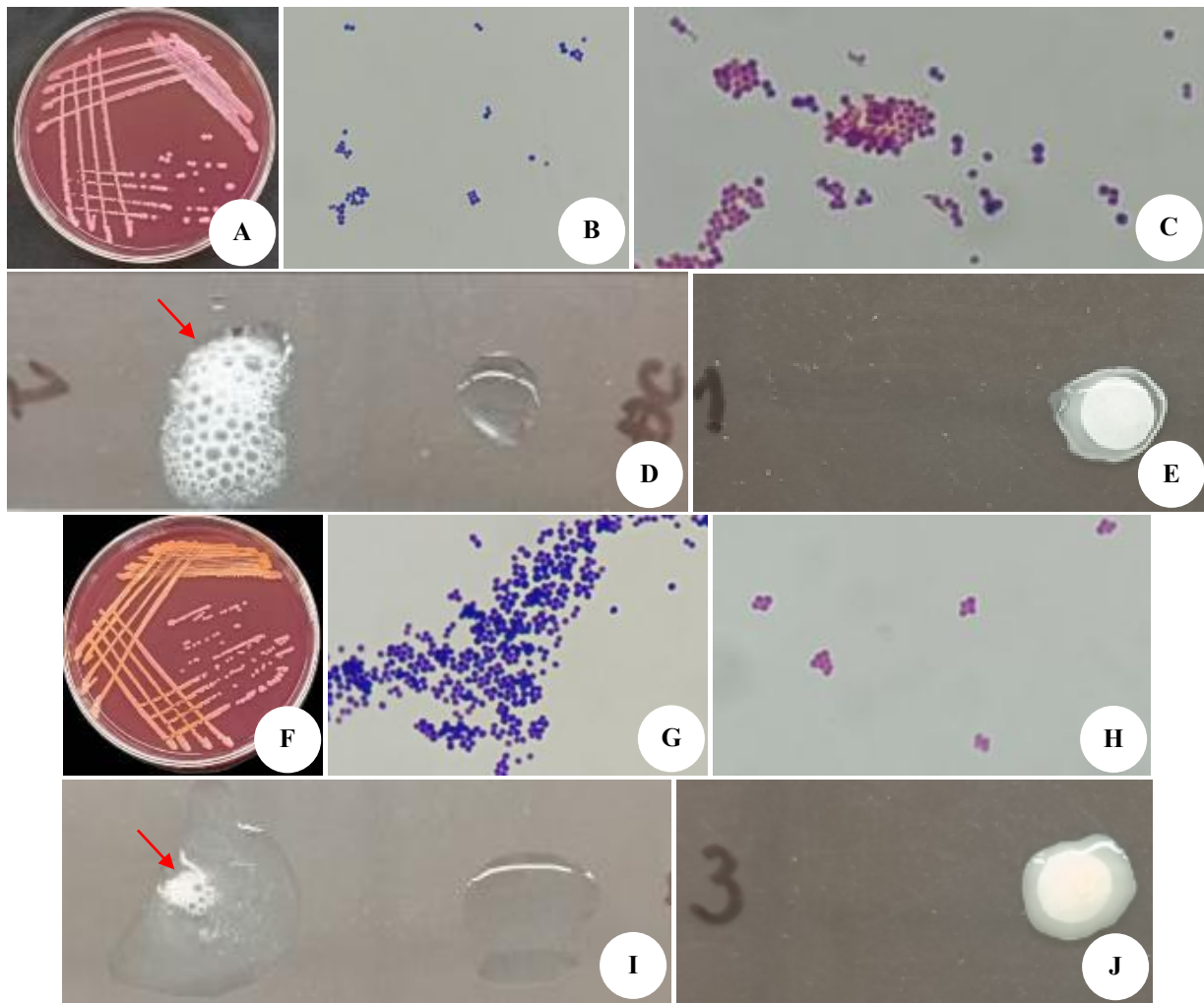
| Bacterial isolate | Colony color | Elevation | Margin | Colony surface | Gram staining | Cell shape | Catalase | Oxidase | Spore |
|-------------------|--------------|-----------|--------|----------------|---------------|------------|----------|---------|-------|
| E1                | Pinkish red  | Convex    | Entire | Smooth         | +             | Coccus     | +        | -       | -     |
| E2                | Pinkish red  | Convex    | Entire | Smooth         | +             | Coccus     | +        | -       | -     |
| E3                | Pinkish red  | Convex    | Entire | Smooth         | +             | Coccus     | +        | -       | -     |
| E4                | Pinkish red  | Convex    | Entire | Smooth         | +             | Coccus     | +        | -       | -     |
| E5                | Pinkish red  | Convex    | Entire | Smooth         | +             | Coccus     | +        | -       | -     |
| E7                | Pinkish red  | Convex    | Entire | Smooth         | +             | Coccus     | +        | -       | -     |

Note: +: Positive reaction, -: Negative reaction

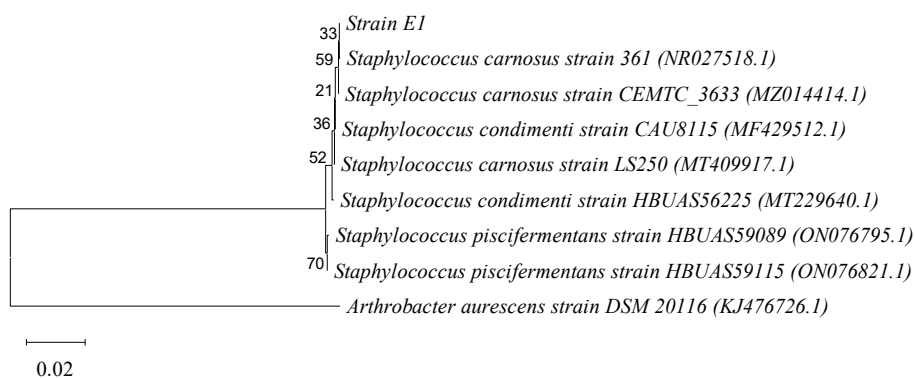
### Isolation of pathogenic bacteria

To survey potential microbial dangers, pathogenic microbes were concurrently separated from the same *Nem chua* tests. Six bacterial strains, assigned E1, E2, E3, E4, E5 and E7, were obtained from MacConkey agar (Table 2). These colonies were circular, smooth, and raised, with whole edges and a characteristic ruddy pigmentation demonstrative of lactose maturation (Figures 2.A and 2.F). All segregates were catalase-positive, spore-negative,

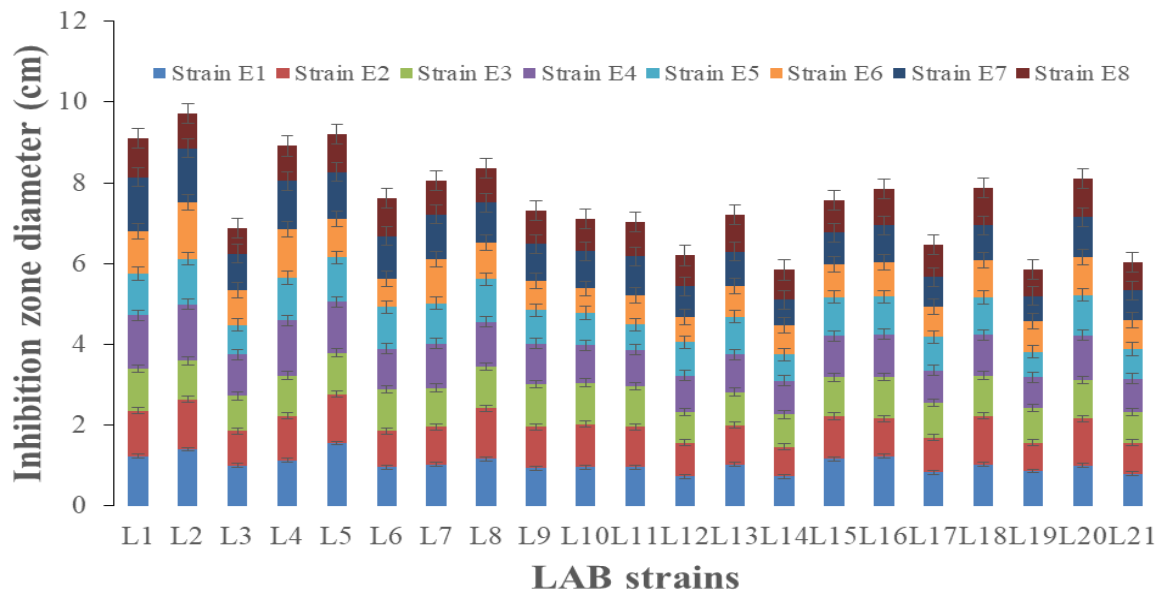
oxidase-negative, and Gram-positive (Figures 2.B-2.E and 2.G-2.J). Molecular analysis of the 16S rRNA gene confirmed that strain E1 belonged to the genus *Staphylococcus*, as supported by the phylogenetic tree analysis (Figure 3). The discovery of *Staphylococcus* from aged meat underscores the significance of screening for astute pathogens in conventional nourishments, as they may start from dealing with or natural defilement.



**Figure 2.** Colony morphology and biochemical characteristics of two representative *Staphylococcus* strains, E1 and E3, isolated from *Nem chua* in Vinh Long Province, Vietnam. From left to right: A. Bacterial colony, B. Gram staining, C. Spore staining, D. Catalase reaction, and E. Oxidase reaction of strain E1, F. Bacterial colony, G. Gram staining, H. Spore staining, I. Catalase reaction, and J. Oxidase reaction of strain E3



**Figure 3.** Phylogenetic tree of the representative pathogenic bacterial strain E1, constructed using the neighbor-joining algorithm with a bootstrap value of 1,000 replicates. *Arthrobacter aureescens* strain DSM 20116 (KJ476726.1) was used as the outgroup



**Figure 4.** Antibacterial activity of LAB strains against pathogenic bacteria strains

### Probiotic characteristics of lactic acid bacteria strains

#### Antibacterial activity

Assessing the antibacterial capacity of LAB confines is basic to decide their capacity to hinder foodborne pathogens and keep up microbial adjust in matured items. All 21 LAB strains displayed quantifiable inhibitory movement against pathogenic microscopic organisms, with hindrance zones extending from  $0.63 \pm 0.05$  cm to  $1.56 \pm 0.05$  cm (Figure 4). Strain L5 appeared the most grounded restraint against *Staphylococcus* strain E1 ( $1.56 \pm 0.05$  cm,  $p < 0.05$ ), while L2, L8, and L16 also demonstrated strong activity ( $1.16$ - $1.40$  cm). In contrast, strains L3, L17, and L21 exhibited lower inhibition ( $0.75$ - $0.89$  cm).

Differences in antibacterial adequacy may reflect varieties within the generation of antimicrobial compounds such as natural acids, hydrogen peroxide, and bacteriocins. Hu et al. (2019) detailed that *L. plantarum* strains separated from dairy items shown particular antibacterial spectra, and Le et al. (2022) watched restraint zones extending from  $1.0$ - $4.0$  mm among LAB from salted vegetables. Natural acids diminish natural pH, stifling pathogen development (Zapašnik et al. 2022), whereas hydrogen peroxide oxidatively harms bacterial layers. Bacteriocins, in spite of the fact that powerful, regularly have a limit inhibitory extend (Telhig et al. 2020) and can be impacted by pH, temperature, and supplement accessibility (Abbasiliasi et al. 2017). The combined generation of these metabolites underlies the antimicrobial potential of LAB in nourishment security settings.

#### Hemolytic ability

Assessing hemolytic activity is essential for confirming biosafety in probiotic candidates. Among 21 LAB isolates, as it were L17 and L21 exhibited  $\beta$ -hemolysis, forming clear zones on blood agar (Figure 5.A), while the remaining strains displayed  $\gamma$ -hemolysis (no lysis; Figure 5.B). According to the European Food Safety Authority

(EFSA), non-hemolytic microorganisms are categorized within the Qualified Presumption of Safety (QPS) group.

This finding adjusts with past reports illustrating low  $\beta$ -hemolytic frequency among LAB (Halder et al. 2017; Abdelhamid et al. 2020; Zhao et al. 2025). Although rare,  $\beta$ -hemolysis can occur in some *Lactobacillus* strains (Kousha et al. 2022) due to virulence-associated genes such as *hly* and *cytLL/S* (Golob et al. 2019). Therefore, LAB strains exhibiting  $\beta$ -hemolysis should be excluded from probiotic development to ensure consumer safety.

#### Low pH tolerance

Survival beneath gastric sharpness may be a prerequisite for probiotic usefulness. Five strains (L1, L2, L5, L8, L16) were assessed at pH 2.0, 3.0, and 4.0 for 12 hours (Figure 6). At pH 2.0, strain L2 maintained the highest  $\Delta OD$  ( $0.17$  at 9 h;  $0.12$  at 12 h), while L8 showed similar tolerance ( $\Delta OD$   $0.15$ ). Strains L1, L5, and L16 were less tolerant ( $0.03$ - $0.11$ ). At H 3.0, all strains exhibited enhanced  $\Delta OD$  ( $0.14$ - $0.20$ ), peaking at 9 hours, while at pH 4.0, development was apparent over all strains.

The stability of  $\Delta OD$  for 6-9 hours corresponds to the typical gastric residence time (2-4 h) reported by Minekus et al. (2014), illustrating corrosive resistance of L2, L8, and L5. Comparative strength beneath acidic conditions has been watched in *L. acidophilus* and *L. reuteri* (Feng et al. 2017; Wang et al. 2018). These results confirm that selected strains can maintain metabolic activity under stomach-like environments, reinforcing their probiotic potential.

#### Bile salt tolerance

Resistance to bile salts reflects the ability of LAB to survive gastrointestinal transit. Five strains (L1, L2, L5, L8, L16) were cultured on MRS agar with  $0.1$ - $1.0\%$  bile salts (Table 3). Strains L1 and L2 supported unmistakable development indeed at  $1\%$  bile concentration, whereas strain

L5 endured up to 0.3%. Strains L8 and L16 were hindered at  $\geq 0.2\%$ .

These findings are reliable with Tokatl et al. (2015) and Ayyash et al. (2021), who reported similar resistance levels in LAB from fermented foods. Mechanistically, bile tolerance is mediated through modifications in membrane lipid composition, efflux pump activation, and stress-response proteins maintaining ion balance (Chen et al. 2022). Therefore, L1 and L2 emerge as strong candidates for gastrointestinal probiotic applications.

#### Antibiotic resistance

Evaluating the anti-microbial resistance of LAB strains is pivotal for assessing their security and compatibility in probiotic applications. Anti-microbial vulnerability testing of LAB segregates uncovered shifted resistance profiles over ten anti-microbials (Figure 7). The most elevated affectability rate was watched for doxycycline (42.10%), whereas 100% resistance happened against vancomycin, streptomycin, ciprofloxacin, and levofloxacin. Resistance to gentamicin and neomycin was also high.

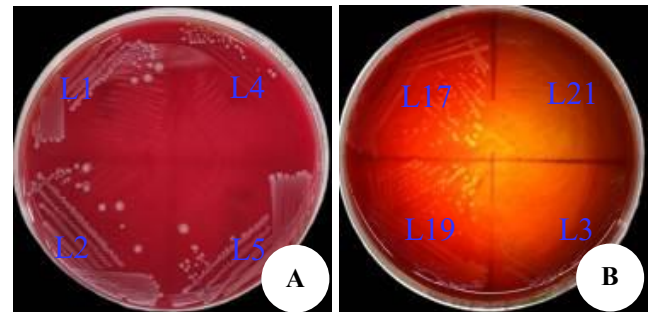
Inborn resistance is commonplace for LAB and is ascribed to film impermeability, modified ribosomal official locales, and efflux instruments (Belay et al. 2024). This characteristic resistance is profitable for keeping up microbial adjust amid anti-microbial treatment (Shahali et al. 2023). As it were portable hereditary components, not chromosomal resistance, posture security concerns (Tong 2021; Zavišić et al. 2023). Strains L2, L8, and L16 exhibited multidrug resistance yet remained susceptible to doxycycline, erythromycin, and ceftazidime. Their capacity to stand up to broad-spectrum anti-microbials but stay

controllable underlines their security and utilitarian potential for keeping up intestine microbiota solidness amid anti-microbial treatment.

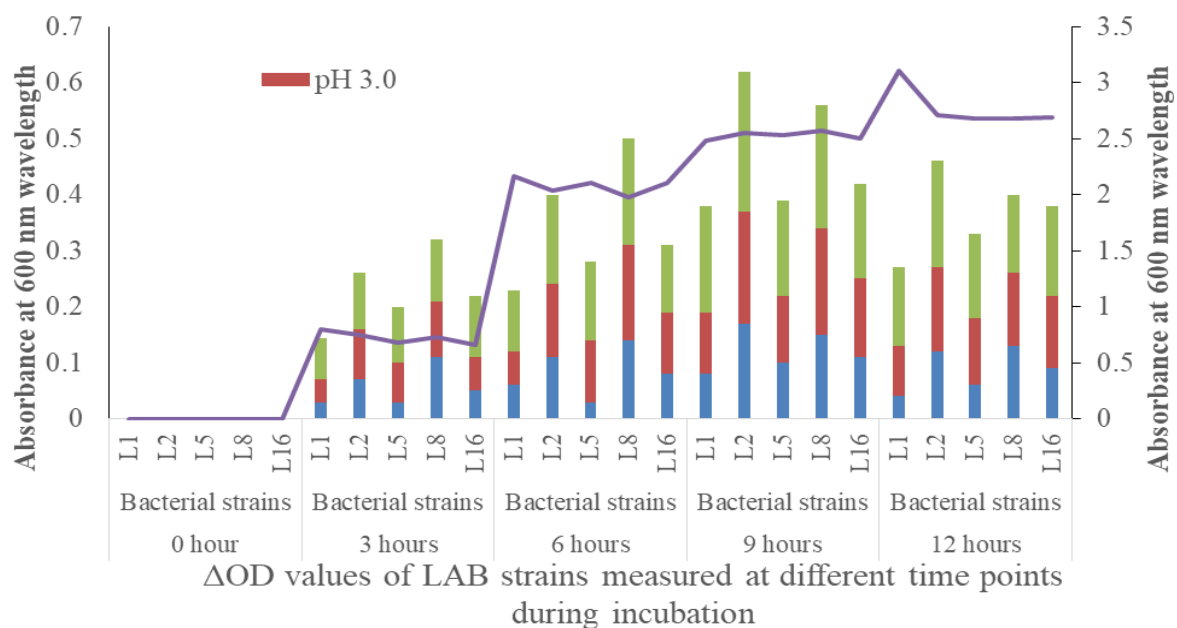
**Table 3.** The survival of some representative LAB strains at different bile salt concentrations

| LAB strains | Bile salt concentrations |      |      |      |      |
|-------------|--------------------------|------|------|------|------|
|             | 0.1%                     | 0.2% | 0.3% | 0.5% | 1.0% |
| L1          | +++                      | +++  | ++   | ++   | ++   |
| L2          | +++                      | +++  | ++   | ++   | ++   |
| L5          | ++                       | +    | +    | -    | -    |
| L8          | +                        | -    | -    | -    | -    |
| L16         | +                        | -    | -    | -    | -    |

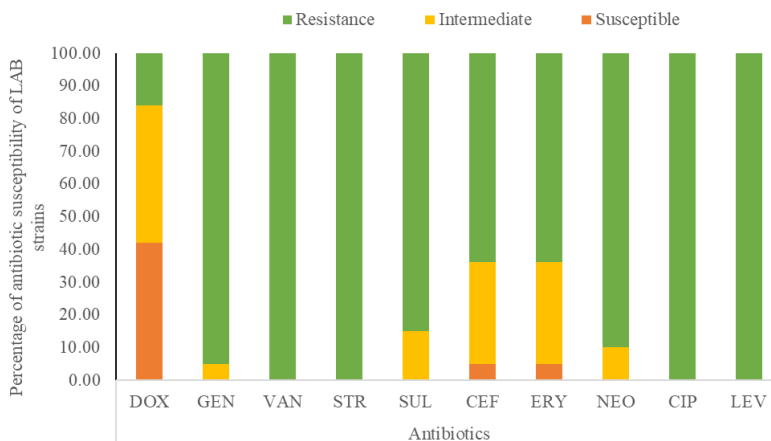
Note: +: Growth, -: No growth



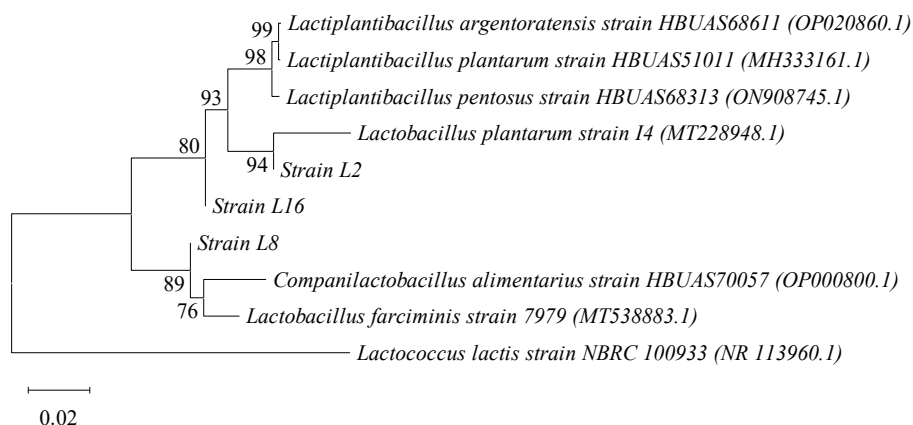
**Figure 5.** Hemolytic activity of representative LAB strains on blood agar. A.  $\gamma$ -hemolytic activity of strains L1, L2, L4, and L5; B.  $\beta$ -hemolytic activity of strains L3, L17, L19, and L21



**Figure 6.** Survival of representative LAB strains under low pH conditions



**Figure 7.** Antibiotic susceptibility of representative LAB strains based on disc diffusion assay



**Figure 8.** A phylogenetic tree was constructed for three representative LAB strains (L2, L8 and L16) using the neighbor-joining algorithm with a bootstrap value of 1,000 replicates. *Lactococcus lactis* strain NBRC 100933 (NR 113960.1) served as the outgroup

### Extracellular enzyme production by lactic acid bacteria strains

The generation of extracellular chemicals contributes to supplement digestion system and stomach related improvement, fortifying probiotic usefulness. Among 15 LAB strains tried, all shown amylase and cellulase exercises, whereas as it were three (L2, L8, L16) created protease (Table 4).

These results are consistent with Ho and Lim (2018), who reported that LAB commonly express polysaccharide-degrading enzymes. Zabidi et al. (2020) and Wang et al. (2021) encourage illustrated that chemical action depends on substrate composition and reflects carbon utilization pathways. The nearness of plant-derived substrates in *Nem chua* likely initiated these enzymatic characteristics.

In contrast, protease activity was strain-dependent, watched as it were in three confines. Toe et al. (2019) and Kieliszek et al. (2021) famous that protease generation shifts by hereditary foundation, with most LAB depending on intracellular peptidases instead of extracellular proteases. Comparative comes about were found by Sulthoniyah et al. (2015) in LAB separated from shrimp paste, where as it were some strains displayed solid proteolysis.

### Molecular identification of lactic acid bacteria strains

The findings from 16S rRNA gene sequencing and phylogenetic tree analysis (Figure 8) demonstrated that three LAB strains, L2, L8, and L16, were identified as *Lactobacillus plantarum* strain I4 (MT228948.1), *L. farciminis* strain 7979 (MT538883.1), and *Lactiplantibacillus argenteratensis* strain HBUAS68611 (OP020860.1), respectively.

Among these, *L. plantarum* is the foremost prevailing and environmentally flexible LAB species regularly separated from matured meat situations (Nguyen et al. 2010; Pérez-Díaz et al. 2022). Its versatile victory has been credited to quick fermentation, productive carbohydrate utilization, and the capacity to create bacteriocins that smother deterioration and pathogenic microscopic organisms (Pilasombut et al. 2015; Todorov and Holzapfel 2019). The predominance of *L. plantarum* in *Nem chua* reflects solid specific weight for strains able of enduring fluctuating salt and pH conditions whereas keeping up metabolic vigor, fundamental for both aging solidness and probiotic usefulness.

**Table 4.** The ability of LAB strains to produce extracellular amylase, protease, and cellulase

| LAB strains | Extracellular enzymes |           |          |
|-------------|-----------------------|-----------|----------|
|             | Amylase               | Cellulase | Protease |
| L1          | +                     | +         | -        |
| L2          | +                     | +         | +        |
| L4          | +                     | +         | -        |
| L5          | +                     | +         | -        |
| L6          | +                     | +         | -        |
| L7          | +                     | +         | -        |
| L8          | +                     | +         | +        |
| L9          | +                     | +         | -        |
| L10         | +                     | +         | -        |
| L11         | +                     | +         | -        |
| L13         | +                     | +         | -        |
| L15         | +                     | +         | -        |
| L16         | +                     | +         | +        |
| L18         | +                     | +         | -        |
| L20         | +                     | +         | -        |

Note: +: Positive activity, -: Negative activity

Similarly, *L. farciminis* strain L8 appeared 99.22% closeness to reference strain 7979 (MT538883.1). This species has been progressively recognized for its gastrointestinal strength and antimicrobial potential. Past considers illustrated that *L. farciminis* segregates from angle sauce and other high-salt matured nourishments displayed solid corrosive and bile resilience, as well as broad-spectrum enmity against enteropathogens (Nguyen et al. 2022; Surono and Hosono 2018). These highlights propose that *L. farciminis* plays an environmental part in keeping up microbial adjust beneath competitive maturation conditions, supporting its application as a biocontrol probiotic in animal-based matured items.

In contrast, *L. argentoratensis* as of late depicted part of the *Lactiplantibacillus* genus (Zheng et al. 2020) has developed as a versatile and practically promising taxon in protein-rich substrates. Genomic and transcriptomic considers uncovered that this species encodes different stress-response proteins and carbohydrate-active proteins, empowering development in acidic, nutrient-dense situations (Yoon et al. 2024; Wang et al. 2023). Its direct proteolytic movement contributes to flavor improvement and amino corrosive turnover amid aging, whereas keeping up redox homeostasis (Li et al. 2022). The event of *L. argentoratensis* in *Nem chua* shows particular improvement of LAB species prepared with both corrosive resistance and enzymatic flexibility key traits for tirelessness and probiotic viability in aged meat biological systems.

In conclusion, a total of 21 LAB isolates were isolated from traditional *Nem chua* samples. Based on 16S rRNA gene sequencing and phylogenetic tree analysis, strains L2, L8, L16, and E1 were identified as closely related to *Lactiplantibacillus plantarum* (formerly *L. plantarum*), *L. farciminis*, *L. argentoratensis*, and *Staphylococcus carnosus*, respectively. All isolated LAB strains displayed antibacterial activity. Among them, five LAB strains (L1, L2, L5, L8, and L16) demonstrated tolerance to acidic conditions. The bile salt tolerance test showed that three out of five LAB strains grew at a concentration of 0.3%.

Regarding antibiotic resistance, the highest susceptibility was observed against doxycycline, while all strains exhibited complete resistance to vancomycin, streptomycin, ciprofloxacin, and levofloxacin. In addition, all LAB strains produced extracellular amylase and cellulase, although only three strains produced extracellular protease. These findings provide a scientific basis for the application of promising strains as probiotics or starter cultures in traditional *Nem chua* fermentation.

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