



Microbial Biodiversity of Vietnamese Pickled Cucumber: Safety-Screening of *Lactiplantibacillus plantarum* DL8 as a Probiotic Candidate

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Abstract

Traditional fermented foods, such as Vietnamese pickled cucumbers, are essential sources of bacterial biodiversity, yet their functional characteristics in Vietnam remain underexplored. This study aimed to identify and evaluate promising probiotic candidates from this matrix. Presumptive lactic acid bacteria (LAB) isolates underwent a multi-stage screening process to assess simulated gastrointestinal tolerance, antimicrobial activity, and safety profiles using both phenotypic and genotypic assays. Identified via 16S rRNA sequencing as *Lactiplantibacillus plantarum*, strain DL8 exhibited remarkable tolerance to gastrointestinal stressors, successfully surviving at pH 3.0 and in 0.4% bile salts. It demonstrated broad-spectrum antimicrobial activity in neutralized cell-free supernatants (CFS) against key foodborne pathogens, including *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella enterica*. Comprehensive safety testing showed that DL8 was non-hemolytic and sensitive to common clinical antibiotics. In addition, PCR-based genotypic screening confirmed the absence of virulence factors, vancomycin resistance genes, and genes encoding biogenic amine production. These *in vitro* findings indicate that *L. plantarum* DL8 represents a promising probiotic candidate and a potential protective starter culture for the regional food industry, requiring further validation through whole-genome sequencing and *in vivo* studies.

Keywords: antimicrobial activity; food safety; lactic acid bacteria; probiotics; traditional fermented foods

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INTRODUCTION

Fermented foods have long been an integral part of human nutrition and are widely recognized as valuable functional foods. The health-promoting properties of these products are mainly attributed to their diverse microbial consortia (Castellone et al., 2021; Marco et al., 2021; Soto-Giron et al., 2021). These consortia improve nutrient bioavailability and produce bioactive compounds, such as antioxidants, immunomodulators, and antimicrobials (Park et al., 2016; Xiang et al., 2019). Consequently, traditional fermented matrices are essential sources for the identification of new probiotic

candidates (live microorganisms that, when administered in adequate amounts, confer a health benefit on the host; FAO and WHO, 2002). These microorganisms can play vital roles in stabilizing the human gastrointestinal environment and reinforcing the intestinal permeability barrier (Sakandar and Zhang, 2021).

These fermentations are mainly carried out by lactic acid bacteria (LAB). Among them, *Lactiplantibacillus plantarum* is a species with high genomic plasticity and ecological adaptability (Bintsis, 2018; Huang et al., 2021). It has long been used in traditional foods and

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designated as “generally recognized as safe” (GRAS) (Yang et al., 2021). The gut flora-regulating, toxin-binding, and pathogenic-bacteria-inhibiting abilities of *L. plantarum* have shown great potential in industrial and clinical applications, as it produces organic acids, hydrogen peroxide, and bacteriocins (Tong et al., 2020; Yu et al., 2021; Divyashree et al., 2024).

The use of probiotics, however, has been increasing commercially and has resulted in an emerging “safety paradox”. LAB have been reported to cause opportunistic infections, particularly in immunocompromised patients (Amachawadi et al., 2018; Siciliano et al., 2021). Moreover, antimicrobial resistance (AMR) is a growing global problem, and a detailed evaluation of probiotic candidates is essential to ensure they do not contain genes for transferable resistance or virulence factors (Raethong et al., 2022; Kiousi et al., 2023). In this context, it is imperative to adopt a stringent approach involving both phenotypic and genotypic screening to ensure the safety of these probiotic candidates before their industrial application (Wang et al., 2021; Yaacob et al., 2022).

While there is a wealth of data on the use of dairy products as sources of probiotic candidates, plant-based candidates from tropical regions remain relatively underexplored. *Dua leo muoi chua* is a traditional Vietnamese pickled cucumber that undergoes spontaneous anaerobic fermentation, characterized by high osmotic stress (3 to 5% NaCl) and rapid acidification (Abriouel et al., 2022). These conditions select for indigenous LAB that are highly tolerant and ecologically adaptable, characteristics that may provide advantages under specific fermentation conditions compared to some traditional dairy starter cultures (Zheng et al., 2020).

The potential use of these specially adapted strains as targeted biopreservatives against foodborne pathogens, however, has yet to be thoroughly investigated by integrating phenotypic and molecular safety assessments of LAB strains isolated from Vietnamese fermented cucumber. Previous studies have assessed the safety of different plant-derived LAB (Nguyen et al., 2023; Hussein et al., 2025). However, very few studies have validated the safety of plant-derived LAB at the strain level with genotypic evidence, such as confirming the presence or absence of biogenic amine-producing, transmissible antibiotic resistance, and virulence genes.

In this context, the aim of this study was to isolate and characterize *L. plantarum* DL8 from

Dua leo muoi chua to fill this research gap and enhance understanding of plant-derived probiotic candidates in tropical regions. The specific goals were: (i) to determine the tolerance of the strain to simulated gastrointestinal stressors; (ii) to evaluate its antagonistic effect against major foodborne pathogens; and (iii) to thoroughly assess its safety profile using integrated phenotypic assays and molecular screening for virulence and resistance genes. Ultimately, this research seeks to identify a safe, well-adapted probiotic candidate that can contribute to regional food safety and potential biopreservation applications.

MATERIALS AND METHOD

Stepwise screening pipeline and study setting

This study was conducted at the School of Agriculture and Aquaculture, Tra Vinh University, Vietnam, from June 2025 to January 2026. In order to identify a high-performance probiotic candidate from traditional Vietnamese pickled cucumber (*Dua leo muoi chua*), a 5-stage hierarchical screening strategy was used. This systematic approach allowed the selection of the most viable and safe isolates for definitive molecular characterization. In Stage I, a total of 96 presumptive acid-producing colonies were isolated and selected. In Stage II, these 96 isolates were phenotypically and biochemically confirmed as LAB. This was followed by Stage III, which entailed testing the 68 confirmed LAB isolates for their tolerance to salinity, acid, and bile. The 15 most promising candidates were chosen and then tested for functionality and safety in Stage IV, including hemolytic activity, antibiotic susceptibility, and antagonistic activity. Finally, in Stage V, the best isolate (DL8) was identified by definitive molecular typing and genotypic safety testing.

Collection of samples and isolation of LAB

Fifty samples of traditional Vietnamese pickled cucumber (*Cucumis sativus* L.), locally known as *Dua leo muoi chua*, were collected from 10 local markets in Vinh Long Province, Vietnam. This sample size (n = 50) was deliberately determined to capture a representative geographical cross-section of local production practices and to account for the natural microbial variations inherent in spontaneous fermentation across the region. To obtain the maximum diversity of autochthonous microbiota, samples were collected from batches with fermentation times of 3 to 7 days, salt concentrations of 3 to 5%

(w/v), and pH values of 3.8 to 4.5. The samples were transported in sterile containers and processed within 4 hours of collection.

For isolation, 10 g of each sample was homogenized in 90 ml of 0.1% (w/v) peptone water (Oxoid-L37, UK). Serial dilutions were prepared up to 10^{-6} , and 100 μ l aliquots were spread-plated in triplicate onto de Man, Rogosa, and Sharpe (MRS) agar (HiMedia, India) supplemented with 1.0% (w/v) CaCO_3 and 1.0% (w/v) NaCl. Plates were incubated anaerobically (AnaeroGen, Oxoid, UK) at 37 °C for 48 hours (Makhlouf et al., 2024). Total viable LAB counts were quantitatively recorded as colony-forming units per milliliter (CFU ml^{-1}).

Phenotypic and biochemical characterization

From plates exhibiting countable colony densities (30 to 300 colonies), 96 presumptive LAB colonies with distinct acid-production halos were selected (Petkova et al., 2021). Only isolates that were Gram-positive, catalase-negative, and non-motile were retained for the screening of urease and citrate activities. The remaining 68 isolates were purified and subsequently identified using carbohydrate fermentation tests (glucose, lactose, and sucrose) (Kang et al., 2020). The confirmed LAB were cryopreserved at -80 °C in MRS broth containing 20% (v/v) glycerol (Guney et al., 2025).

Assessment of probiotic potential

The resilience of 68 isolates to simulated gastrointestinal stressors was evaluated using growth monitoring via optical density (OD_{620}) as a proxy for survival. Overnight cultures were harvested by centrifugation (10,000 $\times$ g, 10 minutes, 4 °C), washed with phosphate-buffered saline (PBS, pH 7.2), and resuspended to an OD_{620} of 0.6 (approximately 10^8 CFU ml^{-1}) (Lu et al., 2024).

Salinity tolerance: The isolates were cultured in MRS broth with different NaCl concentrations of 2.0%, 4.0%, 6.0%, and 8.0% (w/v) for 3 days at 37 °C.

Acid and bile tolerance: To simulate gastric conditions, bacterial suspensions were inoculated into MRS broth adjusted to pH 2.0, 3.0, and 4.0 with 1 M HCl and supplemented with 3 mg ml^{-1} pepsin. To simulate intestinal conditions, suspensions were inoculated into MRS broth adjusted to pH 8.0, supplemented with 1 mg ml^{-1} pancreatin and 0.2%, 0.4%, and 0.8% (w/v) oxgall bile salts (Oxoid, UK). Standard MRS (pH 7.0, 0% bile) was used as a control. After 4 hours of incubation at 37 °C,

viable counts (CFU ml^{-1}) were determined (Ou et al., 2022). This exposure time was carefully selected to reflect the average physiological transit time of ingested materials through the human gastrointestinal tract in accordance with FAO and WHO (2002) guidelines.

Growth intensity was categorized semi-quantitatively as: excellent (+++, $> 10^7$ CFU ml^{-1}), moderate (++ , 10^4 – 10^6 CFU ml^{-1}), weak (+, 10^1 – 10^3 CFU ml^{-1}), and no growth (–). Data were obtained from the average of 3 independent biological replicates (Banik et al., 2023).

The evaluation of safety and antagonistic activity

A total of 15 of the most resilient isolates were then tested for broad-spectrum antimicrobial activity and safety evaluations.

Hemolytic activity and antibiotic susceptibility: Isolates were cultured on blood agar plates supplemented with 5% (v/v) sheep blood and incubated at 37 °C for 24 hours to assess hemolysis. Only γ -hemolytic (non-hemolytic) strains were chosen for further analysis (Unban et al., 2021). Antibiotic susceptibility was determined using the Kirby-Bauer disk diffusion method on MRS agar. Eight clinically relevant antibiotics were tested: doxycycline (30 μ g), ampicillin (10 μ g), streptomycin (10 μ g), kanamycin (30 μ g), gentamicin (10 μ g), erythromycin (15 μ g), ciprofloxacin (5 μ g), and chloramphenicol (30 μ g) (Alebiosu et al., 2017; Nguyen et al., 2023). Results were primarily analyzed based on the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2018). Because CLSI guidelines do not provide specific breakpoints for many *Lactobacillus* species, the interpretative criteria were supplemented with the European Food Safety Authority (Rychen et al., 2018) microbiological cut-off values for assessing antibiotic resistance in probiotic bacteria. Triplicate testing was performed for all tests.

Antagonistic activity: The antimicrobial activity of the isolates against 3 indicator microorganisms was evaluated using the agar well diffusion method (Abubakr, 2018). The indicator strains included *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Salmonella enterica* serovar Typhimurium ATCC 14028. To differentiate the effects of antimicrobial metabolites from those of organic acids, the cell-free supernatant (CFS) was neutralized to pH 7.0 with 1 M NaOH.

Neutralized CFS (100 µl) was then added to 6 mm wells in nutrient agar plates previously seeded with the respective pathogens (10^6 CFU ml^{-1}). Ampicillin (10 µg) and sterile neutralized MRS broth served as positive and negative controls, respectively. After 24 hours of incubation at 37 °C, the diameters of the inhibition zones were measured. All tests were performed in triplicate.

Molecular and genotypic analysis

Genotypic safety was screened via PCR for virulence factors (*esp*, *gelE*, *fsrB*, *asa1*, *cylA*, *cylM*), biogenic amine genes (*hdc1*, *hdc2*, *tdc*), and vancomycin resistance genes (*vanA*, *vanB*). Genomic DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega, USA). PCR was performed using gene-specific primers (Table 1) and DreamTaq Green PCR Master Mix (Thermo Scientific). Standardized PCR conditions included an initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 55 to 60 °C for 30 seconds, and extension at 72 °C for 1 minute, concluding with a final extension at 72 °C for 7 minutes. Validated reference strains known to contain the respective target genes (*Enterococcus faecalis* ATCC 29212) were used

as positive template controls. Sterile nuclease-free water was used as a negative template control. The PCR products were visualized on a 1.5% (w/v) agarose gel (Nguyen et al., 2025).

The superior isolate, DL8, was identified via 16S rRNA gene sequencing using universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') (Liu et al., 2020). The thermal profile consisted of an initial denaturation at 94 °C for 3 minutes, followed by 30 cycles of 94 °C for 45 seconds, 57 °C for 60 seconds, and 72 °C for 90 seconds, with a final extension at 72 °C for 5 minutes. Amplicons were purified using the QIAquick PCR Purification Kit (Qiagen, Germany) and sequenced by Ktest Co., Ltd. (Vietnam). Sequences were analyzed using the National Center for Biotechnology Information (NCBI) BLASTn. Multiple sequence alignment was performed with the ClustalW algorithm for phylogenetic analysis. Evolutionary distances were calculated using the Kimura 2-parameter model, and a phylogenetic tree was obtained by the neighbor-joining method in MEGA6 software, which used the pairwise deletion option for gap filling and 1,000 bootstrap replications to evaluate the robustness of the nodes.

Table 1. Primers used for the detection of virulence factors, vancomycin resistance genes, and biogenic amine-producing genes

Category	Target genes	Primer	Sequences (5' → 3')	Size (bp)	Reference
Virulence factors	<i>esp</i>	Esp F	TTGCTAATGCTAGTCCACGACC	933	Eaton and Gasson (2001)
		Esp R	GCGTCAACACTTGCAATTGCCGAA		
	<i>gelE</i>	GelE F	GCGTCAATCGGAAGAATCAT	213	
		GelE R	CGGGGAAAAAGCTACATCAA		
	<i>fsrB</i>	fsrB F	TTTATTGGTATGCGCCACAA	316	
		fsrB R	TCATCAGACCTTGGATGACG		
	<i>asa1</i>	asa1 F	CCAGCCAACCTATGGCGGAATC	529	
		asa1 R	CCTGTCGCAAGATCGACTGTA		
	<i>cylA</i>	cylA F	ACTCGGGGATTGATAGGC	688	
		cylA R	GCTGCTAAAGCTGCGCTT		
	<i>cylM</i>	cylM F	GATTGGAATGTGGGAATCCTAA	735	
		cylM R	ACTTCCGGCAACCTTTAGTGTA		
Vancomycin resistance	<i>vanA</i>	vanA F	CCCCTTTAACGCTAATACGATCAA	1,030	Dutka-Malen et al. (1995)
		vanA R	CATGAATAGAATAAAAGTTGCAAT		
	<i>vanB</i>	vanB F	GTGACAAACCGGAGGCGAGGA	433	
		vanB R	CCGCCATCCTCCTGCAAAAAA		
Biogenic amines	<i>hdc1</i>	Hdc1 F	AGATGGTATTGTTTCTTATG	367	Le Jeune et al. (1995)
		Hdc1 R	AGACCATACACCATAACCTT		
	<i>hdc2</i>	Hdc2 F	AAYTCNTTYGAYTTYGARAARGARG	435	De Las Rivas et al. (2006)
		Hdc2 R	ATNGGNGANCCDATCATYTTTRTGNC		
	<i>tdc</i>	Tdc F	ACATAGTCAACCATRTTGAA	1,100	Coton et al. (2004)
		Tdc R	CAAATGGAAGAAGAAGTAGG		

Statistical analysis

Each experiment was conducted in 3 independent biological replicates ($n = 3$). Prior to analysis, the normality of data distribution and the homogeneity of variances were statistically confirmed using the Shapiro-Wilk test and Levene's test, respectively. All data are expressed as mean $\pm$ standard deviation (SD). One-way ANOVA followed by Tukey's HSD test was performed using SPSS 22.0 software to determine significant differences at $p < 0.05$ (Ding et al., 2017).

RESULTS AND DISCUSSION

Isolation, preliminary selection, and autochthonous origins

Traditional fermented matrices are important yet underutilized sources of strong local candidates for probiotics (Castellone et al., 2021). In this study, 50 samples of *Dua leo muoi chua*, a traditional pickled cucumber from Vietnam, were collected, and 96 presumptive colonies were isolated. After secondary identification, 68 strains were identified as LAB. All of these isolates were Gram-positive, catalase-negative, urease-negative, citrate-negative, and non-motile. Glucose, sucrose, and lactose were fermented by all 68 strains. Colonies grown on MRS agar were circular, convex, and milky white, which on microscopy were proven to be non-spore-forming rods or coccobacilli (Figure 1).

The isolation of *L. plantarum* DL8 demonstrates its adaptation to high salinity (3 to 5% NaCl) and fast acidification of regional

tropical fermentations (Abriouel et al., 2022). These conditions are favorable to strains that may possess genetic flexibility and ecological adaptability, which could potentially allow them to outperform commercial dairy starter cultures (Zheng et al., 2020). The presence of *L. plantarum* in vegetable fermentations around the world (Bintsis, 2018; Huang et al., 2021), particularly during the peak fermentation stage, reflects its importance in matrix stabilization and flavor development. This also applies to other vegetable fermentations in Asia (Li and Gu, 2019; Banik et al., 2023). Local probiotics, such as the DL8 strain, which thrive in specific environments like high-salt and plant-based diets, may offer functional advantages in specific regional matrices where commercial probiotics are less well adapted (Mao et al., 2021; Nguyen et al., 2023).

High tolerance to simulated gastrointestinal stress

A potential probiotic needs to be able to withstand the harsh biochemical challenges of the human gut. Based on the results of the hierarchical stress screening, 15 of the 68 confirmed isolates grew well at 2.0% and 4.0% NaCl, whereas growth was significantly inhibited at 6.0% NaCl and no growth was observed at 8.0% NaCl. When the isolates were subjected to gastric simulation, none survived at pH 2.0, while 5 isolates (DL3, DL4, DL8, DL10, DL14) had moderate survival at pH 3.0. Interestingly, DL8 proved highly tolerant of oxgall bile salts; at 0.4% oxgall, several other candidates did not grow (Table 2).

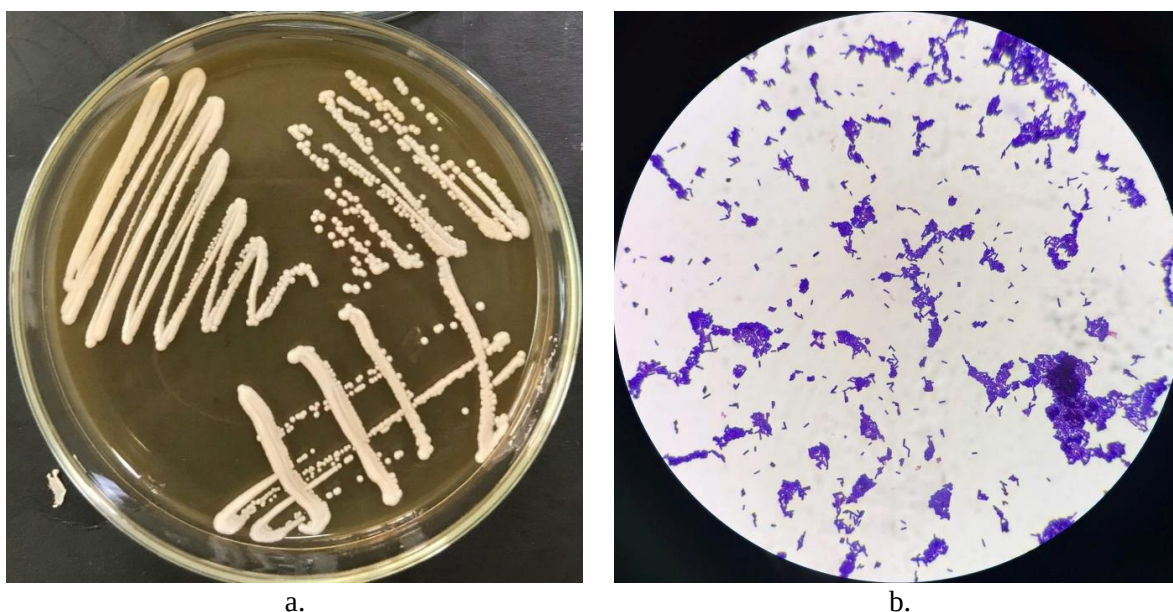


Figure 1. Identification of *Lactiplantibacillus plantarum* DL8: a) Colony morphology; b) Gram staining

Table 2. Resilience of 15 selected LAB isolates to NaCl, pH, and bile salt stressors

Isolate	NaCl (%) tolerance				pH tolerance				Bile (%) tolerance			
	2.0	4.0	6.0	8.0	2.0	3.0	4.0	7.0	0	0.2	0.4	0.8
DL1	+++	+++	+	-	-	-	++	+++	+++	++	-	-
DL2	+++	++	+	-	-	-	+	+++	+++	++	+	-
DL3	+++	+++	++	-	-	++	++	+++	+++	+	+	-
DL4	++	++	+	-	-	++	++	+++	+++	+	-	-
DL5	+++	+++	+	-	-	+	+	++	++	+	+	-
DL6	++	+	+	-	-	-	+	+++	+++	+	+	-
DL7	+++	++	+	-	-	+	+	+++	+++	++	-	-
DL8	+++	+++	++	-	-	++	++	+++	+++	+	+	-
DL9	+++	+++	+	-	-	-	+	++	++	++	+	-
DL10	++	++	+	-	-	++	++	+++	+++	+	+	-
DL11	+++	+++	+	-	-	+	+	+++	+++	+	-	-
DL12	++	+	+	-	-	-	+	++	++	+	-	-
DL13	+++	++	+	-	-	+	+	++	++	++	+	-
DL14	++	++	+	-	-	++	++	+++	+++	+	+	-
DL15	+++	+++	+	-	-	+	+	+++	+++	+	+	-

Note: ‘+’ indicates weak growth; ‘++’ indicates moderate growth; ‘+++’ indicates excellent growth; ‘-’ indicates no growth

This is an encouraging level of survival, especially when compared with the moderate tolerance commonly observed with plant-based LAB, where bile tolerance is typically limited to 0.3% (Banik et al., 2023). The survival rate of DL8 is comparable to established commercial candidates, such as *L. plantarum* ZFM4 (Yan et al., 2023). The mechanism of resistance to low pH conditions may involve an acid tolerance response (ATR) system, which could potentially include the active maintenance of internal pH mediated by the F_1F_0 -ATPase system (Baloch et al., 2019). Moreover, its bile tolerance suggests the possible presence of bile salt hydrolase (BSH) activity, which is known to protect the cell membrane from structural collapse (Lee et al., 2024). Given the harsh fermentation conditions, high osmotic pressure, and low pH resulting from DL8’s indigenous nature, the strain may have undergone selective pressure for these resilient characteristics, ensuring it reaches the colon in

a highly active metabolic state (Khennouf et al., 2023).

Integrated safety profiling: Phenotypic and genotypic assessment

The fast commercialization of the genus *Lactiplantibacillus* has revealed a “safety paradox” that requires careful screening of all candidates for the absence of opportunistic virulence and antibiotic-resistance genes that could be transmitted to other bacteria, such as pathogenic species (Kiouisi et al., 2023). To address this unmet need in the industry, the dual-tier phenotypic and genotypic assessment in this study showed that DL8 is exceptionally safe.

Phenotypically, the top 8 candidates, including DL8, showed γ -hemolysis (non-hemolytic behavior), which is a prerequisite for a bacterium to be used in food manufacturing (Hsu et al., 2022; Ou et al., 2022). Antibiotic susceptibility testing (Table 3; Figure 2) revealed complete

Table 3. Antibiotic susceptibility profiles of the 15 selected LAB isolates

Antibiotic	Sensitive - S (n; %)	Intermediate - I (n; %)	Resistant - R (n; %)
CIP (Ciprofloxacin)	7 (46.7)	4 (26.7)	4 (26.7)
DO (Doxycycline)	5 (33.3)	1 (6.7)	9 (60.0)
CN (Gentamicin)	2 (13.3)	6 (40.0)	7 (46.7)
STR (Streptomycin)	5 (33.3)	0 (0.0)	10 (66.7)
AMP (Ampicillin)	3 (20.0)	4 (26.7)	8 (53.3)
E (Erythromycin)	4 (26.7)	4 (26.7)	7 (46.7)
C (Chloramphenicol)	5 (33.3)	2 (13.3)	8 (53.3)
K (Kanamycin)	3 (20.0)	3 (20.0)	9 (60.0)

Note: Standard interpretation of antimicrobial susceptibility tests of LAB isolates with the disc diffusion method in accordance with CLSI standards

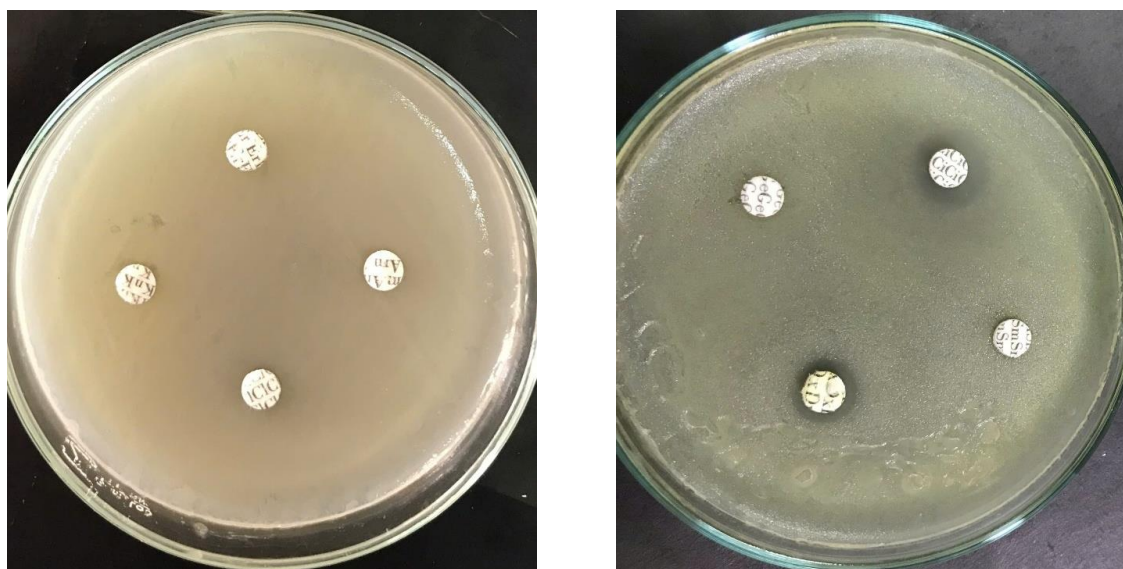


Figure 2. Antibiotic susceptibility test for *Lactiplantibacillus plantarum* DL8

Note: Zones of inhibition indicate sensitivity to doxycycline, ampicillin, streptomycin, kanamycin, gentamicin, chloramphenicol, erythromycin, and ciprofloxacin

Table 4. PCR screening results of representative top candidate LAB isolates (DL8 and DL14) for target virulence, vancomycin resistance, and biogenic amine genes

Category	Target gene	DL8	DL14
Virulence factors	<i>esp, gelE, fsrB, asa1, cyla, cylM</i>	-	-
Vancomycin resistance	<i>vanA, vanB</i>	-	-
Biogenic amines	<i>hdc1, hdc2, tdc</i>	-	-

Note: (-) indicates the absence of the target gene (negative PCR result). No amplicons were detected for any tested genes in these strains

susceptibility of DL8 to crucial clinical antibiotics, such as ampicillin and chloramphenicol. Intrinsic resistance to streptomycin and kanamycin was observed, a trait also noted in 66.7% and 60.0% of the tested isolate pool, respectively. This is a well-documented property of the genus *Lactiplantibacillus* and is not the result of horizontal gene transfer (Varada et al., 2023).

More importantly, high-throughput PCR screening offered definitive genotypic validation. Although 15 isolates underwent molecular screening and all tested negative for the target genes, the results for the 2 most promising candidates, DL8 and DL14, are presented in Table 4. DL8 was completely negative for virulence factors (*esp, gelE, fsrB, asa1, cyla, cylM*), vancomycin resistance markers (*vanA, vanB*), and biogenic amine-producing genes (*hdc1, hdc2, tdc*). The lack of biogenic amine production is an important differentiator for potential commercial scale-up because these toxins are common and often overlooked in uncharacterized regional ferments (Barbieri et al., 2019). The combination of genotypic assays

with phenotypic screening provides a robust framework that explicitly differentiates DL8 from opportunistic lineages before commercialization (Liu et al., 2023; Gizachew and Engidawork, 2024).

Antagonistic activity against a broad spectrum of foodborne pathogens

The antimicrobial activity was significantly different among the isolates ($p < 0.05$), with DL8 showing the highest activity and greatest inhibition zones (Figure 3). Importantly, the inhibitory effects of DL8 cell-free supernatant (CFS) remained intact at a neutral pH of 7.0 (neutralized cell-free supernatant, nCFS). It achieved substantial inhibition zones against *E. coli* ATCC 25922 (25.59 ± 1.38 mm), *S. enterica* ATCC 14028 (21.33 ± 3.51 mm), and *S. aureus* ATCC 25923 (12.88 ± 2.01 mm) (Table 5).

This is a clear analytical milestone, as there was a marked retention of potent antimicrobial activity after neutralization. Because DL8 demonstrated sustained inhibitory activity at pH 7.0, this suggests the possible secretion of resilient

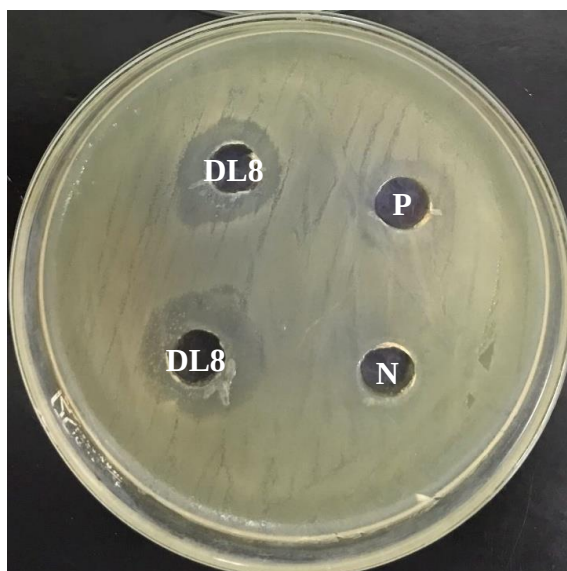


Figure 3. Antimicrobial activity of isolate *Lactiplantibacillus plantarum* DL8

Note: The plate was seeded with *S. aureus* ATCC 25923. DL8 = nCFS from isolate DL8; P = Positive control (ampicillin, 10 µg); N = Negative control (sterile neutralized LB broth)

Table 5. Antimicrobial activity (inhibition zone diameters) of the top LAB isolates against indicator pathogens

Isolates	<i>E. coli</i> ATCC 25922	<i>S. aureus</i> ATCC 25923	<i>S. enterica</i> ATCC 14028
DL1	0.00±0.00 ^c	12.00±1.00 ^b	12.33±2.31 ^c
DL2	0.00±0.00 ^c	11.67±1.15 ^b	13.33±1.15 ^{bc}
DL3	0.00±0.00 ^c	0.00±0.00 ^c	0.00±0.00 ^d
DL4	0.00±0.00 ^c	0.00±0.00 ^c	0.00±0.00 ^d
DL5	0.00±0.00 ^c	12.33±1.15 ^b	0.00±0.00 ^d
DL6	11.33±0.58 ^b	25.00±2.00 ^a	13.33±1.53 ^{bc}
DL7	11.00±1.00 ^b	13.67±6.03 ^b	0.00±0.00 ^d
DL8	25.59±1.38 ^a	12.88±2.01 ^b	21.33±3.51 ^{ab}
DL9	13.00±1.00 ^b	14.00±5.20 ^b	13.33±1.53 ^{bc}
DL10	14.33±4.04 ^b	0.00±0.00 ^b	14.67±2.52 ^{abc}
DL11	15.00±1.00 ^b	8.67±7.77 ^{bc}	12.96±1.06 ^c
DL12	13.67±1.53 ^b	13.00±1.73 ^b	22.25±7.15 ^a
DL13	12.67±1.15 ^b	25.79±5.01 ^a	14.63±2.58 ^{abc}
DL14	16.87±1.55 ^b	0.00±0.00 ^c	14.67±4.73 ^{abc}
DL15	12.93±0.12 ^b	0.00±0.00 ^c	0.00±0.00 ^d

Note: Values are expressed as mean diameter (mm)±SD (n = 3). Different superscript letters in the same column indicate statistically significant differences ($p < 0.05$)

antimicrobial compounds, such as bacteriocins or bacteriocin-like inhibitory substances (BLIS), rather than inhibition resulting solely from organic acid accumulation (Hattem et al., 2024; Hussein et al., 2025). However, as protease treatments and bacteriocin purification were not performed in this study, the exact mechanisms and nature of these metabolites require further experimental validation (Dai et al., 2021; Choovet et al., 2024). DL8's broad-spectrum inhibition of both Gram-negative and Gram-positive foodborne pathogens marks it as a highly promising "clean label" biopreservative

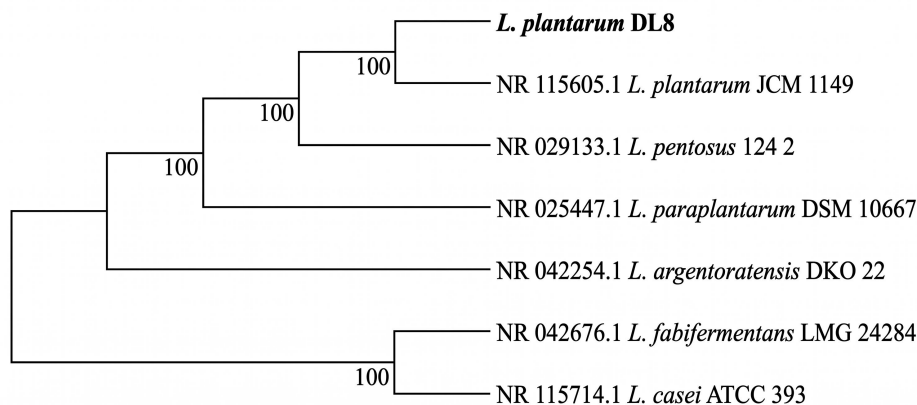
alternative to synthetic food preservatives (Muhammad et al., 2019; Shi et al., 2024).

Molecular identification and study limitations

Molecular characterization of the 16S rRNA gene sequence confirmed the identity of isolate DL8. BLASTn analysis revealed 99.87% sequence identity with *L. plantarum* strain JCM 1149 (Accession No. NR_115605.1) (Table 6). Phylogenetic reconstruction using the neighbor-joining method clustered DL8 within the *L. plantarum* clade with high bootstrap support (Figure 4). The sequence has been deposited in GenBank under accession number PX919727.

Table 6. BLASTn alignment statistics for the 16S rRNA gene sequence of isolate DL8 against the NCBI database

Closely related species	Max score	Query coverage (%)	E-value	Identity (%)	Accession No.
<i>Lactiplantibacillus plantarum</i> strain JCM 1149 16S ribosomal RNA, partial sequence	2,778	90	0.0	99.87	NR_115605.1

Figure 4. Phylogenetic relationships of isolate DL8 and related *Lactiplantibacillus plantarum* based on 16S rRNA gene sequences

Note: The tree was constructed using the neighbor-joining method. Bootstrap values (expressed as percentages of 1,000 replicates) are shown at the nodes. The scale bar represents the number of nucleotide substitutions per site

The *in vitro* results show that DL8 has high potential as a probiotic and a biopreservative, but there are some limitations in the present study that need to be taken into account. First, the use of *in vitro* functional assays requires *in vivo* animal models to conclusively establish the strain's ability to colonize the gut, its immunomodulatory activity, and its health-promoting potential in a complex biological system (Yoon et al., 2022). Second, molecular identification and genotypic screening in this study used 16S rRNA sequencing and targeted PCR. An essential next step is to progress to whole-genome sequencing (WGS). By determining the exact position of any mobile genetic elements, WGS will provide a comprehensive safety profile. In addition, it will enable in-depth genomic insights into the strain's metabolic potential, such as the establishment of specific operons for bacteriocin production, ATR mechanisms, and BSH activity (Gizachew and Engidawork, 2024).

CONCLUSIONS

Lactiplantibacillus plantarum DL8 was successfully isolated from traditional Vietnamese pickled cucumber and demonstrated promising *in vitro* probiotic characteristics, including tolerance to simulated gastrointestinal conditions,

antimicrobial activity against selected foodborne pathogens, and a favorable phenotypic and genotypic safety profile. No tested virulence factors, vancomycin-resistance genes, or biogenic amine-producing genes were detected. These findings suggest that DL8 has potential as both a probiotic candidate and a natural biopreservative for fermented food applications. Nevertheless, further studies involving whole-genome sequencing, adhesion assays, food application trials, and *in vivo* evaluations are required to confirm its safety, functional efficacy, and suitability for commercial use.

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DECLARATION OF GENERATIVE AI USE

The authors used Gemini AI for grammar correction and language refinement.

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