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Research Article

ISOLATION AND CHARACTERIZATION OF A PROBIOTIC FROM JAMUN VINEGAR

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ABSTRACT
The present study aimed to isolate and characterize probiotic lactic acid bacteria (LAB) from jamun vinegar. Thirty-five bacterial isolates were obtained on MRS agar, of which four were Gram-positive and catalase-negative, confirming their classification as LAB. These isolates were screened for probiotic attributes, including acid, bile, and phenol tolerance, antimicrobial activity, antioxidant potential, and safety characteristics. Among them, isolate JV20 demonstrated superior resistance to acidic conditions (pH 2.0; 93 ± 0.17% survivability), bile salts (0.3%; 91 ± 0.12%survivability), and phenol (0.4%; 96 ± 0.13% survivability), indicating robust survival under simulated gastrointestinal conditions. JV20 exhibited strong antagonistic activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*, and along with positive hydrogen peroxide production. Safety evaluation revealed DNase-negative and non-hemolytic characteristics, confirming its non-pathogenic nature. The isolate was resistant to multiple antibiotics, supporting its safety for probiotic use. Biochemical assays showed negative IMViC results and wide carbohydrate fermentation ability, while DPPH assay indicated notable antioxidant activity (52.33 ± 0.36%). Molecular identification through 16S rRNA sequencing confirmed the isolate as *Lactiplantibacillus plantarum* (99% similarity; GenBank accession no. PX106243). These findings highlight jamun vinegar as an underexplored source of potent probiotic candidates. The *L. plantarum* strain JV20 demonstrates promising potential for application in functional foods and nutraceuticals aimed at improving gut health and combating oxidative stress.

Keywords: *Jamun vinegar, Lactiplantibacillus plantarum, probiotic, antioxidant activity, antimicrobial activity, bile tolerance*

INTRODUCTION
Jamun (Syzygium cumini), also known as black plum or Java plum, is a tropical fruit with significant cultural and medicinal importance, particularly in the Indian subcontinent. Its rich composition of anthocyanins, ellagitannins, and other polyphenolic compounds contributes to its deep purple color and potent antioxidant properties, which are beneficial in combating oxidative stress and inflammation, potentially reducing the risk of chronic diseases (Kanta *et al.,* 2023; Tomar *et al.,* 2020). Traditionally, jamun has been used in various forms, including fresh consumption and as a base for products like vinegar, which undergoes a fermentation process involving both alcoholic and acetic acid stages. This fermentation not only enhances the fruit's flavor profile but also fosters a diverse microbial community that may include probiotic strains, offering additional health benefits (Swami *et al.,* 2012; Joshi *et al.,* 2019). The therapeutic potential of jamun is well-documented, with studies highlighting its efficacy in managing diabetes, due to its ability to improve insulin sensitivity and regulate blood sugar levels (Joshi *et al.,* 2019; Adithya *et al.,* 2025). Furthermore, jamun exhibits a range of pharmacological activities, including anti-inflammatory, antimicrobial, and anticancer properties, attributed to its rich phytochemical content, such as flavonoids,

terpenoids, and glycosides (Kumar *et al.,* 2022; Banerjee, 2024). The seeds of jamun, often considered a byproduct, are also rich in bioactive compounds and have been explored for their potential as functional food ingredients, offering benefits like hypoglycemic and antioxidant effects (Kumar *et al.,* 2022; Banerjee, 2024). Despite its numerous health benefits, the consumption of jamun and its derivatives should be moderated, considering individual dietary needs and potential sensitivities (Kanta *et al.,* 2023; Chaudhary & Mukhopadhyay, 2012). The integration of jamun into modern food technologies and its potential as a nutraceutical highlight its value in promoting human health and well-being, although further research is needed to fully understand and harness its bioactive properties (Adithya *et al.,* 2025; Katiyar *et al.,* 2016).

The study of jamun vinegar as a potential source of probiotics is grounded in the rich microbial ecosystem fostered during its natural fermentation process. Jamun, or Syzygium cumini, is a fruit abundant in polyphenols, anthocyanins, and essential nutrients like vitamin C, iron, and potassium, which create a conducive environment for microbial growth, particularly lactic acid bacteria (LAB) (El-Safy *et al.,* 2023; Adithya *et al.,* 2025). The traditional method of producing jamun vinegar involves spontaneous fermentation, allowing indigenous LAB populations to thrive, which are known for their probiotic potential (Satish *et al.,* 2013; Sathe & Mandal, 2016). LAB, such as Lactobacillus and Leuconostoc, are prevalent in vinegar fermentation and contribute to the product's sensory and health benefits through the production of bioactive compounds like organic acids and phenolics (Maske *et al.,* 2023; Hosseini *et al.,* 2025). However, the viability of LAB during vinegar fermentation can be a challenge, as their numbers often fall below the threshold required for probiotic labeling (Maske *et al.,* 2023). Despite this, the initial stages of fermentation see a high incidence of LAB, suggesting that jamun vinegar could serve as a postbiotic product, offering health benefits through the bioactive compounds produced by LAB (Maske *et al.,* 2023). The antioxidant properties of jamun, enhanced by its polyphenolic content, further support the growth and activity of LAB, potentially increasing the probiotic efficacy of the vinegar (El-Safy *et al.,* 2023; Nnko & Mahadhy, 2025). Research into optimizing fermentation conditions and identifying robust LAB strains could enhance the probiotic potential of jamun vinegar, making it a valuable functional food product (Kodba *et al.,* 2024; Nnko & Mahadhy, 2025). Thus, jamun vinegar represents a promising avenue for probiotic research, leveraging the fruit's rich nutrient profile and the traditional fermentation process to potentially deliver health benefits (Satish *et al.,* 2013; El-Safy *et al.,* 2023).

Probiotics are live microorganisms that, when consumed in adequate amounts, confer a range of health benefits to the host (Krishnan *et al.,* 2025). They are often key players in industrial fermentation, particularly LAB that produce lactic acid as a primary byproduct in dairy and non-dairy products. Prominent examples include species from the Lactobacillus and Bifidobacterium genera, which generate bioactive compounds like bacteriocins, exopolysaccharides, organic acids, and antimicrobials (Plessas *et al.,* 2017; Khalil *et al.,* 2018). These substances help curb the growth of harmful bacteria in the gut (Bin Masalam *et al.,* 2018).

To qualify as an effective probiotic, a strain must remain viable and safe, endure bile salts and gastric acids, traverse the digestive tract intact, and adhere to and colonize intestinal epithelial cells (Yu *et al.,* 2013; Angelescu *et al.,* 2019). The rewards are substantial: probiotics bolster immunity, lower lipid and cholesterol levels, offer anti-allergic and anticancer effects, provide antibacterial and antioxidant support, and even exert neuroprotective influences via the gut-brain axis (Cho *et al.,* 2013; Khushboo *et al.,* 2023; Nandal *et al.,* 2025a). Research underscores their efficacy in treating conditions like acute infectious diarrhea, necrotizing enterocolitis, ulcerative colitis, antibiotic-associated diarrhea, irritable bowel syndrome, and Crohn's disease (Krishnan *et al.,* 2025). They also aid in managing liver ailments by maintaining gut barrier function and mitigating inflammation (Plessas *et al.,* 2017). Moreover, probiotics help restore balance in the gut-brain axis, reducing inflammation and infection risks in neurosurgical settings while aiding recovery in neurodegenerative diseases (Nandal *et al.,* 2025a). In inflammatory bowel disease (IBD)—marked by immune dysregulation, colitis flares, and gut inflammation—probiotics modulate microbial communities and offer dietary strategies for intestinal health (Nandal *et al.,* 2025c).

Recent studies have highlighted *Lactobacillus plantarum* as a versatile LAB with standout probiotic attributes (Bin Masalam *et al.,* 2018), including resilience in acidic and bile-laden environments and the ability to outcompete pathogenic gut bacteria (Yu *et al.,* 2013). Innovative approaches like probiotics, prebiotics, and fecal microbiota transplantation are gaining traction for targeting the gut-heart axis in cardiovascular diseases (CVDs), by reshaping microbiota, curbing systemic inflammation, and reducing metabolites like trimethylamine-N-oxide (TMAO) (Nandal *et al.,* 2025b). Building on this, our investigation zeroes in on evaluating the probiotic qualities of strains isolated from jamun vinegar, with a particular emphasis on determining the suitability of *L. plantarum* for integration into functional food products.

MATERIALS AND METHODS

Jamun vinegar is made by crushing the jamun and putting it in a jar with water and salt for fermentation, and after its complete fermentation, the vinegar is processed for isolation. Approximately 50 mL of vinegar per sample was transported in sterile containers at 4°C and processed within 2 h. Samples were homogenized (10:100 v/v) in sterile saline (0.85% NaCl), serially diluted (10^{-1} to 10^{-6}), and 100 µL aliquots were spread-plated on de Man, Rogosa, and Sharpe (MRS) agar (pH 6.5). Plates were incubated for 24-48 h aerobically at 37°C. Colonies displaying LAB morphology were selected, purified by repeated streaking on MRS agar, and stored at -20°C as glycerol stocks (20% v/v glycerol in MRS broth) for further use. This process ensures the isolation of pure cultures by eliminating contaminants through successive transfers, allowing for the selection of strains with typical rod-shaped or cocci morphology characteristic of lactic acid bacteria.

Purified isolates were subjected to Gram staining using a standard protocol where bacterial smears on glass slides were first stained with crystal violet for 1 min, followed by Gram's iodine for 1 min as a mordant, then decolorized with 95% ethanol for 10-20 s, and finally counterstained with safranin for 45 s. The stained slides were observed under light microscopy at 100× oil immersion to confirm Gram-positive reactions, indicated by purple coloration. Catalase activity was tested by adding 3% hydrogen peroxide to a colony on a glass slide; the absence of bubbles confirmed catalase-negative strains, as catalase-positive bacteria would produce oxygen gas bubbles. Only Gram-positive, catalase-negative rods were selected for further probiotic attributes, as these traits are hallmark indicators of lactic acid bacteria, distinguishing them from other microbial groups like Gram-negative or catalase-positive organisms (Nandal *et al.,* 2024).

Acid Tolerance

Isolates were cultivated overnight in MRS broth at 37°C to obtain actively growing cells in the logarithmic phase of growth. These cells were harvested by centrifuging at 7000 rpm for 10 minutes at 4°C to pellet the bacteria without damaging them. The pH of fresh MRS broth was adjusted to 2.0 using 1N HCl to simulate the acidic environment of the stomach, while a control broth was maintained at pH 6.5 to represent neutral conditions. The pelleted cells were resuspended in the pH-adjusted MRS broth and incubated at 37°C to mimic body temperature. Aliquots were withdrawn at 0 and 2 hours to monitor survival over time, serially diluted in PBS to achieve countable colonies, and plated on MRS agar. Plates were incubated at 37°C for 24 hours, and viable cells were quantified using the plate count method, expressed as log CFU/mL. This assay evaluates the strain's ability to withstand gastric acidity, a critical barrier for probiotics to reach the intestines alive, with survival rates calculated as the percentage of viable cells relative to the initial count. Survival rate was calculated as: % Survival = (log CFU/mL at time t / log CFU/mL at time 0) × 100. Unadjusted MRS broth (pH 6.5) served as the control (Talib *et al.,* 2019; Ziyaee *et al.,* 2024; Angelescu *et al.,* 2019; Masalam *et al.,* 2018).

Bile Tolerance

Overnight grown cultures were harvested by centrifugation as described above and resuspended in 10 mL of MRS medium supplemented with 0.3% Oxgall (a bile salt equivalent) to simulate intestinal bile conditions, using plain MRS medium without Oxgall as the control for comparison. The inoculated samples were maintained at 37°C to replicate human body temperature. At 0 and 2 hours, samples were collected,

serially diluted in normal saline to prevent osmotic shock, and 100 µL of appropriate dilutions were spread on MRS agar to enumerate colonies. Plates were incubated at 37°C for 24-48 hours until visible colonies formed, and colony counts were determined at each interval. This test assesses the strain's resistance to bile salts, which can disrupt cell membranes, ensuring potential probiotics can survive the bile-rich environment of the small intestine for colonization and activity. Viable counts were determined as described above, and percentage survival was calculated. Isolates showing >90% survival at 0.5% bile were considered tolerant (Talib; 2019; Plessas *et al.,* 2017; Cho *et al.,* 2013).

Resistance to Phenol

Overnight grown cultures were inoculated at 1% (v/v) into MRS broth containing 0.4% phenol to mimic the phenolic compounds produced in the gut from aromatic amino acid metabolism by microbiota. At 0 and 24 hours, serial dilutions were prepared in saline and plated on MRS agar to assess viable cell numbers through colony counting after incubation at 37°C for 24-48 hours. Phenol resistance is crucial for probiotics to persist in the intestinal environment, where such compounds can inhibit bacterial growth; survival is measured as log CFU/mL over the extended incubation period, indicating the strain's robustness against phenolic stress. Survival was expressed as % growth relative to phenol-free MRS control(Yadav *et al.,* 2016; Somashekaraiah *et al.,* 2019).

Antagonistic Activity

The antimicrobial effects of the isolates against two Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus* MTCC 737) and two Gram-negative (*Escherichia coli* MTCC 443 and *Pseudomonas aeruginosa* MTCC 1688) bacteria were evaluated using the agar well diffusion assay. Pathogen cultures were grown overnight in nutrient broth, adjusted to a turbidity equivalent to 0.5 McFarland standard (approximately 10^{-8} CFU/mL), and 100 µL was mixed into soft agar (0.75% agar) before overlaying on Mueller-Hinton Agar (MHA)/Nutrient agar (NA) plates to create a uniform lawn. Wells (6 mm diameter) were created in the solidified MHA/NA using a sterile borer, and 100 µL of overnight-grown Lactobacillus culture (adjusted to 10^{-8} CFU/mL) was added to each well. Plates were incubated at 37°C for 24–48 hours, and inhibition zones were measured in millimeters around the wells. This method determines the strain's ability to produce inhibitory substances like organic acids, bacteriocins, or hydrogen peroxide, which diffuse into the agar and prevent pathogen growth, highlighting potential for pathogen control in the gut (Yu *et al.,* 2013).

Antibiotic Susceptibility

Susceptibility was determined on MHA/MRS plates using the antibiotic disk diffusion method. Fresh bacterial cultures were adjusted to 0.5 McFarland standard and 100 µL was spread evenly onto solidified MHA/MRS plates at room temperature using a sterile swab for uniform coverage. Antibiotic disks of different antibiotics (Cefepime (30mcg), Levofloxacin (5mcg), Ciprofloxacin (5mcg), Erythromycin (15mcg), Penicillin (10mcg), Gentamicin (10mcg), Amoxycillin/Clavulanic Acid (10/20mcg), Tetracycline(30mcg), Vancomycin (30mcg), Azithromycin (15mcg) and Ampicillin (10mcg) were placed on the plates using sterile forceps, ensuring even spacing to avoid overlapping zones. Plates were incubated at 37°C for 24-48 hours. The inhibition zone diameters were measured with a ruler or antibiotic zone scale and classified as susceptible (large zones), intermediate, and resistant (small or no zones) based on standard interpretive charts. This evaluation ensures the strains do not harbor transferable resistance genes, promoting safety for probiotic use without contributing to antibiotic resistance spread (Ziyaee *et al.,* 2024).

Production of Hydrogen Peroxide (H_2O_2)

The production of hydrogen peroxide was evaluated through the application of 10 µL from overnight cultures onto MRS agar plates enhanced with 2 mg/L of horseradish peroxidase and 0.5 mM of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) serving as detection components. After aerobic incubation at 37°C over a period of 48 to 72 hours, the formation of a blue ring surrounding the colonies served as an indicator of H_2O_2 presence, arising from the peroxidase-driven oxidation of ABTS when H_2O_2 was available. This approach aids in recognizing strains that can generate H_2O_2, a compound with antimicrobial properties that supports the control of pathogens and enhances probiotic performance within oxidative conditions (Yadav *et al.,* 2016).

Hemolytic Activity

Overnight cultures were streaked on blood agar plates (tryptic soy agar supplemented with 5% v/v sheep blood) and incubated at 37°C for 24-48 h. Hemolysis was observed and classified as α (partial hemolysis with greenish discoloration), β (complete hemolysis with clear zones), or γ (no hemolysis). Non-hemolytic (γ) strains are preferred for probiotics, as hemolytic activity indicates potential virulence and unsafety for human consumption (Pieniz *et al.,* 2014).

DNase Activity

The evaluation of DNase activity involved the streaking of isolates onto DNase agar plates, followed by incubation at 37°C over a period of 24 to 48 hours. The appearance of clear areas surrounding the colonies served as an indicator of DNase presence, resulting from the breakdown of DNA. The lack of such areas (negative outcomes) verifies the non-production of extracellular DNase, which is recognized as a possible virulence element, thereby supporting the safety of the strain (Gupta and Malik, 2007).

IMViC Tests

Standard protocols were followed for biochemical differentiation. For the Indole test, tryptone broth was inoculated with overnight culture and incubated for 48 h, then tested with Kovac's reagent; a red ring at the surface indicated positivity. The Methyl Red test used MR-VP broth incubated for 48 h, followed by addition of methyl red indicator; a red color signified positivity for acid production. The Voges-Proskauer test involved the same broth with Barritt's reagents added after 48 h incubation; a red color indicated acetoin production. The Citrate test used Simmons citrate agar slants incubated for 48 h; a blue color change showed citrate utilization. These tests profile metabolic pathways, confirming non-pathogenic LAB characteristics like negative indole and citrate reactions (Nandal *et al.,* 2025d; Nandal *et al.,* 2024).

Carbohydrate Fermentation

For carbohydrate fermentation patterns evaluation, 12 different sugars were tested: lactose, glucose, xylose, sucrose, ribose, fructose, dextrose, mannitol, galactose, maltose, and trehalose. MRS basal medium was prepared with 0.0189 mg of phenol red indicator per 100 mL of distilled water as a pH-sensitive dye. This medium was distributed into flasks, and each received 0.5% of one specific carbohydrate, labeled accordingly. The mixtures were autoclaved at 115°C for 15 minutes to sterilize without degrading sugars. Overnight cultures were inoculated at 1% (v/v) into each labeled flask and incubated at 37°C for 16–24 hours. Fermentation was verified after 24 hours by observing a color shift from red to yellow, indicating acid production from sugar breakdown. This assay reveals metabolic versatility, essential for probiotic adaptation in diverse gut environments. Results were compared to Bergey's Manual for LAB classification (Kodba *et al.,* 2024; Holt *et al.,* 1994).

Antioxidant Activity

The assessment of antioxidant potential involved the application of the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. Centrifugation of overnight cultures occurred at 10,000 rpm over a duration of 10 minutes to yield cell-free supernatant (CFS). Subsequently, 100 µL of the CFS was combined with 900 µL of a 0.1 mM DPPH solution prepared in methanol within test tubes. Following a 30-minute incubation in darkness at room temperature to facilitate the reaction's completion, the absorbance was determined at 517 nm via a spectrophotometer, with methanol serving as the blank. The scavenging activity was determined using the formula (%) = [(A_control - A_sample) / A_control] × 100, in which A_control represents the absorbance of the DPPH solution in the absence of CFS. This approach measures the capacity of the strain to counteract free radicals, thereby supporting health advantages related to antioxidation, such as mitigating oxidative stress within the host (Yadav *et al.,* 2016).

Molecular Identification and Phylogentic analysis

Genomic DNA was isolated using a HiPurA® commercial Bacterial Genomic DNA Purification Kit following the manufacturer's instructions, involving cell lysis with lysozyme and proteinase K, followed by binding to a column, washing to remove impurities, and elution in buffer. The 16S rRNA gene was amplified using a 16S rRNA Semi-Q PCR Kit (HiMedia, MBPCR094). The PCR reaction mix included 25 µL of 2X PCR TaqMixture (containing Taq polymerase, dNTPs, and buffer), 2 µL of 16S Primer Mix, 2 µL

of template DNA, and Molecular Biology Grade Water to a final volume of 50 µL. Amplification was performed in a thermal cycler with conditions: initial denaturation at 94°C for 5 min (1 cycle), followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, extension at 72°C for 45 seconds, and a final extension at 72°C for 5 min (1 cycle). The PCR product was visualized on a 1% agarose gel with ethidium bromide staining, purified using a gel extraction kit and sent for Sanger sequencing (Eurofins Genomics, India). The sequence obtained was trimmed for quality using R-packages, and compared using BLASTn against the NCBI database. A similar multiple sequences were aligned using CLUSTAL W for alignment of different sequences. BLAST is used for sequence alignment. MEGA-12 uses the Neighbour-joining method for Phylogenetic tree construction (Nandal *et al.,* 2024).

Statistics

All experiments were conducted in triplicate to ensure reproducibility, with results reported as the mean ± standard deviation (SD) derived from the three independent replicates. Where necessary, means and SDs were computed by inputting the raw data into statistical functions using Microsoft® Excel 2019 software.

RESULTS

Isolation and Preliminary Screening

A total of 35 LAB isolates were obtained from Jamun vinegar samples, of which 15 were successfully sub-cultured. Four isolates were Gram-positive rods and catalase-negative, confirming their classification as lactic acid bacteria (LAB). These characteristics suggest their suitability for probiotic evaluation (Table 1).

Tolerance Tests

The tolerance ability of isolates was assessed under simulated gastrointestinal stress conditions, as shown in Table 2. Acid tolerance (pH 2.0, 2 h): JV20 showed the highest survivability (93 ± 0.17%), while JV02, JV13, and JV17 exhibited significantly lower rates (0%, 12 ± 0.12%, and 5 ± 0.15%, respectively). Bile salt tolerance (0.3%, 2 h): JV20 retained the maximum survivability (91 ± 0.12%), with the others showing reduced viability (0%, 15 ± 0.19%, and 7 ± 0.14%, respectively). Phenol tolerance (0.4%, 24 h): JV20 demonstrated strong resistance with 96 ± 0.13% survivability, whereas the other isolates showed poor performance (9%, 10 ± 0.26%, and 10 ± 0.19%, respectively). Due to the poor performance of JV02, JV13, and JV17 in these initial tolerance tests, they were not processed further for subsequent evaluations.

Antagonistic Activity

Antimicrobial activity against bacteria was evaluated for JV20, which showed strong inhibition against *Bacillus subtilis* (13 mm), *Staphylococcus aureus* (14 mm), and *Escherichia coli* (15 mm), with moderate inhibition against *Pseudomonas aeruginosa* (7 mm) (Table 3).

Antibiotic Susceptibility Testing (AST)

AST revealed that JV20 was resistant to all mentioned antibiotics (Table 4).

H_2O_2 Production

H_2O_2 production was assessed using MRS-ABTS agar. JV20 showed a clear blue halo, indicating positive H_2O_2 production (Table 5).

Safety Assessment

JV20 exhibited non-hemolytic (γ-hemolysis) and DNase-negative profiles, confirming its safety (Table 5).

Biochemical Tests

JV20 was negative for IMViC tests, consistent with LAB metabolism and non-pathogenic profiles.

Carbohydrate Fermentation

JV20 fermented a wide variety of carbohydrates efficiently, confirming superior metabolic adaptability (Table 6).

Antioxidant Activity

DPPH radical scavenging activity for JV20 displayed high activity (52.33 ± 0.36%) (Table 5).

Molecular Identification

The selected strain JV20 was confirmed as *Lactiplantibacillus plantarum* via 16S rRNA sequencing (99% identity). The sequence was submitted to GenBank (accession number PX106243). Neighbour joining phylogenetic tree of *Lactiplantibacillus plantarum* JV20 in figure 1.

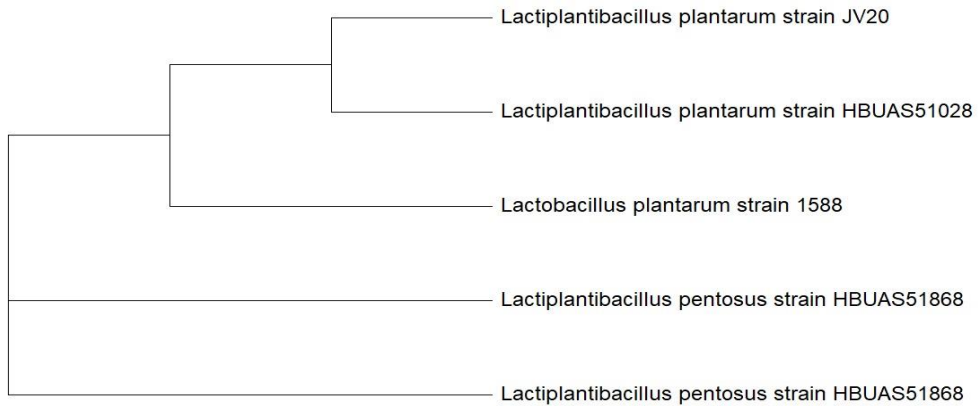

Figure 1: showing Phylogenetic dendrogram of *Lactiplantibacillus plantarum* JV20 and related lactic acid bacterial species based on the 16sRNA sequence similarities.

Table 1. Gram staining and catalase test of LAB isolates.

Isolate	Gram Stain	Catalase Test
JV 02	Positive	Negative
JV 13	Positive	Negative
JV 17	Positive	Negative
JV 20	Positive	Negative

Table 2. Acid, bile salt, and phenol tolerance of LAB isolates.

Isolate	% of survivability at pH 2.0	% of survivability in Bile Salt	% of survivability in Phenol
JV 02	0	0	9±0.18
JV 13	12±0.12	15±0.19	10±0.26
JV 17	5±0.15	7±0.14	10±0.19
JV 20	93±0.17	91±0.12	96±0.13

Values are mean ± SD of triplicates of each sample.

Table 3. Antagonistic activity of LAB isolate JV20 against pathogens.

Pathogen	JV20
Bacillus subtilis	13 mm
Staphylococcus aureus	14 mm
Pseudomonas aeruginosa	7 mm
Escherichia coli	15 mm

Table 4: carbohydrate fermentation and AST of isolate JV20

Test Type	**Name**	**Result (JV-20)**
Sugars	Glucose	Positive
	Galactose	Positive
	Fructose	Positive
	Xylose	Positive
	Sucrose	Positive
	Maltose	Positive
	Lactose	Positive
	Dextrose	Positive
	Ribose	Positive
	Trehalose	Positive
	Mannitol	Positive
Antibiotics	Cefepime	Resistant
	Levofloxacin	Resistant
	Ciprofloxacin	Resistant
	Erythromycin	Resistant
	Penicillin	Resistant
	Gentamicin	Resistant
	Amoxycillin/Clavulanic Acid	Resistant
	Tetracycline	Resistant
	Vancomycin	Resistant
	Azithromycin	Resistant
	Ampicillin	Resistant

Table 5. Antioxidant activity (DPPH scavenging), Hemolytic activity, DNase activity, and H_2O_2 production of LAB isolate JV20.

Isolate	Scavenging Activity (%)	Hemolytic Activity	DNase Activity	H_2O_2 Production
JV20	52.33±0.36	Non-Hemolytic (γ-hemolysis)	Negative	Positive

Values are mean ± SD of triplicates of each sample.

DISCUSSION

The initial screening and tolerance tests were critical in selecting a robust isolate. Out of the initial 35 isolates, only JV20 demonstrated exceptional resilience to simulated gastrointestinal conditions. Its high survival rates in acidic pH (93%), bile salts (91%), and phenol (96%) are paramount, as these are primary barriers for probiotics to colonize and exert their beneficial effects in the host (Zago *et al.,* 2011). The significant attrition of other isolates (JV02, JV13, JV17) under these stresses underscores that origin alone does not guarantee probiotic suitability, and rigorous in vitro screening is essential.

The ability of probiotics, particularly Lactobacillus strains, to inhibit pathogenic bacteria is well-documented and involves multiple mechanisms. The broad-spectrum antagonistic activity of JV20 against both Gram-positive and Gram-negative pathogens, such as Bacillus subtilis, Staphylococcus aureus, and Escherichia coli, is likely due to the production of antimicrobial compounds, including bacteriocins and hydrogen peroxide (H_2O_2), as evidenced by the ABTS agar test (Rushdi, 2022). Probiotic lactobacilli, such as Lactobacillus acidophilus and Lactobacillus plantarum, have been shown to produce substances that inhibit the growth of various pathogens, including E. coli and S. aureus, through mechanisms like competition for nutrients and adhesion sites, and the production of inhibitory compounds (Kiousi *et al.,* 2024; Sukiasyan *et al.,* 2023). The moderate inhibition observed against Pseudomonas aeruginosa by JV20 suggests that the antimicrobial effects of probiotics can be strain- and pathogen-specific, which aligns with findings that different Lactobacillus strains exhibit varying levels of antagonistic activity against pathogens like *Pseudomonas* and *S. aureus* (Zielińska *et al.,* 2021; Letizia *et al.,* 2022). Additionally, probiotics can disrupt quorum sensing pathways, which are crucial for bacterial communication and virulence, further reducing the pathogenic potential of infectious agents (Vinayamohan *et al.,* 2024). The strain-specific nature of these effects underscores the importance of selecting appropriate probiotic strains for targeted therapeutic applications, as different strains may exhibit unique antimicrobial properties and efficacy against specific pathogens (Letizia *et al.,* 2022).

The comprehensive antibiotic susceptibility profile of JV20, which shows resistance to a wide range of antibiotics, highlights the importance of understanding intrinsic resistance mechanisms in bacteria. Intrinsic resistance, as discussed in various studies, often results from cellular impermeability and the activity of efflux pumps, but it can also involve numerous proteins from diverse functional categories, as seen in *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Fajardo *et al.,* 2008; Vestergaard *et al.,* 2016). The genetic basis of such resistance can be species-specific, as demonstrated by the hypersusceptibility of Acinetobacter baylyi mutants to β-lactam antibiotics when certain genes are disrupted (Gomez & Neyfakh, 2006). This resistance can be advantageous for JV20, allowing it to survive in antibiotic-treated environments, potentially aiding in gut colonization and mitigating antibiotic-associated dysbiosis (Hollenbeck & Rice, 2012). The safety profile of JV20, characterized by the absence of hemolytic and DNase activity and a negative IMViC profile, aligns with the Generally Recognized As Safe (GRAS) status of L. plantarum, indicating its non-pathogenic nature (Hollenbeck & Rice, 2012). Furthermore, the strain's robust carbohydrate fermentation profile suggests high metabolic adaptability, crucial for thriving in the competitive gut environment (Hollenbeck & Rice, 2012). The significant in vitro antioxidant activity of JV20, with a 52.33% DPPH scavenging rate, is a promising functional attribute, as oxidative stress is implicated in numerous gastrointestinal disorders. Probiotics with antioxidant capabilities can help

modulate the host's redox balance, offering additional health benefits beyond basic probiotic functions (Poole, 2010). Understanding the genetic basis of JV20's resistance is essential to rule out the presence of transferable genes, which could pose a risk of spreading resistance to other bacteria (Willson, 1990). This understanding could also inform the development of adjuvants that augment the activity of current antibiotics, potentially rendering resistant bacteria susceptible once again (Melander & Melander, 2017). Overall, the intrinsic resistance of JV20, coupled with its safety and functional attributes, positions it as a potential candidate for therapeutic applications in managing antibiotic-associated dysbiosis.
Finally, the molecular identification via 16S rRNA sequencing confirmed the isolate as *Lactiplantibacillus plantarum* JV20, a species widely documented for its probiotic properties. The phylogenetic analysis provides a clear taxonomic placement for future comparative studies.

CONCLUSION

In conclusion, this study demonstrates that Jamun vinegar is a viable source for novel probiotic isolates. Among the isolates, *Lactiplantibacillus plantarum* JV20 emerged as the most promising candidate based on a comprehensive in vitro evaluation. It satisfies fundamental probiotic prerequisites, including high tolerance to gastrointestinal stresses, antimicrobial activity, safety, and beneficial functional properties like antioxidant capacity. Its broad antibiotic resistance profile requires further genetic analysis to ensure safety. Nonetheless, the collective results strongly suggest that *L. plantarum* JV20 has significant potential for development as a probiotic. Future research should focus on in vivo studies to validate its efficacy, stability, and health benefits in animal models or human trials.

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