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Research Article

Novel Insights into the isolation and *in vitro* probiotic characterization of *Weissella confusa* from Navara Rice in Kerala, India

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Abstract
Fermented foods are important reservoirs of probiotic lactic acid bacteria (LAB), which confer health benefits by modulating gut microbiota. Navara, a medicinal rice from Kerala, India, valued in Ayurveda, is an underexplored source of these bacteria. This study aimed to isolate, identify, and conduct the first in-vitro probiotic characterization of LAB from fermented Navara rice. A bacterial isolate was obtained on MRS agar and identified via 16S rRNA gene sequencing as *Weissella confusa* strain PK18202456.1. Its probiotic potential was evaluated using standard *in vitro* assays. The isolate demonstrated strong antagonistic activity against enteric pathogens (e.g., 12 mm zone vs. MRSA), robust coaggregation (76.67% with E. coli), and high cell-surface hydrophobicity (93.92% at 4 h). It survived simulated gastrointestinal stress, tolerating pH 3.0 and 0.3% bile salts, and exhibited bile salt hydrolase activity (22.7 ± 2.5 mm zone). Antibiotic profiling showed susceptibility to ampicillin, tetracycline, erythromycin, and clindamycin, with resistance to vancomycin and gentamicin. This work provides the first evidence for the isolation and comprehensive probiotic characterization of a *Weissella confusa* strain from Navara rice, establishing it as a promising candidate for future functional food applications.

Keywords: Antibiotic, Bacteria, Health Navara, Probiotic, Rice

INTRODUCTION

Probiotics have gained significant attention in the current scenario (Mohammed *et al*., 2025) since their principal role in modulating immunity and maintaining gut health, thereby diminishing the risk of gastrointestinal disorders (Mercado *et al*., 2025), has been verified in earlier studies. The integration of probiotics into functional foods has rapidly accelerated over the past few years, and the scientific validation of their health-promoting potential has increased consumer demand for natural solutions. Since traditional fermented foods are particularly significant in this context, as they principally reflect ancient biotechnological practices, in which microbial consortia extend shelf life and improve sensory qualities, nutritional values, and therapeutic relevance (Ray *et al.*, 2016). Across Southeast Asia, rice serves as a staple grain and is central to an extensive range of traditional fermented foods in various provinces (Singh *et al*., 2025). Household-level preparations, including idli, dhokla, uttapam, babru, ambeli, vada, appam and adai, remain culturally relevant and nutritionally valuable. Furthermore, the presence of various minerals, including B-complex vitamins and vitamin K, in the fermented rice dishes made it an energy-rich source. They are well documented for regulating body temperature, lowering blood pressure, and enhancing digestion, and earlier studies have linked their consumption with improved bowel function and the prevention of gastrointestinal ailments (Choi *et al*., 2014).The reported beneficial effects are primarily attributed to lactic acid bacteria (LAB) that dominate the microbial communities of fermented rice (Huang *et al*., 2025). The following microbial strains are previously known to produce the beneficial effects: *Lactiplantibacillus plantarum, Lactobacillus delbrueckii subsp. bulgaricus, Lacticaseibacillus casei, Pediococcus acidilactici, Streptococcus thermophilus*, and species of *Weissella* and *Bifidobacterium* (Chunguang and Dapeng, 2008; Lu *et al*., 2008; Song *et al.,* 2013). The aforesaid microbial consortia not only contribute to desirable sensory qualities but also confer probiotic traits, including tolerance to gastrointestinal stress and subsequent antimicrobial activity against pathogens. Despite long-standing consumption of fermented rice across various regions of India, systematic evaluations of its probiotic potential remain scarce in the current context. Much of the earlier research has principally been conducted across the northeastern states reported the extensive microbial diversity in sour rice and rice wine fermentations (Bora *et al*, 2016; Shukla and Goyal, 2011), while none of the previous studies have investigated the fermented rice in Kerala, with special reference to the introduced inferences. This represents a critical research gap, as such differences may yield strains with novel functional properties. Therefore, this study is the first to specifically target the isolation and systematic in-vitro probiotic assessment of LAB from fermented Navara rice, a valued medicinal food staple in Kerala, with a focus on safety.

Simultaneously, concerns regarding the safety of certain microbial genera, specifically Weissella, which have been previously reported in both food fermentations and clinical samples, justify the need for precise evaluation at the strain level (Fairfax *et al*., 2014; Fessard and Remize, 2017). The present study was intended to explore the probiotic potential of fermented cooked rice prepared in households in Kerala. The primary focus of the present study was to isolate and identify lactic acid bacteria with respect to auto-aggregation, antimicrobial activity, and hydrophobicity. Furthermore, safety assessments such as haemolytic activity and antibiotic resistance profiling were accomplished.

MATERIALS AND METHODS

Collection of samples, bacterial isolation and identification

The sample specimens of Navara (*Oryza sativa* L.) sourced from Palakkad, Kerala, India. Fermented sour rice was collected aseptically in sterile containers and immediately transported to the laboratory for microbiological analysis. For bacterial isolation, the samples were serially diluted (10^{-2}–10^{-4}) using phosphate-buffered saline (PBS). From each dilution, 0.1 ml was spread onto de Man–Rogosa–Sharpe (MRS) agar plates (Himedia, Mumbai, India). The inoculated plates were incubated at 37 °C for 24–48 hours for bacterial growth. Distinct and unique colonies were subcultured and examined by Gram staining, colony characteristics, and standard biochemical tests.

Molecular identification

Genomic DNA was extracted using a bacterial DNA isolation kit (Bangalore Genei, India) and analyzed by agarose gel electrophoresis. The 16S rDNA gene was amplified with specific primers as instructed by the manufacturer, under standard PCR conditions, and the products were sequenced (Xcelris Labs, India). Consensus sequences were aligned and compared with NCBI GenBank using BLAST. Phylogenetic analysis was performed using the neighbour-joining method with 1000 bootstrap replicates, and sequence relationships were visualized in MEGA.

Probiotic traits assessment

An acid tolerance test was done according to the method suggested by (Ko *et al*., 2022). The Biofilm Formation Assay was performed according to Yumnam *et al*., (2025).

Bile tolerance test

The bile tolerance test was performed according to the method described by Nath *et al* (2020). Overnight-grown cultures were inoculated into MRS broth with 0.3% bile salts (Himedia, India) and incubated at 37 °C. Viability was evaluated hourly by plating on MRS agar, while broth without bile served as the control.

Test for resistance to low pH

Acid tolerance was evaluated according to the method described by (Kirtzalidou *et al*. (2011), by incubating bacterial suspensions in PBS adjusted to pH 3 and 7.2 at 37 °C for 3 h, mimicking gastric conditions. Viability was assessed at hourly intervals by plating on MRS agar, and results were expressed as log10 CFU/ml.

Antimicrobial activity assay

Antimicrobial activity was evaluated using the agar well diffusion method. Cell-free supernatant (CFS) from the isolate was obtained by centrifugation (10,000 × g, 10 min, 4°C) and adjusted to pH 7.0 to neutralize organic acids. Wells (6 mm diameter) were cut into Mueller-Hinton agar plates seeded with a standardized suspension (0.5 McFarland) of the target pathogen. Then, 100 µL of the pH-neutralized CFS was added to the wells. A well containing sterile, pH-adjusted MRS broth served as the negative control on each plate. Plates were incubated at 37°C for 24 hours, after which the diameter of the inhibition zone (including the well diameter) was measured in millimeters.

Simulated gastric juice tolerance test

Simulated gastric juice was prepared with pepsin (3 g/L), KCl (7 mM), $NaHCO_3$ (45 mM), and NaCl (125 mM) adjusted to pH 3 (Archer and Halami, 2015). Overnight cultures were harvested, washed, and resuspended in PBS before incubation in gastric juice at 37 °C for 3 h.

PBS at neutral pH served as a control. Viability was assessed by plating on MRS agar, and survival was expressed as $\log_{10}$ CFU/ml and survival percentage.

Equation 1:

$$\text{Bacterial survival (\%)} = \frac{CFU\ assay}{CFU\ control} x\ 100$$

CFU assay: Cell count post-incubation in simulated gastric juice at pH 3,

CFUcontrol: Cell count after incubation in PBS solution.

Pancreatin tolerance test

Bacterial cultures were inoculated into MRS broth with 0.5% pancreatin (Himedia, India) and incubated at 37 °C for 48 h. Viability was evaluated by plating on MRS agar, with broth without pancreatin as a control (Koll *et al*., 2010).

Bile salt hydrolase (BSH) activity assay

BSH activity was determined as described by (Dashkevicz and Feighner, 1989) with modifications. Briefly, the isolate was spot-inoculated on MRS agar plates supplemented with 0.5% (w/v) sodium taurodeoxycholate (TDCA; Himedia, India) and 0.37 g/L calcium chloride. The plates were incubated anaerobically at 37°C for 72 h. The formation of a clear, opaque halo of precipitated bile acids around the bacterial colonies was considered positive for BSH activity. The diameter of the hydrolysis zone was measured in triplicate.

Cell surface hydrophobicity

Cell surface hydrophobicity was assessed using the method described by Pan and Liu (2006). Cultures grown in MRS broth (37 °C, 24 h) were harvested, washed with PBS (pH 7.2), and resuspended. Absorbance at 600 nm was recorded before and after mixing with hydrocarbons (n-hexadecane and toluene) and allowing phase separation. The percentage hydrophobicity was then calculated using the following equation (Shangpliang *et al*., 2017).

Equation 2:

Percentage Hydrophobicity (%)=

$$\frac{OD\ initial - OD\ Final}{OD\ Initial} x\ 100$$

Cellular autoaggregation

Cellular Autoaggregation was determined according to the method described by Xu *et al*., (2009) . Overnight cultures grown at 37 °C were harvested, washed, and resuspended in PBS (pH 7.2). Absorbance at 600 nm was recorded before and after 2 h incubation at 37 °C, and the percentage of autoaggregation was calculated.

Equation 3:

Percentage Cellular autoaggregation (%) =

$$\frac{OD\ initial - OD\ Final}{OD\ Initial} x\ 100$$

Safety assessment

Antibiotic susceptibility test

Antibiotic sensitivity was determined using the disk diffusion method (Baccer *et al*., 1966). The isolates were spread on Mueller Hinton agar (MHA) and incubated at 37 °C for 24–48 h. The zone of inhibition was measured and interpreted according to standard guidelines (Nath et al., 2019). Commercial discs (HiMedia, India) of gentamicin, vancomycin, tetracycline, ampicillin, clindamycin, erythromycin, and chloramphenicol were used for the present assessment.

Detection of H_2O_2 production

Detection of H_2O_2 production was performed using the method described by Jeyagowri *et al*., (2015).The absorbance of the reaction mixture was measured spectrophotometrically at 400 nm. A reaction mixture containing all components except the bacterial culture served as the negative control.

Statistical analysis

All the experiments were performed in triplicate, and the results are noted as mean ± standard deviation. Data were initially entered in Microsoft Excel 2007 and exported to R version 4.4.2 (2024-10-31) -- "Pile of Leaves". A t-test with the following levels of significance was used to determine statistical significance: $p < 0.001$, $p < 0.01$, and $p < 0.05$.

RESULTS

Identification

This study documented the prevalence of *Weissella confusa* in traditional fermented rice. The isolate produced colonies on MRS agar plates. The biochemical analysis determined the following results (Table 1). As shown in Fig. 1, the evolutionary history was inferred using the Maximum Likelihood method and the Tamura -Nei model, and the tree with the highest log likelihood (-8294.29) is shown. The analysis involved 19 nucleotide sequences, and the following codon positions were included in this investigation: 1st+2nd+3rd+Noncoding. There were a total of 1210 positions in the final dataset. The isolate of the present study named “PK18202456.1” produced strong similarity with *Weissella confusa* with strong bootstrap support. The molecular identification validates the taxonomic identity of the isolate PK18202456.1.

Detection of H_2O_2 production

The isolate from Navara rice demonstrated clear hydrogen peroxide production, yielding a significant absorbance of 0.995 ± 0.003 at 400 nm. In contrast, the negative control showed no detectable absorbance (0.000 ± 0.000). This confirms that the measured signal is specifically due to H_2O_2 production by the isolate, a desira-

Fig. **1**. *Phylogenetic analysis of Weissella confusa isolate PK18202456.1. The evolutionary tree was constructed using the Maximum Likelihood method based on 16S rRNA gene sequences, showing the isolate's close relationship with reference Weissella confusa strains*

ble probiotic trait for its role in suppressing pathogenic microbes. The low standard deviation indicates consistent measurement within this experimental setup.

Co-Aggregation assay with pathogens

The co-aggregation assay unveiled that the *W. confusa* isolate showed 53.64% aggregation towards *E. coli* and 48.96% towards *S. aureus* at 0 hours. After 24 hours of exposure, the co-aggregation elevated to 76.67% with *E. coli* and 77.21% with *S. aureus*. These results verify that the strain shows co-aggregation ability in a time-dependent manner, which may significantly contribute to the competitive exclusion of pathogens (Fig. 2).

Antimicrobial activity

Antimicrobial activity was evaluated using the well diffusion approach against methicillin-resistant *Staphylococcus aureus* and E. *coli* ATCC 25922. The negative control (sterile MRS broth) produced no zone of inhibition, confirming that the observed antagonism was specifically due to antimicrobial compounds produced by the isolate. The *W. confusa* isolate (PK18202456.1) produced a 12 mm zone of inhibition, indicating a moderate antibacterial potential.

Cell surface hydrophobicity

Cell hydrophobicity was assessed at different time intervals. The isolate produced hydrophobicity values of 52% (2 h), 93.92% (4 h), 21.6% (6 h), and 42.5% (24 h) at the respective times indicated in brackets. The highest value was observed at 4 hours, and it justifies strong surface interactions. The variation observed at later time points suggests that hydrophobicity may fluctuate with incubation time (Fig. 3).

Auto-Aggregation assay

The auto-aggregation potential of the *W. confusa* isolate (PK18202456.1) was assessed over a 24-hour period. The isolate showed a progressive increase in aggregation over time, indicating strong cell-to-cell interaction capacity. At the 2nd hour, the aggregation was documented at 12%, which gradually elevated to 21% and 28% at the 4th and 6th hours, respectively. The 8th hour showed 34% aggregation, while the 24th hour reported a significant increase to 56%. These findings suggest that the studied *W. confusa* isolate (PK18202456.1) showed a considerable potential for auto-aggregation, which is recognized as an important probiotic trait (Fig. 4).

Co-Aggregation with Other Pathogens

When tested with other bacterial strains, the *W. confusa* isolate (PK18202456.1) produced strong co-aggregation in the following manner: 80%, 78%, 76% and 74% with *S. typhimurium*, non-typhoidal *Salmonella*, *V. cholerae* and *Shigella sonnei*, respectively. These results revealed that the isolate in the present study interacts strongly with multiple pathogens, a relevant probiotic feature (Fig. 5).

Biofilm formation assay

The ability of the isolate to form biofilms was assessed. The *W. confusa* isolate (PK18202456.1) showed a

Table 1. Biochemical characteristics of *Weissella confusa* isolate PK18202456.1

Parameter	Observations
Indole test	+
Methyl red test	+
Nitrate reduction test	–
Catalase test	–
Citrate utilisation test	–
Starch hydrolysis test	–
Oxidase test	–

mean biofilm absorbance of 0.800, though with considerable variability among replicates (SD = ± 0.777). The negative control recorded 0.221 ± 0.025. While this indicates a capacity for biofilm formation, the high variability suggests the phenotype under these assay conditions may be inconsistent or influenced by unmeasured factors.

Acid tolerance (pH 3.0)

Optical density measurements indicated a trend of tolerance to acidic conditions. The W. confusaisolate maintained stable absorbance values (0.058–0.059) over the 6-hour incubation at pH 3.0. In contrast, the absorbance of the uninoculated control medium decreased from 0.044 to 0.036 over the same period (Table 2). This suggests the isolate's potential to survive transit through the acidic gastric environment.

Bile salt tolerance (0.3% Bile)

The isolate demonstrated tolerance to bile salts, a key requirement for intestinal persistence. The optical density of the *W. confusa* culture increased from 0.104 at 0 hours to 0.177 at 6 hours in the presence of 0.3% bile. The uninoculated control medium showed lower absorbance values throughout the incubation (0.061 to 0.129) (Table 3).

Pancreatin tolerance (0.5% Pancreatin)

The isolate showed an increasing trend in optical density when exposed to the digestive enzyme pancreatin. The absorbance of the *W. confusa* culture rose from 0.079 to 0.188 over 6 hours, while the uninoculated control exhibited lower values, increasing from 0.057 to 0.137 (Table 4). This indicates the isolate's capacity to withstand enzymatic digestion in the small intestine.

Bile salt hydrolase (BSH) Activity

BSH activity was confirmed with measurable clear zones in triplicate [23 mm, 20 mm, and 25 mm (22.7 ± 2.5)]. The findings demonstrate active bile salt hydrolysis in vitro, a biochemical trait that has been associated with cholesterol-lowering and bile salt deconjugation

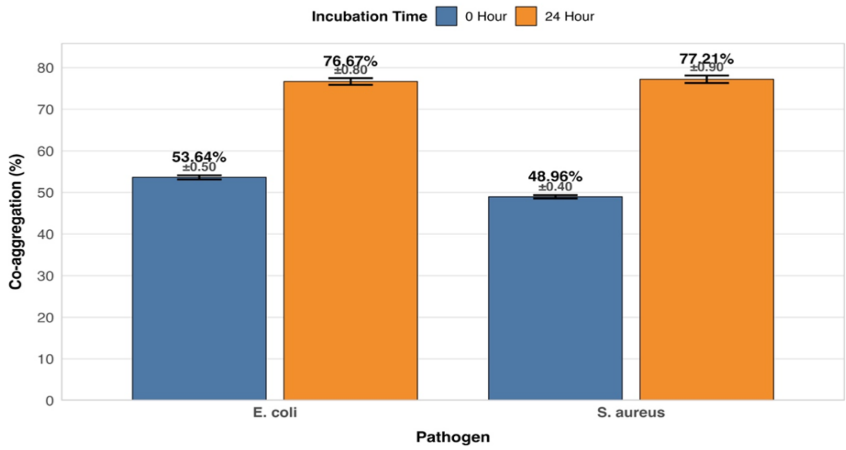

Fig. 2. *Co-aggregation of Weissella confusa PK18202456.1 with enteric pathogens; Percentages represent mean co-aggregation ± standard deviation (n=3) with Escherichia coli and Staphylococcus aureus after 0 and 24 hours of incubation*

Table 2. Acid tolerance of *Weissella confusa* isolate PK18202456.1. Control: Uninoculated MRS broth at pH 3.0. Data are mean optical density (OD_{600}) ± SD (n=3).

Time	Control (Mean ± SD)	*W. confusa* Isolate (Mean ± SD)
0 Hour	0.044 ± 0.001	0.058 ± 0.001
2 Hour	0.040 ± 0.002	0.053 ± 0.002
4 Hour	0.037 ± 0.003	0.059 ± 0.002
6 Hour	0.036 ± 0.002	0.059 ± 0.003

Table 3. Bile salt tolerance of *Weissella confusa* isolate PK18202456.1. Control: Uninoculated MRS broth with 0.3% (w/v) bile salts. Data are mean optical density (OD_{600}) ± SD (n=3)

Time	Control (Mean ± SD)	*W. confusa* Isolate (Mean ± SD)
0 Hour	0.061 ± 0.004	0.104 ± 0.010
2 Hour	0.061 ± 0.004	0.107 ± 0.003
4 Hour	0.083 ± 0.005	0.138 ± 0.013
6 Hour	0.129 ± 0.001	0.177 ± 0.009

Table 4. Pancreatin tolerance of *Weissella confusa* isolate PK18202456.1. Control: Uninoculated MRS broth with 0.5% (w/v) pancreatin. Data are mean optical density (OD_{600}) ± SD (n=3)

Time	Control (Mean ± SD)	*W. confusa* Isolate (Mean ± SD)
0 Hour	0.057 ± 0.005	0.079 ± 0.014
2 Hour	0.059 ± 0.001	0.077 ± 0.005
4 Hour	0.081 ± 0.007	0.100 ± 0.007
6 Hour	0.137 ± 0.003	0.188 ± 0.019

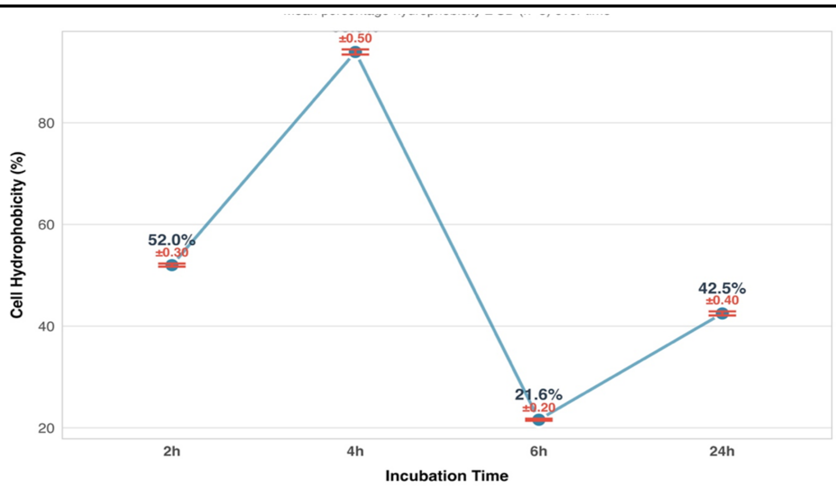

Fig. 3. *Cell surface hydrophobicity of Weissella confusa PK18202456.1 over time. Values show mean hydrophobicity percentage ± SD (n=3), demonstrating a transient peak at 4 hours followed by stabilization.*

effects in probiotic bacteria.

Survival in simulated gastric juice

The isolate from Navara rice survived at pH 2.0 for 2 hours, confirming its potential to tolerate gastric stress. This survival ability is a critical requirement for strains considered as probiotics.

Antibiotic susceptibility test

The *W. confusa* isolate (PK18202456.1) showed varied susceptibility to antibiotics. It was susceptible to ampicillin, tetracycline, erythromycin, chloramphenicol, and clindamycin, while the strain is resistant to gentamycin and vancomycin. These results suggest that the studied strain retains sensitivity to several clinically significant antibiotics but also harbours resistance traits that require careful consideration (Fig. 6).

Antimicrobial activity against enteric pathogens

The isolate demonstrated measurable inhibition zones against selected pathogens. The *Vibrio cholerae, Shigella sonnei* and *Salmonella typhi* produced a zone of inhibition of 15 mm, 17 mm and 15 mm, respectively, while no inhibition was recorded against non-typhoidal *Salmonella*. These findings justify selective antagonism against enteric pathogens.

DISCUSSION

The bacterial isolate *Weissella confusa* investigated in this study from Navara rice was characterized by colony morphology, molecular taxonomy (Fig. 1), and biochemical properties, and demonstrated functional probiotic traits. It is important to note that the probiotic properties reported here are specific to the character-

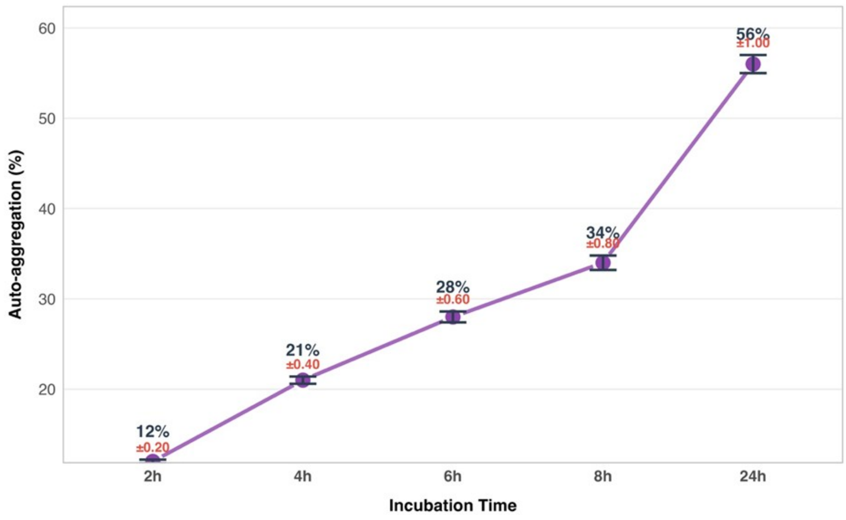

Fig. 4. *Time-dependent auto-aggregation of Weissella confusa PK18202456.1. Auto-aggregation capacity increased progressively from 2 to 24 hours, with mean percentages ± SD (n=3) indicating strong cell self-adherence*

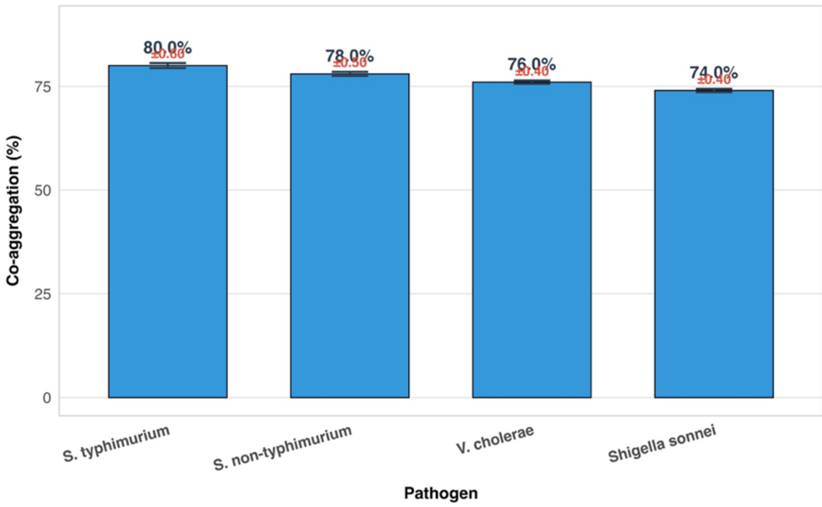

Fig.5. *Co-aggregation of Weissella confusa PK18202456.1 with multiple enteric pathogens. The isolate showed strong co-aggregation capacity (>70%) with Salmonella typhimurium, Vibrio cholerae, and Shigella sonnei*

ized isolate, *Weissella confusa* PK18202456.1, and may not be generalizable to all strains of the species or from this food source. The implementation of 16S rRNA gene sequencing provided definitive species-level identification as *Weissella confusa* (strain PK18202456.1), representing the first report of this species from Navara rice (Fig. 1). The biochemical profiling unveiled the presence of lactic acid bacteria, with positive results for indole and methyl red tests, and negative results for catalase, citrate utilization, and oxidase activities. Similar observations were reported in earlier studies by (Jeyagowri *et al*., 2015; Shukla and Goyal, 2011). The aforesaid biochemical patterns usually correlate with non-pathogenic lactic acid bacteria adapted to plant-derived substrates. Specifically, the positive methyl red test indicates mixed acid fermentation, a metabolic trait common in Weissella species. The negative catalase and oxidase results are characteristic of obligate fermentative LAB that thrive in anaerobic environments, such as fermented foods and the gut. The negative citrate utilization indicates that the strain cannot use citrate as a sole carbon source, which aligns with typical carbohydrate fermentation patterns of food-associated LAB. These biochemical traits collectively support both the taxonomic identification and the strain's adaptation to plant-based fermentation niches.

Functional probiotic potential

One of the major findings was the potential of the *W. confusa* isolate (PK18202456.1) to produce hydrogen peroxide. The significant production of H_2O_2 (0.995 ± 0.003) in a null control indicates an antimicrobial strategy that could contribute to inhibiting pathogens in vitro, a trait relevant for gut health. The production of hydrogen peroxide has previously been documented in lactic acid bacteria as a predominant trait that assists their

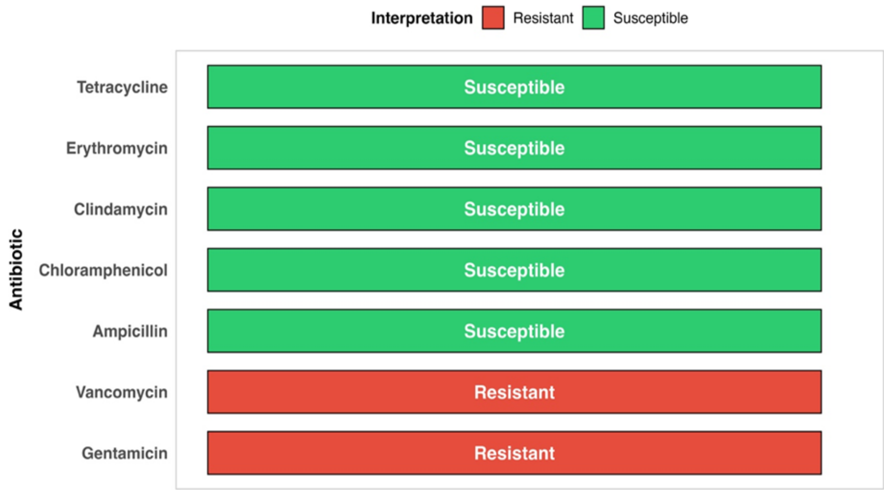

Fig.6. *Antibiotic susceptibility profile of Weissella confusa PK18202456.1. The isolate was susceptible to most tested antibiotics but showed resistance to gentamicin and vancomycin, consistent with typical Weissella species patterns*

antagonistic activity against enteric pathogens (Patel and Ljungh, 2014; Quattrini and Fortina, 2020). The co-aggregation assay further produced a strong interaction of the isolate towards the pathogenic microbial consortia. The isolate from the Navara rice showed co-aggregation with E. coli (53.64% → 76.67%) and S. aureus (48.96% → 77.21%) within 24 hours in a time-dependent manner. Co-aggregation with pathogens is crucial as it facilitates competitive exclusion, thereby reducing pathogen adhesion to host epithelial cells (Angmo *et al*., 2016). Similar to earlier studies on Weissella confusa and Lactobacillus plantarum, our isolate also showed substantial co-aggregation with multiple pathogens, including Salmonella typhi, Vibrio cholerae, and Shigella sonnei, strengthening its potential as a protective probiotic strain (Adesulu-Dahunsi *et al*., 2018). The antimicrobial activity of the isolate from Navara rice was verified by inhibition zones ranging from 12 to 17 mm against E. coli, methicillin-resistant S. aureus (MRSA), V. cholerae, Shigella sonnei, and S. typhi. The antimicrobial activity, observed against multiple pathogens and validated against a sterile negative control, suggests the production of inhibitory metabolites including bacteriocins. The observed antagonism suggests the production of inhibitory metabolites. While the exact nature of these compounds was not characterized in this study, they may include organic acids, bacteriocins, or other antimicrobial substances, as is commonly reported for lactic acid bacteria. Future studies should characterize these compounds through pH adjustment, protease treatment, and chromatographic analysis. In line with the present observations, previous studies have demonstrated that Weissella and Lactobacillus species produce antimicrobial compounds with inhibitory activity against pathogenic bacteria. For instance, Tenea and Lara (2019) reported that Weissella confusa Cys2-2 inhibits Gram-negative bacteria through antimicrobial metabolite production, while Ahmadova *et al*., (2013) documented the inhibitory spectrum and probiotic potential of Lactobacillus curvatus A61.

Surface and adhesion-related properties

The isolate showed variable cell-surface hydrophobicity, with the highest value (93.92%) recorded at 4 h. This finding suggests strong transient adhesion potential. Although fluctuations were observed at later times, the levels of overall hydrophobicity remained above the threshold (>40%), generally indicating adhesive capacity (Nostro *et al*., 2004). Complementing this observation, auto-aggregation was progressively increased, reaching 56% at 24 h, which is comparable with that reported for other probiotic LAB strains in fermented foods (Han *et al*., 2017). Altogether, the aforesaid traits emphasize the potential of the studied strains to adhere to intestinal mucosa and competitively exclude pathogens. Biofilm formation further confirmed the colonization potential of the isolate. A significantly higher absorbance than the negative control indicates a structured biofilm-forming ability. This trait is beneficial for persistence in the gastrointestinal tract and for resistance to a plethora of environmental stresses, echoing earlier findings from LAB strains in fermented foods in the Indian scenario reported by Sharma *et al*. (2018).

Gastrointestinal stress tolerance

Survival under acidic and enzymatic conditions is vital for probiotics. The *W. confusa* isolate (PK18202456.1) survived significantly at pH 3.0 for 6 h. This finding indicates a strong acid tolerance and a prerequisite for passage through gastric environments (Lee *et al*., 2012). Bile salt tolerance was also evident in this study, with growth maintained up to 0.3% bile. The BSH activity is relevant, as it represents a biochemical capability associated with cholesterol metabolism in probiotic strains (Hamon *et al*., 2011).The isolate also exhibited resilience against the studied digestive enzyme, pancreatin, with survival significantly higher than that of controls. Such tolerance aligns with earlier findings in Weissella and Lactobacillus strains that demonstrate variable but promising resistance to gastrointestinal enzymes (Oh et al.,2018). Furthermore, survival in simulated gastric juice at pH 2.0 confirms the isolate's potential to withstand gastric transit, further reinforcing its probiotic traits.

Antibiotic susceptibility profile

The isolate was also susceptible to clinically relevant antibiotics, including ampicillin, tetracycline, erythromycin, and clindamycin, while resistant to vancomycin and gentamycin. Resistance to vancomycin is consistent with reports on Weissella and other LAB, where intrinsic resistance is not considered a safety concern because it is typically non-transmissible (Kamboj *et al*.,, 2015). Nevertheless, genomic assessment of antibiotic resistance genes is warranted to unveil horizontal gene transfer potential. For probiotic safety assessment, future studies should include genomic analysis to confirm whether these resistances are intrinsic (chromosomal) or potentially transferable via mobile genetic elements. Taken together, the *W. confusa* isolate (PK18202456.1) exhibited a broad spectrum of probiotic traits, including acid and bile tolerance, enzymatic resistance, adhesion capacity, pathogen exclusion, antimicrobial activity, and biofilm formation. The reported findings are consistent with earlier studies focusing on LAB isolated from Indian fermented foods such as idli batter and dairy products, which have reported similar functional characteristics (Shukla *et al*., 2011). The present study thus highlights Navara rice as a promising reservoir of potential probiotic bacteria with functional significance for human health.

Study limitations

This study has certain limitations. The analysis of the time-course tolerance data (acid, bile, pancreatin) was descriptive. While the optical density trends clearly indicate robust survival, a repeated-measures ANOVA would be the statistically rigorous approach for future studies to confirm differences and interactions between treatment and time formally. Additionally, the variability observed in the biofilm formation assay suggests that protocol optimization could yield more consistent measurements for this specific trait.

Conclusion

Fermented Navara rice is considered a traditional, nutritious food with health benefits. The isolated strain Weissella confusa PK18202456.1 showed key probiotic traits, including survival under acidic and bile conditions, strong adhesion, and antimicrobial activity, with a safety profile warranting further genomic characterization of its antibiotic resistance markers. These results suggest its potential as a probiotic and starter culture for functional foods. Further, it is suggested that research should focus on: (i) *in vivo* validation of cholesterol modulation and pathogen exclusion in animal models, (ii) purification and characterization of the antimicrobial compounds responsible for the observed activity, and (iii) whole-genome sequencing to fully assess biosafety, including antibiotic resistance gene context and absence of virulence factors.

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Conflict of interest

The authors declare that they have no conflict of interest.

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