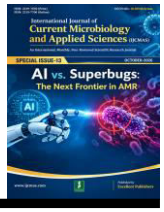
Int.J.Curr.Microbiol.App.Sci (2026) *Special Issue-13: 341-354*

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Special Issue-13 [October-2026]**
Journal homepage: http://www.ijcmas.com



Original Research Article https://doi.org/10.20546/ijcmas.2026.sp13.036

Isolation, Functional Characterization and Molecular Identification of a Fish Gut-Derived *Lactobacillus plantarum* with Probiotic Potential

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Keywords

Salmon fish gut; lactic acid bacteria; probiotic; *Lactobacillus plantarum*; acid tolerance; bile tolerance; antibacterial activity; 16S rRNA.

Article Info

Received:
20 August 2026
Accepted:
29 September 2026
Available Online:
10 October 2026

A B S T R A C T

The gastrointestinal tract of fish represents an important reservoir of microorganisms adapted to the host environment and therefore provides a useful source for the selection of autochthonous probiotic candidates. The present study aimed to isolate lactic acid bacteria from the gut of healthy salmon fish, screen the isolates for probiotic-associated traits, and identify the most promising isolate by 16S rRNA gene sequencing. Gut material was aseptically collected, homogenized and serially diluted before plating on de Man, Rogosa and Sharpe (MRS) agar. Four morphologically distinct isolates, designated C1–C4, were recovered. Isolate C1 was selected after preliminary characterization because it was a Gram-positive rod and showed negative catalase and oxidase reactions. The isolate tolerated exposure to pH 2.5 and showed moderate growth in the presence of 0.3% bile salts. It also exhibited γ-haemolysis on blood agar. Cell-free supernatant of C1 inhibited Salmonella Typhi, Escherichia coli, Proteus vulgaris and *Acinetobacter baumannii*, with inhibition zones of 18.0, 13.5, 13.0 and 10.5 mm, respectively. Molecular characterization generated an approximately 1,500-bp 16S rRNA amplicon. BLAST comparison showed 99.6% identity with *Lactobacillus plantarum* strain ATCC 14917, 99.4% with strain WCFS1 and 99.2% with strain JDM1, and the phylogenetic analysis placed isolate *L. plantarum* clade. The isolate therefore showed a combination of gastrointestinal stress tolerance, non-haemolytic phenotype and antagonistic activity that supports its consideration as a potential fish-feed probiotic.

Introduction

Aquaculture has become a major component of global aquatic food production and is increasingly important for food security, livelihoods and the supply of animal protein. Intensification of production, however, also increases the biological pressure on cultured fish. High stocking density, repeated handling, artificial feeding, fluctuating water quality and exposure to opportunistic microorganisms can disturb host–microbe equilibrium and increase susceptibility to disease. The expansion of aquaculture has consequently been accompanied by continued interest in preventive approaches that can maintain fish health while reducing dependence on antimicrobial chemotherapy. Reviews of aquaculture health management have identified probiotics as one of the biological tools that can contribute to disease prevention, growth and maintenance of intestinal microbial balance (Nayak, 2010; Hai, 2015; El-Saadony *et al.,* 2021).

The use of antibiotics in aquatic production has also raised environmental and public-health concerns. Antibiotic residues and resistant bacteria may enter aquatic environments and create selective pressure favouring resistant populations. Cabello (2006) and Martínez (2009) emphasized that intensive antimicrobial use in aquaculture can affect environmental microbial communities and may contribute to the wider antimicrobial-resistance problem. These concerns have encouraged the development of non-antibiotic strategies based on biological control, improved husbandry, vaccination and functional feeds. Probiotics are particularly attractive because their activity can occur within the intestinal microbial ecosystem rather than relying on broad chemical suppression of microorganisms. The classical concept of a probiotic refers to a live microorganism that, when administered in an adequate amount, confers a health benefit on the host.

In aquaculture, the concept is broader in application because candidate microorganisms may be administered through feed or water and may influence growth, feed utilization, disease resistance, immune responses, gut microbial communities and, in some cases, water quality (Verschuere *et al.,* 2000; Balcazar *et al.,* 2006; Hai, 2015). The effects are strain dependent. Consequently, the presence of a bacterium in the fish gut does not automatically make it a probiotic, and a systematic screening process is required before a strain is selected for application.

The fish gastrointestinal tract is a dynamic microbial habitat. Its microbial composition is influenced by the host species, age, diet, water environment, season, rearing system and physiological condition. Gut microorganisms participate in nutrient transformation, digestion, intestinal barrier function and interactions with the host immune system (Ringo *et al.,* 2016). Some intestinal bacteria produce extracellular enzymes and metabolites that may complement host digestion, whereas others can compete with opportunistic organisms for nutrients and attachment sites. This ecological complexity explains why host-derived microorganisms are frequently investigated as sources of candidate probiotics.

Lactic acid bacteria have received particular attention because many members are associated with carbohydrate fermentation, acid production and antagonistic activity. *Lactobacillus plantarum* has been widely studied because of its metabolic flexibility and tolerance to environmental stresses. De Vries *et al.,* (2006) reviewed the functional properties of *L. plantarum* and highlighted its capacity to survive under diverse conditions. Hai (2015) further emphasized that intestinal isolates can be evaluated for host-specific application through a combination of in vitro and in vivo tests. It is important to note that the genus Lactobacillus was taxonomically reorganized in 2020; the species formerly known as *Lactobacillus plantarum* is now placed in the genus Lactiplantibacillus (Zheng *et al.,* 2020). The traditional name remains widely used in the literature, and both names are therefore encountered in aquaculture publications.

An effective probiotic candidate should be able to remain viable under conditions that resemble the gastrointestinal tract. Acid tolerance is commonly used as an initial screening characteristic because microorganisms entering the digestive tract may encounter acidic conditions. Bile tolerance provides a second functional criterion because bile salts can damage bacterial membranes and influence microbial survival in the intestine. Gilliland *et al.,* (1984) demonstrated the importance of bile tolerance for dietary lactobacilli, while Charteris *et al.,* (1998) developed in vitro approaches to examine gastrointestinal transit tolerance. In fish, these assays should be interpreted as preliminary indicators rather than direct simulations of the complete digestive process.

Safety screening is equally important. A candidate strain intended for feed application should not exhibit obvious virulence-associated characteristics. Haemolytic activity

is frequently examined because haemolysis may indicate production of factors capable of damaging erythrocytes. The absence of haemolysis is therefore a useful screening observation, although it does not by itself establish complete strain safety. FAO/WHO (2002) guidance on probiotic evaluation emphasizes the need for a systematic characterization process, and contemporary safety assessment increasingly includes antimicrobial susceptibility, genomic screening and evaluation of transferable resistance or virulence determinants. Antagonistic activity against pathogens is another important functional criterion. Probiotic bacteria may inhibit competing microorganisms through acidification, production of hydrogen peroxide, bacteriocin-like substances and other metabolites, as well as through nutrient competition and interference with pathogen adhesion. Verschuere *et al.,* (2000) described these mechanisms as ecological interactions that can help maintain microbial balance. Schillinger and Lucke (1989) demonstrated that lactobacilli can produce inhibitory activities against other bacteria, illustrating the value of cell-free supernatants in preliminary screening. In aquaculture, the relevance of antagonism should nevertheless be interpreted in relation to the actual pathogens affecting the intended fish host.

Phenotypic characterization provides the first level of bacterial identification, but phenotypic traits alone are not sufficiently discriminatory for species assignment. Gram reaction, cell morphology, catalase and oxidase reactions are useful screening tests, while molecular identification provides a more reliable taxonomic framework. The 16S rRNA gene contains conserved and variable regions that permit comparison with reference sequences and is widely used for bacterial identification (Clarridge, 2004). In the present work, a nearly full-length 16S rRNA amplicon was obtained and compared with sequences in the NCBI database. The resulting identification was further supported by phylogenetic analysis.

The study was therefore designed to follow a sequential selection strategy: recovery of gut-associated bacteria on MRS agar, preliminary phenotypic screening, assessment of acid and bile tolerance, safety screening by haemolysis, evaluation of antibacterial activity, and molecular identification of the selected isolate. The specific objective was to characterize the most promising isolate from the salmon fish gut and determine whether its observed properties support its further development as a fish-feed probiotic candidate.

Materials and Methods

Fish sample collection and gut preparation

Healthy Oncorhynchus species (salmon fish) were obtained from a local fish market and transported to the laboratory under cooled conditions. The external surface was washed with sterile distilled water and disinfected with 70% ethanol to reduce contamination from the skin and surrounding surfaces. Under aseptic conditions, the abdominal cavity was opened using sterile instruments and the intestinal tract was removed. The gut material was transferred to sterile physiological saline and homogenized thoroughly to obtain a uniform microbial suspension.

Isolation and purification of lactic acid bacteria

The homogenized gut suspension was serially diluted in sterile saline to obtain dilutions from 10^{-1} to 10^{-6}. Aliquots from appropriate dilutions, particularly 10^{-5} and 10^{-6}, were spread on MRS agar and incubated at 37 °C for 24–48 h under anaerobic or microaerophilic conditions. Distinct colonies were selected according to colony morphology and purified by repeated subculture on MRS agar. (De Man *et al.,* 1960; Cappuccino and Welsh, 2017).

Colony morphology and preliminary characterization

Purified isolates were examined for colony shape, colour, margin and elevation. Gram staining was performed and cells were observed under oil immersion. Catalase activity was assessed using 3% hydrogen peroxide, with immediate effervescence interpreted as a positive reaction. Oxidase activity was examined using an oxidase reagent and interpreted according to the standard microbiological procedure (Cappuccino and Welsh, 2017; MacFaddin, 2000).

Acid tolerance assay

An overnight MRS broth culture of the selected isolate was harvested by centrifugation and the cells were washed with sterile phosphate-buffered saline. The washed cells were resuspended in medium adjusted to pH 2.5. After incubation at 37 °C, aliquots were serially diluted and plated on MRS agar. Growth after exposure to the acidic condition was interpreted as evidence of

acid tolerance. The assay was based on established approaches for evaluating gastrointestinal stress tolerance of potentially probiotic lactic acid bacteria (Havenaar *et al.,* 1992; Charteris *et al.,* 1998).

Bile salt tolerance assay

The selected isolate was inoculated into MRS medium containing 0.3% bile salts and incubated at 37 °C. Following exposure, the culture was diluted and plated on MRS agar. Growth was compared with the corresponding control culture without bile salts. The presence of colonies in the bile-containing treatment was considered evidence of tolerance to the tested concentration. The use of 0.3% bile salt as a screening concentration follows the conventional probiotic screening approach reported for lactobacilli (Gilliland *et al.,* 1984; Charteris *et al.,* 1998).

Haemolytic activity

Safety screening was performed on blood agar containing 5% sheep blood. The selected isolate was streaked onto the medium and incubated at 37 °C for 24 h. Plates were examined for no haemolysis.

Antibacterial activity of cell-free supernatant

The selected isolate was grown overnight in MRS broth and the culture was centrifuged to remove cells. The supernatant was filtered through a 0.22-µm membrane to obtain a cell-free preparation. The antibacterial activity was evaluated by agar-well diffusion against *Acinetobacter baumannii, Escherichia coli, Proteus vulgaris* and *Salmonella typhi*. Mueller–Hinton agar plates seeded with the test organisms were prepared, wells were filled with the cell-free supernatant and plates were incubated at 37 °C for 24 h. Zones of inhibition were measured in millimetres.

Genomic DNA extraction and 16S rRNA amplification

Genomic DNA was extracted from the selected isolate using a chloroform: isoamyl alcohol procedure. Bacterial cells were harvested, lysed and subjected to phase separation with chloroform: isoamyl alcohol (24:1). DNA in the aqueous phase was precipitated with cold isopropanol, washed with 70% ethanol and dissolved in nuclease-free water (Sambrook and Russell, 2001). The bacterial 16S rRNA gene was amplified using universal primers 27F and 1492R. PCR products were separated by agarose gel electrophoresis and visualized under ultraviolet illumination (Sambrook and Russell, 2001).

Sequencing, BLAST analysis and phylogenetic identification

The purified PCR product was submitted for sequencing. The resulting sequence was compared with reference sequences in the NCBI database using BLAST (Altschul *et al.,* 1990). Closest matches, query coverage and percentage identity were recorded. A phylogenetic tree was generated to assess the relationship between isolate and reference sequences. The molecular identification was interpreted together with the phenotypic and functional observations.

Results and Discussion

Isolation of gut-associated bacterial isolates

The fish specimen and aseptic gut dissection used for bacterial isolation are shown in Fig. 1 and Fig. 2, respectively. Serial dilution of the homogenized gut sample produced dense growth at lower dilutions and discrete colonies at 10^{-5} and 10^{-6}. The recovered colonies were predominantly small, smooth and cream to pale yellow, demonstrating successful recovery of culturable gut-associated bacteria on MRS agar. The spread-plate culture showing different colony morphotypes is presented in Fig. 3.

Colony morphology and preliminary screening

Four distinct colony types, C1–C4, were obtained on MRS agar (Table 1). C1 formed circular, cream-coloured colonies with smooth margins and raised elevation. C2 produced circular pale-yellow colonies with smooth margins and a flat elevation. C3 was irregular, cream-coloured and flat, while C4 formed circular, light-cream colonies with smooth margins and slight elevation. Representative microscopic images of the four isolates are shown in Fig. 4.

Gram staining showed that all four isolates were Gram-positive rods, although their arrangements differed (Table 2). C1 appeared as Gram-positive rods occurring singly or in short chains and was catalase-negative and oxidase-negative. C2, C3 and C4 were also catalase-

negative but showed oxidase-positive reactions. On the basis of this preliminary profile, C1 was selected for further functional and molecular characterization. Representative microscopic images are provided in Fig 4.

Acid and bile tolerance

Isolate C1 retained visible growth on MRS agar after exposure to pH 2.5, demonstrating survival under the tested acidic condition (Fig. 5). Moderate colony formation was observed in the presence of 0.3% bile salts compared with the corresponding control (Fig. 6). The combined acid- and bile-tolerance profile indicated that C1 could withstand two important gastrointestinal stresses used in preliminary probiotic screening.

Haemolytic phenotype

C1 grew on blood agar without a clear zone around the colonies, and the agar remained red (Fig. 7). The isolate was therefore classified as γ-haemolytic under the conditions tested. The absence of detectable haemolysis provided a favourable preliminary safety characteristic for further probiotic evaluation.

Antibacterial activity

The cell-free supernatant of C1 produced visible inhibition zones against all four test organisms (Fig. 8). The largest zone was observed against Salmonella typhi (18.0 mm), followed by E. coli (13 mm), P. vulgaris (13.0 mm) and *A. baumannii* (10 mm) (Table 3). The pattern demonstrated that the isolate or its extracellular products had measurable antagonistic activity under the assay conditions. Because replicate numbers and variance estimates were not documented in the thesis table, the present manuscript reports the measured zone diameters descriptively and does not retain the unsupported p-value statement from the thesis.

Molecular identification of the selected isolate

Genomic DNA extracted from C1 produced an intact preparation suitable for amplification (Fig. 9). PCR using universal 16S rRNA primers 27F and 1492R produced an amplicon of approximately 1,500 bp. Agarose gel electrophoresis showed a distinct band in the sample lane, with a corresponding positive control band and no visible product in the negative control (Fig. 10). These observations supported successful and specific amplification of the bacterial 16S rRNA gene.

BLAST comparison showed that the sequence of isolate 005_FISLAC matched *Lactiplantibacillus plantarum* reference strains with high identity. The closest match was *L. plantarum* ATCC 14917 (NR_104927.1) with 100% query coverage and 99.6% identity, followed by strain WCFS1 (NR_075041.1) with 100% coverage and 99.4% identity and strain JDM1 (CP001617) with 99% coverage and 99.2% identity (Table 4). Phylogenetic analysis placed the isolate within the *L. plantarum* cluster (Fig. 11). On the basis of the combined BLAST and phylogenetic evidence, isolate 005_FISLAC was identified as *Lactiplantibacillus plantarum*.

The present study followed a stepwise strategy for selecting a potentially useful probiotic bacterium from the fish gastrointestinal tract. The initial isolation of four morphologically distinct colonies on MRS agar confirms that the sampled gut contained culturable bacteria able to grow under the selected conditions. MRS agar is widely used for the recovery of lactic acid bacteria because its nutrient composition and selective characteristics favour these organisms. The recovery of several colony morphotypes also illustrates an important point in probiotic discovery: a host gut is not a single-organism habitat, and selection must proceed through several levels of screening rather than assuming that the first recovered colony is beneficial.

The C1 isolate was selected because it combined a Gram-positive rod morphology with negative catalase and oxidase reactions. This phenotypic profile is compatible with lactic acid bacteria and provided a logical basis for additional testing. Gatesoupe (2008) described LAB as important members of fish-associated microbiota and noted that their occurrence and abundance vary among fish species and environmental conditions. The current findings are consistent with the use of fish intestinal material as a source of candidate LAB, although phenotypic characteristics alone cannot distinguish *L. plantarum* from closely related taxa.

The acid-tolerance result was one of the most relevant functional observations. C1 retained growth following exposure to pH 2.5, indicating that a viable population survived the tested acidic stress.

Table.1 Colony morphological characteristics of bacterial isolates recovered from the fish gut.

Isolate	Shape	Colour	Margin	Elevation
C1	Circular	Cream	Smooth	Raised
C2	Circular	Pale yellow	Smooth	Flat
C3	Irregular	Cream	Irregular	Flat
C4	Circular	Light cream	Smooth	Slightly raised

Table.2 Phenotypic screening of bacterial isolates recovered from the fish gut.

Isolate	Gram reaction	Cell morphology	Arrangement	Oxidase	Catalase
C1	Positive	Rod	Single/short chains	Negative	Negative
C2	Positive	Rod	Single/short chains	Positive	Negative
C3	Positive	Short rod	Single	Positive	Negative
C4	Positive	Rod	Short chains	Positive	Negative

Table.3 Antibacterial activity of cell-free supernatant from isolate C1 against selected bacterial test organisms.

Test organism	Zone of inhibition (mm)
Salmonella Typhi	18
Escherichia coli	13
Proteus vulgaris	13
Acinetobacter baumannii	10

Table.4 BLAST comparison of the 16S rRNA sequence of isolate 005_FISLAC with reference sequences.

Closest match	Accession number	Query coverage	Identity
***Lactiplantibacillus plantarum* ATCC 14917**	NR_104927.1	100%	99.6%
***Lactiplantibacillus plantarum* WCFS1**	NR_075041.1	100%	99.4%
***Lactiplantibacillus plantarum* JDM1**	CP001617	99%	99.2%

Figure.1 Salmon Fish (Oncorhynchus sp) specimen used for isolation of gut-associated bacteria.



Figure.2 Aseptic dissection and removal of the intestinal tract.



Figure.3 Recovery of morphologically distinct gut-associated bacterial colonies on MRS agar.

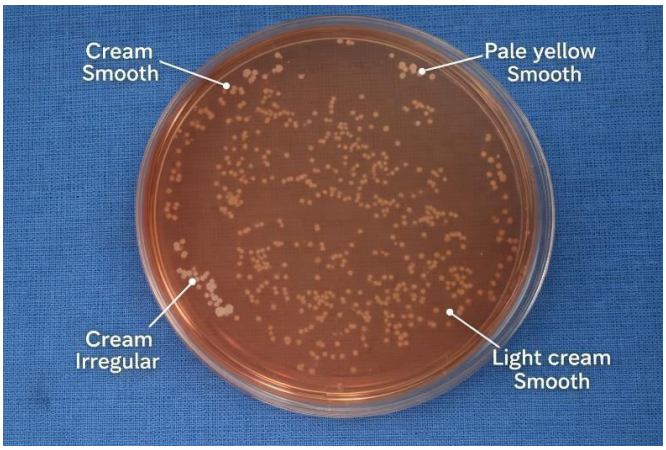


Figure.4 Gram-stained preparations of isolates C1, C2, C3 and C4.

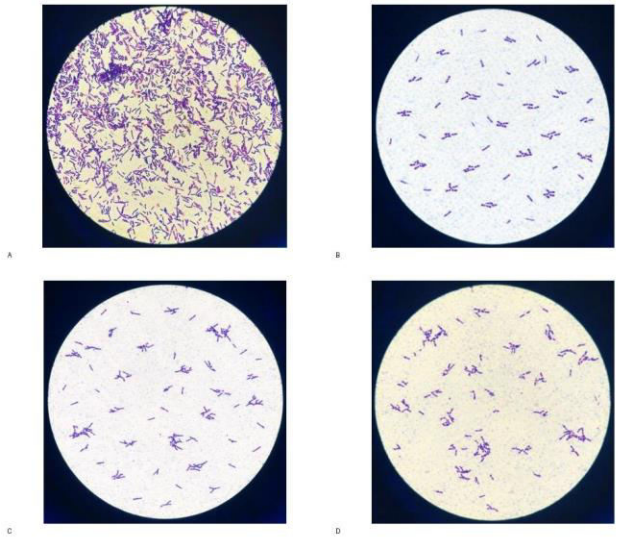


Figure.5 Acid tolerance of isolate C1 at pH 2.5.



Figure.6 Bile salt tolerance of isolate C1 in the presence of 0.3% bile salts.

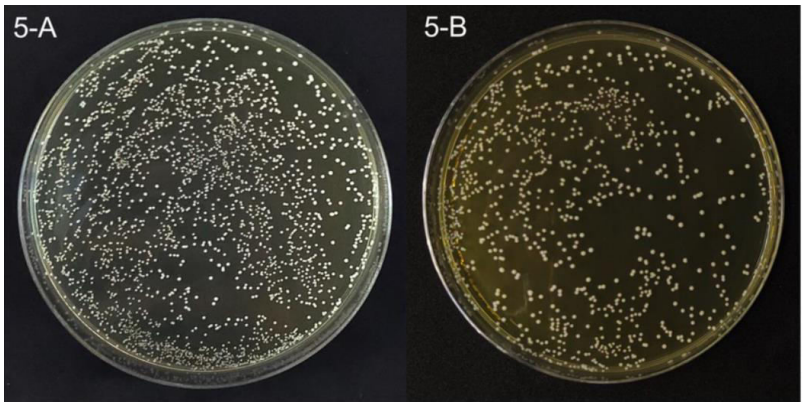


Figure.7 C1 on blood agar showing absence of a visible haemolytic zone.



Figure.8 Antibacterial activity of isolate C1 cell-free supernatant against (A) Salmonella Typhi, (B) Escherichia coli, (C) Proteus vulgaris and (D) *Acinetobacter baumannii*.

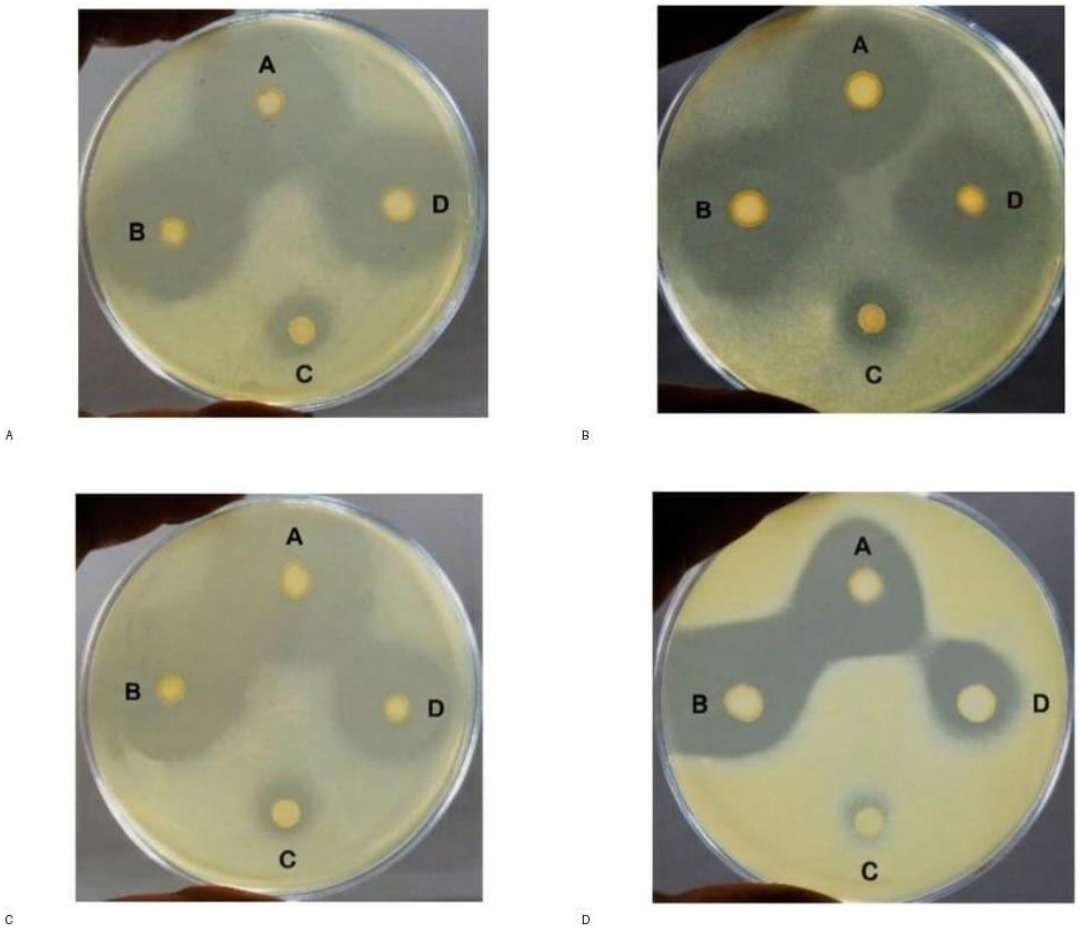


Figure.9 Genomic DNA preparation from the selected probiotic candidate.



Figure.10 Agarose gel electrophoresis of the approximately 1,500-bp 16S rRNA PCR product from isolate 005_FISLAC.

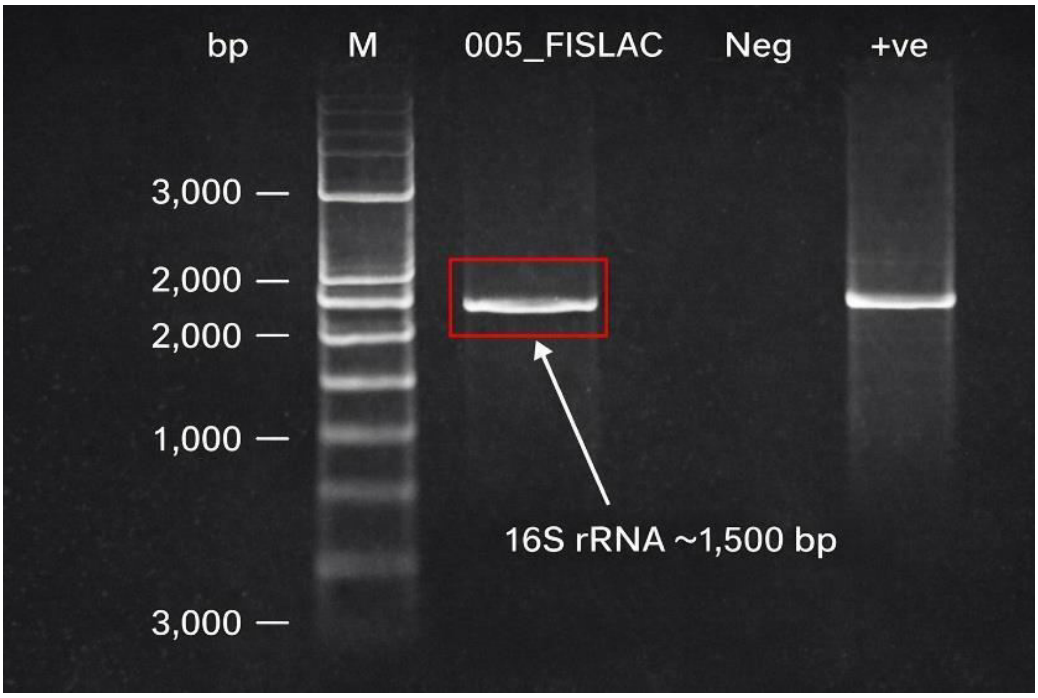
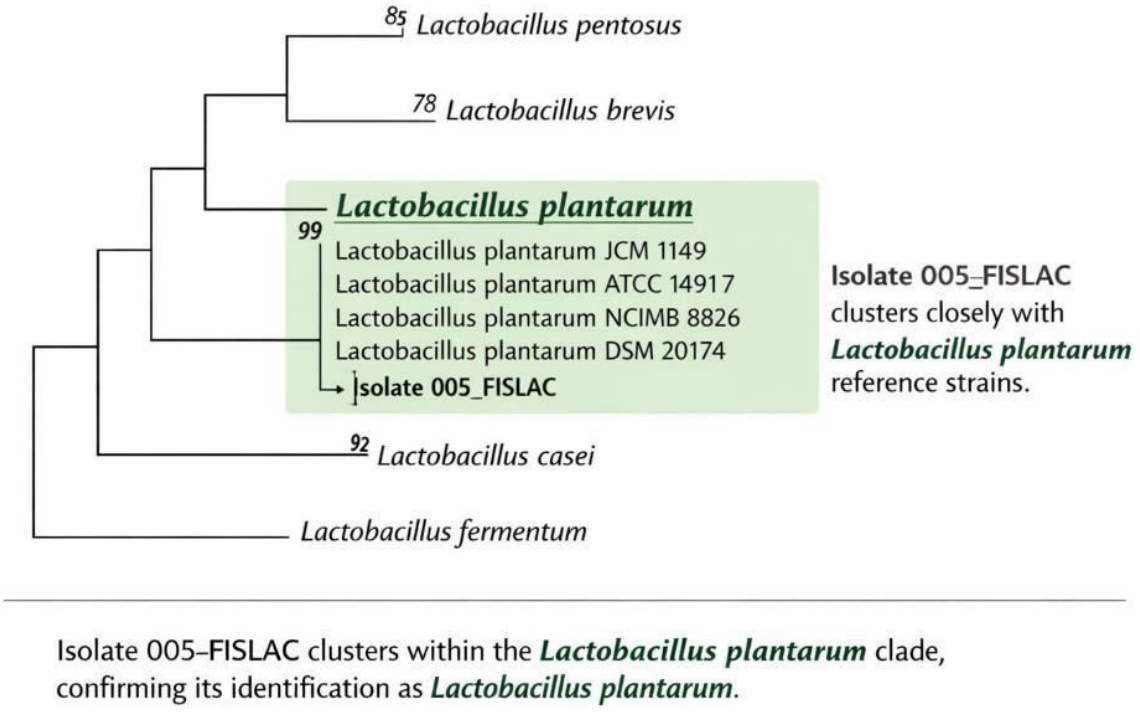


Figure.11 Phylogenetic placement of isolate 005_FISLAC within the *Lactobacillus plantarum* group based on 16S rRNA sequence comparison.



Similar pH values are routinely used in screening studies of potentially probiotic lactobacilli, and *L. plantarum* strains are known for relatively broad environmental tolerance. Corcoran *et al.,* (2008) showed that survival of probiotic lactobacilli can vary substantially under acidic conditions, demonstrating why tolerance should be assessed experimentally rather than inferred from species identity. In the present study, the observation should therefore be interpreted as evidence that the selected isolate has the physiological capacity to withstand the specific acid challenge used in vitro.

Bile tolerance provided a second line of evidence. C1 showed moderate colony formation in the presence of 0.3% bile salts. Bile salts can alter bacterial membranes and impose a significant physiological stress on microorganisms entering the intestine. Gilliland *et al.,* (1984) established the relevance of bile tolerance for dietary lactobacilli, and later probiotic-screening protocols have commonly used approximately 0.3% bile as a practical challenge concentration. The present result indicates that C1 survived the tested bile exposure, although quantitative survival data such as log CFU reduction were not recorded. Future work should therefore quantify the percentage survival or change in viable counts rather than relying only on qualitative colony formation.

The haemolysis assay showed no detectable haemolytic activity. This is an important preliminary observation because haemolytic strains are less desirable for probiotic development. Nevertheless, the absence of haemolysis cannot by itself establish that an isolate is non-pathogenic. Modern probiotic safety evaluation considers antimicrobial resistance, virulence determinants, toxin-associated genes, mobile genetic elements and genome-level characteristics in addition to basic phenotypic tests. Accordingly, C1 should be described as non-haemolytic under the conditions tested rather than categorically declared safe solely from the blood-agar result.

The antibacterial assay showed that the C1 cell-free supernatant inhibited all four test organisms. The largest zone was recorded against *Salmonella typhi*, while the smallest was observed against *A. baumannii*. Such variation is biologically plausible because susceptibility to organic acids, bacteriocin-like compounds, hydrogen peroxide and other metabolites differs among bacterial species. Schillinger and Lucke (1989) demonstrated that lactobacilli can produce extracellular inhibitory activities, while Verschuere *et al.,* (2000) described antimicrobial production and competitive exclusion as important probiotic mechanisms. However, the present experiment did not fractionate the supernatant or neutralize organic acids; therefore, the inhibition cannot be attributed specifically to bacteriocins. Further studies using pH-neutralized supernatant, catalase treatment and protease sensitivity assays would help distinguish acid-mediated activity from proteinaceous antimicrobial compounds.

The inhibition of *Salmonella typhi* is particularly notable from a food and aquaculture perspective because Salmonella is an important enteric pathogen. However, the test organism used in the thesis was identified as *Salmonella typhi*, and the experimental system was an in vitro agar diffusion assay. The result should not be interpreted as proof of disease prevention in fish. Rather, it demonstrates that extracellular products from C1 can inhibit the growth of the selected test organism under laboratory conditions. Challenge experiments using relevant fish pathogens, such as Aeromonas hydrophila or Vibrio species according to the host system, would be more directly informative for aquaculture application.

Molecular identification strengthened the phenotypic classification. The approximately 1,500-bp 16S rRNA product and the high sequence identity to *L. plantarum* reference strains supported placement of the isolate within the *L. plantarum* group. Clarridge (2004) described 16S rRNA sequencing as a valuable approach for bacterial identification because it provides a standardized sequence marker for comparison among taxa. The current nomenclature should also be considered when preparing the manuscript. Zheng *et al.,* (2020) reorganized the Lactobacillus genus into multiple genera and placed *L. plantarum* in the genus Lactiplantibacillus.

An important limitation is that 16S rRNA sequencing alone may not always provide definitive species-level resolution among closely related lactobacilli. Species-specific markers or whole-genome approaches can provide stronger discrimination. Lee *et al.,* (2021), for example, highlighted limitations of 16S rRNA for distinguishing closely related Lactiplantibacillus taxa and discussed more discriminatory sequence targets. Consequently, the present molecular result should be reported as a strong 16S-based identification rather than as complete genomic confirmation. This distinction is especially important if the strain is intended for regulatory or commercial development.

The combination of host origin, acid tolerance, bile tolerance, non-haemolytic phenotype, antibacterial activity and molecular identity gives C1 a coherent preliminary probiotic profile. The results are also consistent with the broader literature showing that probiotic selection should integrate origin, safety, stress tolerance, antagonistic activity and molecular identity. Hai (2015) emphasized that probiotic screening should be species- and strain-conscious and should include both in vitro and in vivo evaluation. Dawood *et al.,* (2016) similarly stressed that probiotic efficacy is influenced by strain characteristics, dosage, host species and experimental conditions.

The principal value of the present study therefore lies in the identification of a fish-gut-derived *L. plantarum* strain with multiple desirable preliminary properties. The isolate is not yet fully validated as a commercial probiotic because the study did not include a complete antimicrobial-resistance profile, genome-based safety assessment, quantitative adhesion testing, enzyme profiling, defined viable-cell dosage, intestinal colonization analysis or a pathogen challenge trial. These are logical next steps for strengthening the evidence base. Nevertheless, the strain is sufficiently characterized to justify subsequent feed-incorporation experiments, which form the second research component derived from the thesis.

In conclusion, A lactic acid bacterial candidate was successfully recovered from the gut of healthy salmon fish and screened through phenotypic, functional and molecular approaches. Four colony types were obtained, of which isolate C1 was selected because of its Gram-positive rod morphology and negative catalase and oxidase reactions. C1 survived the tested acidic condition of pH 2.5, showed moderate tolerance to 0.3% bile salts, and exhibited no detectable haemolysis on blood agar. Its cell-free supernatant inhibited *Salmonella typhi, Escherichia coli, Proteus vulgaris and Acinetobacter baumannii*, with the greatest inhibition against *Salmonella typhi*. 16S rRNA sequencing generated an approximately 1,500-bp amplicon, and BLAST analysis showed up to 99.6% identity with reference *Lactiplantibacillus plantarum* sequences.

Phylogenetic analysis further placed the isolate within the *L. plantarum* clade. Collectively, these observations support the selection of isolate 005_FISLAC as a promising fish gut-derived probiotic candidate for subsequent feed-based evaluation.

Author Contributions

Preethi H: Contributed in the research work and writing the content. Preethi K: Contribued in the Experimental worksand documentation. Priya Dharshini M: Documentation of results. Priyadarshini R: Effectively participated in the sample collection and processing. Parkavi R: Contributed in the experimental work. Gomathi N: Conceived the original idea and designed the model and wrote the manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990) 'Basic local alignment search tool', *Journal of Molecular Biology*, 215(3), pp. 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Balcázar, J.L., de Blas, I., Ruiz-Zarzuela, I., Cunningham, D., Vendrell, D. and Múzquiz, J.L. (2006) 'The role of probiotics in aquaculture', *Veterinary Microbiology*, 114(3–4), pp. 173–186. https://doi.org/10.1016/j.vetmic.2006.01.009

Cabello, F.C. (2006) 'Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment', *Environmental Microbiology*, 8(7), pp. 1137–1144. https://doi.org/10.1111/j.1462-2920.2006.01054.x

Cappuccino, J.G. and Welsh, C.T. (2017) *Microbiology: A Laboratory Manual*. 11th edn. Boston: Pearson.

Charteris, W.P., Kelly, P.M., Morelli, L. and Collins, J.K. (1998) 'Development and application of an in vitro methodology to determine the transit tolerance of potentially probiotic *Lactobacillus* and *Bifidobacterium* species in the upper human gastrointestinal tract', *Journal of Applied Microbiology*, 84(5), pp. 759–768. https://doi.org/10.1046/j.1365-2672.1998.00407.x

Clarridge, J.E. (2004) 'Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases', *Clinical Microbiology Reviews*, 17(4), pp. 840–862. https://doi.org/10.1128/CMR.17.4.840-862.2004

Corcoran, B.M., Stanton, C., Fitzgerald, G.F. and Ross, R.P. (2005) 'Survival of probiotic lactobacilli in acidic environments is enhanced in the presence of metabolizable sugars', *Applied and Environmental Microbiology*, 71(6), pp. 3060–3067. https://doi.org/10.1128/AEM.71.6.3060-3067.2005

Dawood, M.A.O., Koshio, S. and Esteban, M.Á. (2018) 'Beneficial roles of feed additives as immunostimulants in aquaculture: a review', *Reviews in Aquaculture*, 10(4), pp. 950–974. https://doi.org/10.1111/raq.12209

De Man, J.C., Rogosa, M. and Sharpe, M.E. (1960) 'A medium for the cultivation of lactobacilli', *Journal of Applied Bacteriology*, 23(1), pp. 130–135. https://doi.org/10.1111/j.1365-2672.1960.tb00188.x

de Vries, M.C., Vaughan, E.E., Kleerebezem, M. and de Vos, W.M. (2006) '*Lactobacillus plantarum*—survival, functional and potential probiotic properties in the human intestinal tract', *International Dairy Journal*, 16(9), pp. 1018–1028. https://doi.org/10.1016/j.idairyj.2005.09.003.

Denev, S., Staykov, Y., Moutafchieva, R. and Beev, G. (2009) 'Microbial ecology of the gastrointestinal tract of fish and the potential application of probiotics and prebiotics in finfish aquaculture', *International Aquatic Research*, 1, pp. 1–29.

El-Saadony, M.T., Alagawany, M., Patra, A.K., Kar, I., Tiwari, R., Dawood, M.A.O., Dhama, K. and Abdel-Latif, H.M.R. (2021) 'The functionality of probiotics in aquaculture: an overview', *Fish & Shellfish Immunology*, 117, pp. 36–52. https://doi.org/10.1016/j.fsi.2021.07.007

FAO/WHO (2002) *Guidelines for the Evaluation of Probiotics in Food*. Report of a Joint FAO/WHO Working Group. London, Ontario.

Gatesoupe, F.J. (2008) 'Updating the importance of lactic acid bacteria in fish farming: natural occurrence and probiotic treatments', *Journal of Molecular Microbiology and Biotechnology*, 14(1–3), pp. 107–114. https://doi.org/10.1159/000106089

Gilliland, S.E., Staley, T.E. and Bush, L.J. (1984) 'Importance of bile tolerance of *Lactobacillus acidophilus* used as a dietary adjunct', *Journal of Dairy Science*, 67(12), pp. 3045–3051. https://doi.org/10.3168/jds.S0022-0302(84)81670-7

Hai, N.V. (2015) 'The use of probiotics in aquaculture', *Journal of Applied Microbiology*, 119(4), pp. 917–935. https://doi.org/10.1111/jam.12886

Havenaar, R., Brink, B.T. and Huis in't Veld, J.H.J. (1992) 'Selection of strains for probiotic use'. In: Fuller, R. (ed.) *Probiotics: The Scientific Basis*. London: Chapman & Hall, pp. 209–224.

Lee, J.M., Park, S.H., Jin, C.Z., Kang, M.K., Park, D.J. and Kim, C.J. (2021) 'The *groESL* ISR sequence-based species-specific identification of GRAS and non-GRAS *Lactiplantibacillus* as an alternative to 16S rRNA sequencing', *LWT*, 147, 111504. https://doi.org/10.1016/j.lwt.2021.111504

MacFaddin, J.F. (2000) *Biochemical Tests for Identification of Medical Bacteria*. 3rd edn. Philadelphia: Lippincott Williams & Wilkins.

Martínez Cruz, P., Ibáñez, A.L., Monroy Hermosillo, O.A. and Ramírez Saad, H.C. (2012) 'Use of probiotics in aquaculture', *ISRN Microbiology*, 2012, 916845. https://doi.org/10.5402/2012/916845

Martínez, J.L. (2009) 'Environmental pollution by antibiotics and by antibiotic resistance determinants', *Environmental Pollution*, 157(11), pp. 2893–2902. https://doi.org/10.1016/j.envpol.2009.05.051

Nayak, S.K. (2010) 'Probiotics and immunity: a fish perspective', *Fish & Shellfish Immunology*, 29(1), pp. 2–14. https://doi.org/10.1016/j.fsi.2010.02.017

Ringø, E., Zhou, Z., Vecino, J.L.G., Wadsworth, S., Romero, J., Krogdahl, Å., Olsen, R.E., Dimitroglou, A., Foey, A., Davies, S., Owen, M., Lauzon, H.L., Martinsen, L.L., De Schryver, P., Bossier, P., Sperstad, S. and Merrifield, D.L.

(2016) 'Effect of dietary components on the gut microbiota of aquatic animals. A never-ending story?' *Aquaculture Nutrition*, 22(2), pp. 219–282. https://doi.org/10.1111/anu.12346

Sambrook, J. and Russell, D.W. (2001) *Molecular Cloning: A Laboratory Manual*. 3rd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory PresS.

Schillinger, U. and Lücke, F.K. (1989) 'Antibacterial activity of *Lactobacillus sake* isolated from meat', *Applied and Environmental Microbiology*, 55(8), pp. 1901–1906. https://doi.org/10.1128/aem.55.8.1901-1906.1989

Verschuere, L., Rombaut, G., Sorgeloos, P. and Verstraete, W. (2000) 'Probiotic bacteria as biological control agents in aquaculture', *Microbiology and Molecular Biology Reviews*, 64(4), pp. 655–671. https://doi.org/10.1128/MMBR.64.4.655-671.2000

Zheng, J., Wittouck, S., Salvetti, E., Franz, C.M.A.P., Harris, H.M.B., Mattarelli, P., O'Toole, P.W., Pot, B., Vandamme, P., Walter, J., Watanabe, K., Wuyts, S., Felis, G.E., Gänzle, M.G. and Lebeer, S. (2020) 'A taxonomic note on the genus *Lactobacillus*: description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae', *International Journal of Systematic and Evolutionary Microbiology*, 70(4), pp. 2782–2858. https://doi.org/10.1099/ijsem.0.004107

How to cite this article:

Preethi H., Preethi K., Priya Dharshini M., Priyadarshini R., Parkavi R. and Gomathi Natrajan. 2026. Isolation, Functional Characterization and Molecular Identification of a Fish Gut-Derived *Lactobacillus plantarum* with Probiotic Potential. *Int.J.Curr.Microbiol.App.Sci.* Special Issue-13: 341-354.
doi: https://doi.org/10.20546/ijcmas.2026.sp13.036