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IN VITRO PROBIOTIC CHARACTERIZATION AND MOLECULAR IDENTIFICATION OF *LACTIPLANTIBACILLUS PLANTARUM* FROM GERMINATED WHEAT AND CAULIFLOWER SOURDOUGH

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ABSTRACT

This study aimed to develop a functional sourdough incorporating germinated wheat flour and dehydrated cauliflower flour, and to isolate, evaluate, and characterize the dominant lactic acid bacteria (LAB) obtained from the fermented product. Microbial isolation and morphological characterization revealed *Lactiplantibacillus plantarum* as the dominant strain. Molecular identification employing 16S rRNA gene sequencing and BLAST analysis confirmed the isolate as *Lactiplantibacillus plantarum* (GenBank accession: PV342492.1), showing 98–100% similarity with reference strains in the NCBI GenBank database. The isolate exhibited strong amylolytic activity with an amylolytic index of 1.9, indicating effective starch degradation during fermentation. Protease and lipase activities were also detected, suggesting the isolate's role in protein and lipid hydrolysis, which improves the flavour and textural quality of sourdough products. In vitro probiotic assessments demonstrated that the isolate maintained high viability under acidic conditions (77.78% at pH 2.0) and in the presence of bile salts, showing a survival rate of 74.16% after 24 h at 0.3% bile concentration. *Antimicrobial activity was observed against foodborne pathogens including Staphylococcus aureus, Escherichia coli, and Salmonella species.* Phylogenetic analysis confirmed the close evolutionary relationship of the isolate with other L. plantarum strains, validating its dominance in the vegetable-enriched sourdough ecosystem. The incorporation of cauliflower flour appeared to support robust growth and stability of L. plantarum during fermentation without hindering microbial proliferation. These in vitro findings suggest that the formulated sourdough has potential for the development of high-fibre fermented bakery products; however, in vivo validation is required before specific gastrointestinal health claims can be substantiated.

Keywords: Plant-based sourdough, *Lactiplantibacillus plantarum*, Molecular characterization, Probiotics, Germinated wheat, Cauliflower flour

INTRODUCTION

Sourdough is considered one of the oldest natural starters widely used in leavened bread making, relying on the natural activity of lactic acid bacteria (LAB) and yeasts. These microorganisms are responsible for acid production, dough fermentation, and the development of flavour and texture in sourdough products **(Arora *et al.*, 2021; Behera *et al.*, 2018)**. Compared to baker's yeast fermentation, sourdough fermentation enhances shelf life, improves nutritional quality, and reduces antinutritional factors **(Paramithiotis, 2025)**. Lactic acid bacteria are the dominant microbial group in sourdough systems. Among them, Lactiplantibacillus plantarum is frequently reported due to its ability to survive acidic conditions and utilise a wide range of carbohydrates. This species contributes to dough stability, flavour formation, and inhibition of spoilage microorganisms. Additionally, several studies have highlighted its probiotic and antimicrobial properties, making it suitable for functional food applications **(Ibrahim *et al.*, 2021)**. There is growing interest in the nutritional advantages offered by sourdough fermentation compared with other leavening agents. Germination of wheat activates enzymes that improve starch and protein availability, while vegetables such as cauliflower provide dietary fibre and bioactive compounds that may support microbial growth during fermentation **(Drabinska *et al.*, 2021)**. However, there is limited information on isolation and characterisation of probiotics from germinated cereals combined with dehydrated vegetables in sourdough formation. Sourdough has emerged recently in the Indian bakery market, driven by global culinary trends and growing consumer interest in healthier, naturally fermented foods **(Laatikainen, 2023; Pallant, 2020)**. Despite increasing interest, adoption is limited by high production costs, labour-intensive processes, prolonged fermentation, and starter maintenance **(Siepmann *et al.*, 2023)**. The dense texture and tangy flavour of sourdough further restrict its acceptance among Indian consumers **(Amr & Alkhamaiseh, 2022)**. Sourdough occupies a niche market, appealing mainly to health-conscious and artisanal-focused individuals. Thus, adding dehydrated cauliflower florets is a promising strategy to enhance sensory appeal and consumer acceptance of sourdough, thereby boosting its market position in the Indian bakery sector. Given the nutritional interest in vegetable-enriched functional foods and the need to ensure microbial viability during fermentation, this research aims to assess the in vitro probiotic potential and molecular identification through 16S rRNA gene sequencing of L. plantarum isolated from sourdough incorporated with dehydrated cauliflower flour. In particular, it addresses whether vegetable flour inclusion affects the growth dynamics and probiotic attributes of the LAB strain.

MATERIALS AND METHODS

The raw materials used were wheat grains (*Triticum aestivum L.*), fresh cauliflower, and sterile distilled water. Wheat grains and cauliflower were procured from a local supermarket in Walajabad. All materials were stored in food-grade containers under suitable conditions prior to use. The study was conducted at the Department of Home Science, SDNB Vaishnav College for Women, Chromepet, Chennai – 44. The wheat grains were authenticated by the Siddha Central Research Institute, Ministry of AYUSH, Government of India (Authentication Code: T09122402A). Prior to fermentation, the wheat grains were germinated and milled into flour, while the cauliflower was tray-dried and ground into a fine powder.

Preparation of Sourdough Starter Culture

The flow chart in Figure 1 depicts the preparation of the plant-based sourdough. Germinated wheat flour and dehydrated cauliflower flour were mixed in equal quantities (100 g each) with 100 mL of sterile water in a sterilized jar. The germination of wheat was carried out as per the method suggested by **Nkhata *et al.* (2018)**. The sourdough was prepared following the protocol reported by **Lhomme *et al.* (2016)** with slight modifications. After 24 hours of initial fermentation, an additional 100 g of each flour and 100 mL of water were added. The mixture was stirred daily and allowed to ferment for seven days before use. Fermentation was carried out under ambient laboratory conditions (28–30°C) in loosely covered sterilized containers to allow gas exchange while minimizing contamination. The sourdough was stirred manually once daily to ensure uniform distribution of microorganisms and substrates. Microbial viability was assessed by total viable count (TVC) enumeration on MRS agar at the end of the seven-day fermentation period.

Microbial Isolation

The bacterial isolation was done by serially diluting the sourdough sample with sterilised saline water. One millilitre (1 mL) of the dough was pour-plated on MRS agar (containing per litre: beef extract, 2 g; tryptone, 10 g; yeast extract, 4 g; glucose, 10 g; K2HPO4·3H2O, 2.5 g; sodium acetate, 5 g; tris ammonium citrate, 2 g; MgSO4·7H2O, 200 mg; manganese sulfate, 50 mg; Tween 80, 1 mL), previously sterilised by autoclaving at 121°C for 15 minutes. The plates were incubated at 37°C for 48 hours. Distinct colonies were screened based on colony morphology.

CFU/mL = Number of colonies × Dilution factor / Volume loaded in the culture plate

Screening of Isolates

The amylolytic activity of LAB isolates was evaluated by the agar-diffusion method on starch agar. Wells (10 mm) were filled with 100 µL of 24-h MRS-cultured bacteria, incubated at 37°C for 48 h, and treated with iodine. Clear zone diameters were measured, and the amylolytic index (AI) was calculated as AI = R/r (where R is the clear zone diameter and r is the colony diameter). Isolate D2 was selected for comprehensive probiotic characterisation based on its significantly superior amylolytic index (AI = 1.9) compared to D1 (AI = 1.2) and D3 (AI = 1.4). High amylolytic activity was used as the primary selection criterion due to its direct technological relevance in starch-rich sourdough fermentation systems, where efficient starch hydrolysis is essential for fermentable sugar supply and dough acidification kinetics.

Morphological and Biochemical Identification

Morphological Features (Gram Staining, Catalase Test)

The macroscopic characteristics of bacterial colonies were examined on MRS agar following incubation at 37°C for 48 h. Colony size, shape, texture, and colour were recorded. Bacterial cells were heat-fixed and stained sequentially with crystal violet for 1 min, Lugol's iodine for 1 min, and decolorised with 90% alcohol. Safranin was applied for 30–45 s, followed by washing and drying. Slides were examined under a light microscope at 100× magnification. Catalase activity was examined by adding hydrogen peroxide to bacterial cultures; no visible effervescence was produced.

Carbohydrate Fermentation Profiles

Carbohydrate utilisation was assessed using the KB009™ HiCarbo Kit (KB009A). The bacterial isolate was inoculated into the test wells provided in the kit. After incubation at 37°C for 24–48 hours, carbohydrate fermentation was determined based on visible colour changes in the reaction medium.

Survival under Simulated Gastrointestinal Condition

The survival of L. plantarum (D2) was evaluated under acidic and bile stress. For acid tolerance, MRS broth was adjusted to pH 2.0 and 3.0, while MRS at pH 7.0 served as the environmental control. For bile tolerance, MRS was supplemented with 0.3% (w/v) ox-bile, while bile-free MRS served as the control. The baseline control (0 h) was established by measuring the initial population immediately upon inoculation, representing 100% viability. Samples were incubated at 37°C, and growth was monitored by measuring Optical Density (OD) at 560 nm at 3, 6, and 24 h. The percentage viability was calculated as: % Viability = (OD of Stress Sample / OD of 0 h Control) × 100. All experiments were performed in triplicate (n = 3).

Gas Production during Fermentation

Gas production of the LAB isolates was assessed in modified MRS broth containing 1% glucose with inverted Durham tubes. Overnight cultures were inoculated and incubated at 37°C for 5 days. Gas accumulation in the Durham tubes was subsequently observed to determine CO_2 production.

Enzymatic Activity

Enzymatic activities of the LAB isolate were assessed using standard assays. Amylase activity was determined by incubating 0.5 mL of enzyme with 1 mL of 1% starch and 0.5 mL phosphate buffer (pH 7) at 35°C for 10 min, stopping the reaction with 3,5-dinitrosalicylic acid (DNSA), and measuring reducing sugars. Protease activity was measured by incubating 0.25 mL of enzyme with 1% casein at 37°C for 30 min, precipitating proteins with trichloroacetic acid (TCA), and reading absorbance of the filtrate at 275 nm. Lipase activity was determined by incubating 0.1 mL enzyme with 0.1 mL p-nitrophenyl acetate and 0.8 mL phosphate buffer (pH 7) at 30°C for 30 min, terminating with sodium carbonate, and measuring p-nitrophenol at 410 nm. One unit of each enzyme was defined as the amount catalysing 1 µmol of product per minute.

Antimicrobial Activity

The antibacterial activity of the samples was evaluated by the agar well diffusion method. Bacterial suspensions (10^6 CFU/mL) were spread on brain heart infusion agar, and 8 mm wells were filled with cell-free supernatant at 100, 75, 50, and 25 µL. Plates were allowed to diffuse at room temperature for 2 h and incubated at 37°C for 24 h. Inhibition zone diameters were subsequently measured in millimetres.

Molecular Identification

Genomic DNA was extracted from 1 mL of overnight bacterial culture centrifuged at 6,000 × g for 10 min. The pellet was resuspended in 200 µL TE buffer, lysed with 200 µL lysis buffer, and incubated at 65°C for 10–15 min. After cooling, 400 µL chloroform:isoamyl alcohol (24:1) was added, mixed, and centrifuged at 12,000 × g for 10 min. The aqueous phase was transferred, and DNA was precipitated with 1 mL isopropanol at −20°C for 30 min, centrifuged, washed with 70% ethanol, air-dried, and suspended in TE buffer. The 16S rRNA gene was amplified using universal primers 27F: 5′-AGAGTTTGATCCTGGCTCAG-3′ and 1492R: 5′-GGTTACCTTGTTACGACTT-3′. PCR was carried out with initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 5 min. Amplicons were separated on 1% agarose gel at 100 V for 60 min, purified, and sequenced. Sequences were analysed for homology using CLUSTAL X and compared with GenBank references. Phylogenetic relationships were inferred using the neighbour-joining method.

Statistical Analysis

Student's t-test was applied to compare mean values between two groups, specifically for enzyme activity assays (isolate D2 vs. sterile uninoculated control) and antimicrobial inhibition zone measurements (each pathogen vs. negative control). One-way ANOVA was applied for comparisons involving three or more groups, specifically for the amylolytic index comparison across isolates D1, D2, and D3, and for viability data across multiple time points under simulated gastrointestinal conditions. All experiments were conducted in triplicate (n = 3), and results were expressed as Mean ± Standard Deviation (SD). Data were analysed using jamovi software (Version 2.6). A p-value of <0.05 was considered statistically significant.

RESULTS

Microbial Isolation from Germinated Wheat Sourdough Incorporated with Dehydrated Cauliflower Flour

Microbial isolation from germinated wheat sourdough enriched with dehydrated cauliflower flour demonstrated a robust microbial population, indicating an active fermentation process. The total viable count (TVC) was determined to be 4.0×10^6 CFU/mL. These results are in agreement with ***Viiard et al. (2016)***, who reported LAB populations in the range of 10^6 to 10^9 CFU/g in traditional rye sourdoughs. This high microbial density is critical for ensuring fermentation stability, rapid acidification, and the synthesis of bioactive metabolites in the sourdough matrix.

Screening of Isolates

Screening of isolates demonstrated variation in amylolytic activity, with isolate D2 exhibiting the highest amylolytic index (1.9), followed by D3 (1.4) and D1 (1.2). Amylolytic LAB play a critical role in starch hydrolysis, generating fermentable sugars necessary for microbial metabolism and dough leavening. Similar findings have been reported for *Lactiplantibacillus plantarum* and *Pediococcus pentosaceus,* which enhance fermentation efficiency and bread quality through starch degradation ***(Arora et al., 2021; Petkova et al., 2021).***

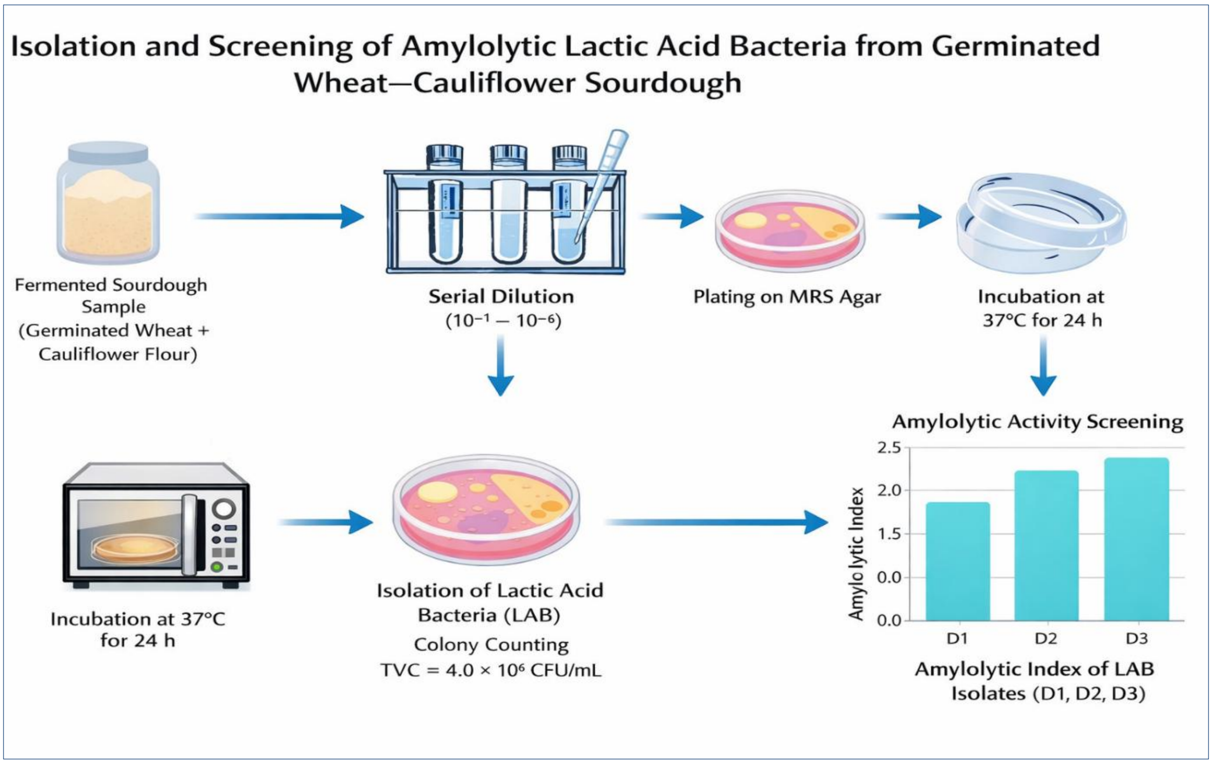

Figure 1 Schematic workflow of the isolation and screening of amylolytic lactic acid bacteria from germinated wheat–cauliflower sourdough. The process includes preparation of fermented sourdough, serial dilution (10^{-1}–10^{-6}), plating on MRS agar, incubation at 37 °C for 24 h, and enumeration of total viable count (TVC = 4.0×10^{6} CFU/mL). Isolated strains (D1, D2, and D3) were further evaluated for amylolytic activity, and the amylolytic index was determined, with D2 showing the highest activity followed by D3 and D1.

Morphological Characteristics

The isolates obtained from the functional sourdough exhibited characteristic LAB morphology. Colonies on MRS agar were circular, smooth, and moist, with a slightly raised elevation and entire margins. Microscopic characterisation revealed Gram-positive, non-motile, rod-shaped cells arranged singly or in short chains. These features are consistent with the morphological profile of *Lactiplantibacillus plantarum*, as previously described by ***Behera et al. (2018)***. The isolates were catalase-negative and oxidase-negative, which are defining biochemical traits of the *Lactobacillaceae* family ***(Sevgili et al., 2023)***. The morphological and biochemical characteristics are summarised in Table 1.

Table 1 Morphological Features of the Isolates

S.No	Morphological Feature	Observation
1.	Colony Shape	Circular
2.	Colony Size	Small to Medium (~1–2 mm)
3.	Colony Texture	Smooth and moist
4.	Colony Elevation	Slightly raised
5.	Colony Margin	Entire (smooth edges)
6.	Cell Shape	Rod-shaped (Bacillus type)
7.	Cell Arrangement	Single or short chains
8.	Motility	Non-motile
9.	Gram Reaction	Gram-positive
10.	Catalase Test	Negative

Carbohydrate Fermentation Profile

he metabolic versatility of isolate *L. plantarum* (D2) was evaluated using the Hi-Carbo Kit (HiMedia, India). The isolate demonstrated a robust fermentation profile, successfully utilising a wide array of carbohydrates including lactose, maltose, fructose, dextrose, raffinose, trehalose, sucrose, L-arabinose, and mannose. Acid production during fermentation resulted in a distinct colour transition of the medium from red to yellow. Conversely, the isolate was unable to ferment xylose, galactose, and melibiose. This broad fermentative capacity is a hallmark of *Lactiplantibacillus plantarum* and is essential for its dominance in the sourdough matrix **(Calvert *et al.*, 2021).**

Survival under Simulated Gastrointestinal Conditions

The isolate *L. plantarum* (D2) exhibited significant resilience to simulated gastrointestinal stressors (Table 2). At pH 2.0, the strain maintained a viability of 77.78 ± 0.35% after 3 h. Although a decline to 75.00 ± 0.28% was observed at 6 h, the survival remained statistically significant ($p < 0.001$). Under bile stress (0.3%), the isolate showed robust tolerance with 76.19 ± 0.42% viability at 3 h and 74.16 ± 0.31% after 24 h ($p < 0.05$).

Table 2 Survival of *Lactiplantibacillus plantarum* (D2) under simulated gastrointestinal conditions

Condition	Time (h)	Viability (%) (Mean ± SD)	p-value
Acid tolerance (pH 2.0)	0 (Control)	100.00 ± 0.00	–
	3	77.78 ± 0.35	< 0.001***
	6	75.00 ± 0.28	< 0.001***
Bile tolerance (0.3%)	0 (Control)	100.00 ± 0.00	–
	3	76.19 ± 0.42	< 0.05*
	24	74.16 ± 0.31	< 0.05*

**Values are expressed as mean ± SD (n = 3). Statistical significance was determined relative to the control (0 h): *p < 0.05; **p < 0.001. "–" indicates no comparison (control baseline).*

Gas Production

L. plantarum (D2) followed a strictly homofermentative metabolic pathway with no gas production observed in Durham tubes after 5 days of incubation, indicating exclusive lactic acid production via the Embden–Meyerhof–Parnas (EMP) pathway (Figure 2).

Figure 2 Gas Production during Fermentation of Sourdough

Enzymatic Activity

The isolate exhibited prominent amylolytic activity (3.91 ± 0.05 U/mL), along with protease (0.76 ± 0.02 U/mL) and lipase (0.78 ± 0.01 U/mL) activities (Table 3). Enzymatic yields were significantly higher ($p < 0.001$) than the negative controls, confirming the microbial origin of these enzymes.

Table 3 Enzymatic potential of *Lactiplantibacillus plantarum* (D2)

Enzyme Assay	Control (U/mL)	Isolate D2 (U/mL) (Mean ± SD)	p-value
Amylase	0.00 ± 0.00	3.91 ± 0.05	< 0.001***
Protease	0.00 ± 0.00	0.76 ± 0.02	< 0.01**
Lipase	0.00 ± 0.00	0.78 ± 0.01	< 0.01**

**Control = Sterile uninoculated MRS broth. **p < 0.01 and ***p < 0.001 indicate highly significant activity compared to control. Student's t-test used.*

Antimicrobial Activity

The isolate exhibited significant antagonistic activity against both Gram-positive and Gram-negative bacteria. The highest zone of inhibition was observed against *Staphylococcus aureus* (14.00 ± 0.20 mm), followed by *Escherichia coli* (12.80 ± 0.30 mm) and *Salmonella* spp. (12.00 ± 0.20 mm) (Table 4). All results were statistically significant compared to the negative control ($p < 0.01$).

Table 4 Antimicrobial activity of *Lactiplantibacillus plantarum* (D2) against foodborne pathogens

Test Pathogen	Gram Reaction	Trial 1	Trial 2	Trial 3	Mean ± SD (mm)	p-value
Staphylococcus aureus	Positive (+)	14.2	13.8	14.0	14.00 ± 0.20	< 0.001***
Escherichia coli	Negative (−)	12.5	13.1	12.8	12.80 ± 0.30	< 0.01**
***Salmonella* spp.**	Negative (−)	11.8	12.2	12.0	12.00 ± 0.20	< 0.01**

**Values expressed as Mean ± SD (n=3). Inhibition zone diameter includes well diameter (6 mm). **p < 0.01 and ***p < 0.001 compared to negative control (sterile distilled water).*

Molecular Identification

High-quality genomic DNA was successfully extracted and the 16S rRNA gene amplified using universal primers 27F/1492R, producing a distinct band at approximately 1500 bp on agarose gel electrophoresis. BLAST analysis revealed 98–100% identity with reference strains of *Lactiplantibacillus plantarum* in the NCBI GenBank database. The 16S rRNA gene sequence was deposited under accession number PV342492.1 (https://www.ncbi.nlm.nih.gov/nuccore/PV342492.1/). Phylogenetic analysis using the Neighbour-Joining method with 1,000 bootstrap replicates confirmed close evolutionary clustering of isolate D2 with established *L. plantarum* type strains (Figure 3).

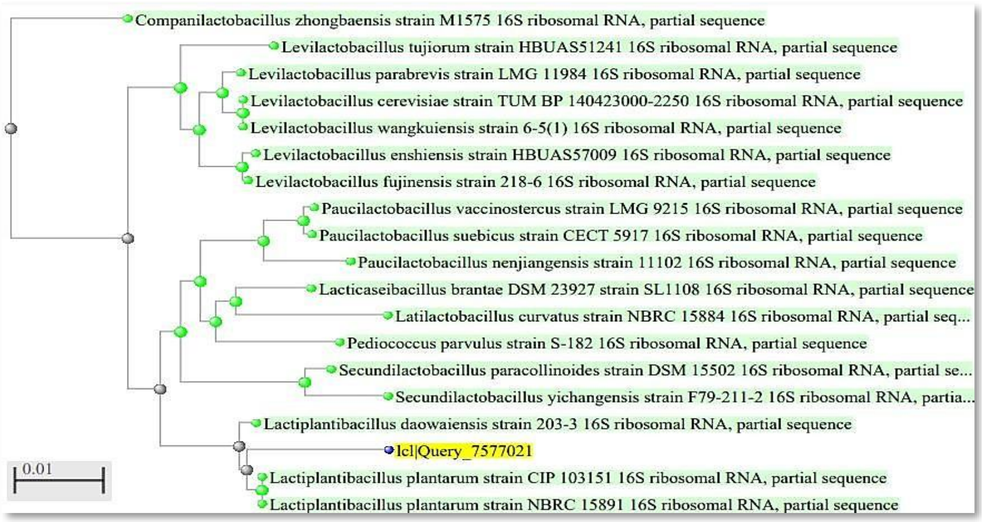

Figure 3 Phylogenetic tree based on 16S rRNA gene sequences illustrating the evolutionary relationship of isolate D2 with related *Lactiplantibacillus* species. The tree was constructed using the Neighbor-Joining method. Numbers at the nodes represent bootstrap percentages based on 1000 replicates. The scale bar indicates the number of nucleotide substitutions per site.

DISCUSSION

The present study isolated and characterised *Lactiplantibacillus plantarum* (D2) from a novel germinated wheat–cauliflower sourdough, demonstrating strong probiotic and technological properties. The TVC of 4.0×10^6 CFU/mL is consistent with LAB populations reported in vegetable-enriched sourdoughs **(Viiard *et al.*, 2016)**, confirming active fermentation.

The homofermentative metabolism of isolate D2 was confirmed by the absence of gas production in Durham tubes. This is technologically advantageous in a fiber-rich matrix such as cauliflower sourdough: because cauliflower flour weakens the gluten network, excessive CO_2 production could create large unstable air pockets causing loaf collapse during baking. The absence of gas production ensures a consistent, fine-textured crumb with structural integrity **(Ognean, 2015; Pino *et al.*, 2021).**

The amylase activity of L. plantarum (D2) (3.91 ± 0.05 U/mL) is particularly relevant in a germinated wheat matrix, where partial starch hydrolysis during germination increases substrate availability, enabling the isolate to sustain rapid acidification via the Embden–Meyerhof–Parnas (EMP) pathway. Protease activity (0.76 ± 0.02 U/mL) contributes to partial gluten network modification and the liberation of free amino acids that serve as aroma precursors for aldehydes, alcohols, and esters important to sourdough flavour development. Lipase activity (0.78 ± 0.01 U/mL) facilitates the release of free fatty acids, which further enrich the aromatic profile of the sourdough. Together, these enzymatic activities indicate that D2 actively contributes to both dough conditioning and sensory quality improvement in the fibre-rich cauliflower sourdough system **(Akamine *et al.*, 2023).**

The observed in vitro acid and bile tolerance of isolate D2 meets key criteria for probiotic candidacy, as the strain must survive gastric transit (pH 1.5–2.0) and bile exposure (0.3%) to reach the intestinal mucosa in viable form **(Bartkiene *et al.*, 2022)**. The viability of 77.78% at pH 2.0 and 74.16% after 24 h bile exposure are comparable with values reported for established *L. plantarum* strains used in commercial probiotic products. The antimicrobial activity against *S. aureus*, *E. coli*, and *Salmonella* spp. may be attributed to the production of organic acids, bacteriocins, and hydrogen peroxide by LAB, as well as the presence of glucosinolate-derived bioactive compounds from cauliflower flour **(Iosca et al, 2023).**

Cauliflower contains glucosinolates and their hydrolysis products, including isothiocyanates and volatile sulphur compounds, which may generate characteristic off-odours in food matrices. In the present study, the inclusion of dehydrated cauliflower flour at equal ratio with germinated wheat flour did not produce objectionable sensory changes during fermentation. This may be attributed in part to the metabolic activity of L. plantarum (D2), whose rapid lactic acid production causes a swift pH drop that may suppress endogenous myrosinase activity, thereby limiting glucosinolate hydrolysis and the associated release of sulphur volatiles **(Amann *et al.*, 2022)**. Future studies should include GC-MS quantification of volatile sulphur compounds and trained sensory panel evaluations across varying cauliflower inclusion levels to objectively assess odour modulation and optimal cauliflower flour supplementation levels.

The present study did not include a separate non-germinated or non-enriched sourdough control for direct fermentation comparison. Therefore, the independent effects of germination and cauliflower flour on LAB performance cannot be isolated. The control used in specific assays (e.g., amylolytic index and viability studies) refers only to assay-level comparisons and does not represent a full

formulation control. Future studies should include parallel control formulations to enable direct comparative evaluation.
The molecular identification by 16S rRNA gene sequencing and phylogenetic analysis (Figure 3) confirmed the dominance of *Lactiplantibacillus plantarum* in this sourdough system. The high bootstrap support values and clustering with established type strains validate the taxonomic assignment **(Zielinska & Kostrzewska, 2024; Russo *et al.*, 2022)**. The deposition of the sequence in GenBank (PV342492.1) ensures reproducibility and comparability with future studies.
Despite these insights, this study has several limitations that should be acknowledged. First, only the dominant isolate (D2) was subjected to full probiotic characterisation; a comprehensive metagenomic or high-throughput sequencing analysis of the entire sourdough microbiome was beyond the scope of this work, limiting ecological interpretation of the microbial community dynamics. Second, fermentation was performed under fixed ambient temperature (28–30°C) and duration (7 days); the effects of varying hydration ratios, fermentation temperature ranges, or back-slopping frequency on microbial performance and product quality were not investigated. Third, the absence of a control sourdough formulation without cauliflower flour means the independent contribution of the vegetable ingredient to LAB growth and probiotic properties cannot be precisely determined from the current data. Fourth, all probiotic assessments were conducted in vitro; in vivo validation in animal models or human clinical trials is required before gastrointestinal health claims can be substantiated.

CONCLUSION

The present study demonstrated that sourdough prepared with germinated wheat flour and dehydrated cauliflower flour effectively supported the growth and functional activity of lactic acid bacteria without negatively affecting the fermentation process. Molecular identification employing 16S rRNA gene sequencing and BLAST analysis confirmed the identity of the dominant isolate as *Lactiplantibacillus plantarum* (D2) (GenBank: PV342492.1), showing high similarity with reference strains. The isolate exhibited notable enzymatic activities (amylase, protease, and lipase) indicating roles in starch degradation, protein breakdown, and flavour development during fermentation. In vitro evaluations demonstrated satisfactory tolerance to acidic conditions (77.78% viability at pH 2.0) and bile salts (74.16% viability at 24 h), along with antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* species. Phylogenetic analysis confirmed the close evolutionary relationship of the isolate with other *L. plantarum* strains. Importantly, the incorporation of dehydrated cauliflower flour did not reduce bacterial viability and appeared to provide a supportive substrate for microbial growth. These in vitro findings are promising and indicate the technological suitability of *L. plantarum* (D2) as a functional sourdough starter culture. However, these results do not constitute clinical evidence of gastrointestinal health benefits; in vivo studies and human clinical trials are required to validate the probiotic efficacy of this strain under physiological conditions. Further studies focusing on in vivo validation, complete microbiome characterisation, and shelf-life assessment are recommended to support the practical application of this sourdough formulation.

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Conflict of interest: The authors declare no conflict of interest.

Ethics statement: The research work was approved by the Institutional Human Ethics Committee (IHEC) of Shrimathi Devkunvar Nanalal Bhatt Vaishnav College for Women (Autonomous), Chromepet, Chennai – 600 044, affiliated with the University of Madras. The IHEC approval number is SDNBVC/IHEC/24/01, dated 07/10/2024. All ethical guidelines were followed in the conduct of this study.

Informed consent statement: This study did not involve human participants, and therefore, informed consent was not required.

Author's contribution:
R. Vijaya Vahini – Conceptualisation, Writing – Review & Editing, Supervision, Project Administration.
T. Divyasri – Formulation, Laboratory Analysis, Original Draft Preparation.

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