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Characterization of indigenous lactobacilli from dairy fermented foods of Haryana as potential probiotics utilizing multiple attribute decision-making approach

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Abstract

The interest in region-specific ethnic fermented foods and their functional microbiota is rising. The demands for functional foods are continuously rising, so research is going on to develop nutritious food with many beneficial attributes and low safety concerns. The present study was designed to isolate and characterize lactobacilli probiotic candidates from locally resourced fermented foods (*dahi*, *lassi*, and *raabadi*) to make ready-to-eat fermented functional products later. Cultures were isolated from 82 fermented food samples collected from different villages. The initial experiments of gram staining, catalase test, and carbohydrate fermentation were assessed for the morphology, purity, and primary characterization on the genus level, which was verified through molecular characterization using PCR. Seven lactobacilli strains (no. MS001-MS007) were then assessed for safety, probiotic candidacy, phytase degradation, and biofilm forming abilities. All seven bacterial cultures showed no hemolytic activity and antibiotic sensitivity against more than 14 antibiotics out of 20. All seven lactobacilli isolates were able to tolerate pH 3.0, 0.3% bile 0.5% pancreatin, lysozyme (100 mg/L to 300 mg/L) and also shown possessed phytase degradation ability. All the cultures showed antioxidative potential and biofilm formation ability. Culture MS007 showed considerably higher bile salt hydrolase activity among all the isolates, whereas MS005 possessed excellent phytate degradation ability among others. Bacterial strains were identified using 16S rRNA gene sequencing. Moreover, the order of preference of isolates was calculated using the multidimensional Technique for Order of Preference by Similarity to Ideal Solution (TOPSIS) based on probiotic and other functional properties. The most promising attributes showing cultures were recognised as *Limosilactobacillus fermentum* MS005 and *Lactiplantibacillus plantarum* MS007, which could be further used for functional food product development.

Keywords Probiotics, *Lactobacillus*, Antioxidative attributes, Biofilm formation, TOPSIS, 16S rRNA sequencing

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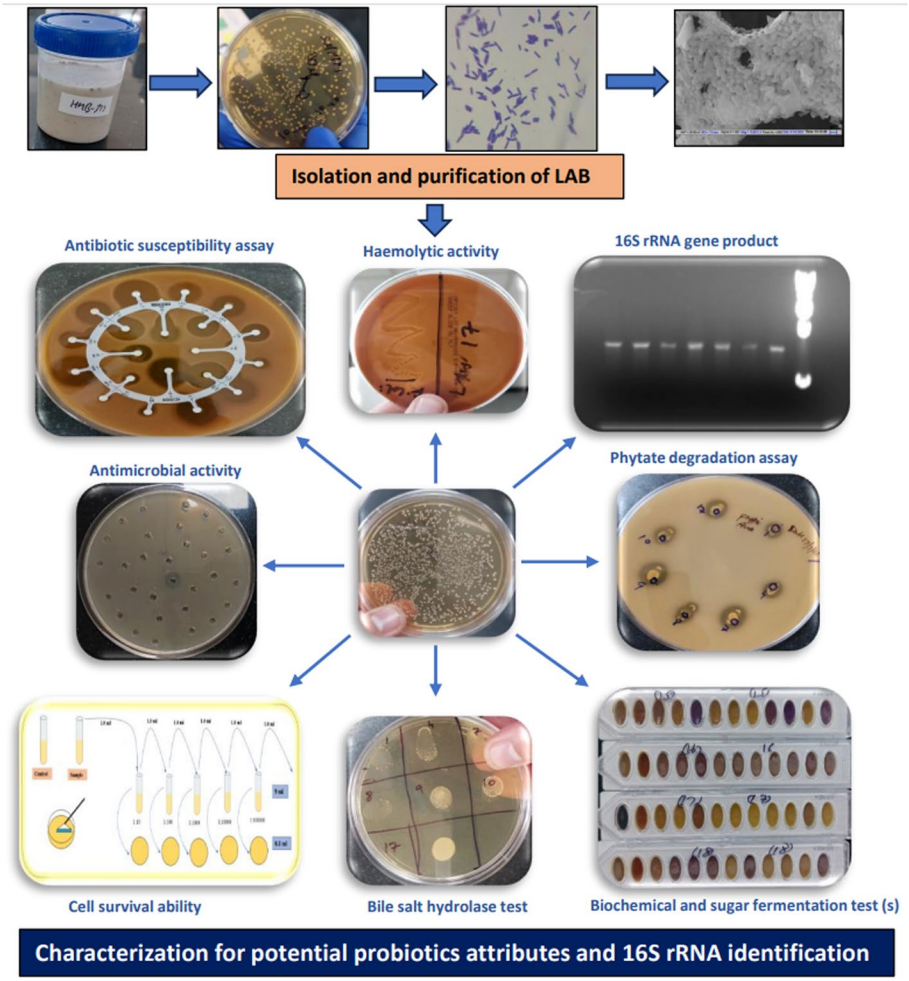
Graphical Abstract



Introduction

Probiotics are 'live microorganisms which, when administered in adequate amount confer health benefits on the host' (FAO/WHO 2002). Several sources, such as vegetables, rotten fruits, sausages, bovine and human faecal samples, mother's milk, fermented foods, etc., have been used to isolate and characterize probiotics cultures (Bhat et al. 2017). Among other lactic acid bacteria (LAB), lactobacilli are the prominent microorganisms used in dairy based fermented probiotic products as starters or adjunct cultures. Due to their non-pathogenic nature, most of them are classified as "generally recognised as safe" (GRAS) organisms (Kumari et al. 2022). Due to probiotics' diverse inherent health-enhancing properties, researchers' inquisitiveness has enhanced for isolating the novel strains having potential functional properties (Bhat & Bajaj 2019; Samtiya et al. 2022). The *Lactobacillus* genus is the most prominent type of LA bacteria used as a starter culture in food fermentation (Kumari et al. 2022). Presently, autochthonous *Lactobacillus* cultures having possible probiotics attributes are being progressively used in dairy foodstuffs as a potential starter culture to retain traditional foodstuffs typicality to enhance their health-promoting properties (Kumari et al. 2022; Saliba et al. 2021). Besides exploiting the conventional technological and probiotic capabilities, the recent research on characterizing the isolates with additional phytase activity is also in demand. In context of high popularity of cereal-based foods in India, such functionality might give an extra edge to the food starters of choice. Fermentation with such strains could enhance the

bioavailability of minerals (i.e., Fe, Zn, and Ca) through phytase production and decreasing phytic acid constituents in plant foods. Pearl millet-based fermented foods can be the food of choice to isolate phytase-producing lactobacilli (Samtiya et al. 2021). For the in vitro screening of potential strains of probiotics, several parameters are recommended by the Indian Council of Medical Research and Department of Biotechnology (ICMR-DBT) guidelines, such as resistivity against gastric acidity, tolerance to bile acid, antimicrobial activity, bile salt hydrolase activity, and for safety evaluation of hemolytic potential and antibiotic resistance patterns (Ganguly et al. 2011). Several other parameters, such as biofilm formation capacity, auto-aggregation ability, lysozyme tolerance, cell surface hydrophobicity, antioxidative capacity, phytase activity, ethanol tolerance, etc., could also be used for the evaluation of probiotic potentials (Bhushan et al. 2021; Pradhan & Tamang 2021; Shivangi et al. 2020). A scarcity has been seen in reports on isolating and characterizing safe probiotic lactobacilli starters with additional potential of reducing anti-nutrients. So, making a cereal-based fermented product with improved micronutrient bioavailability could be a promising approach that can help mitigate micronutrient deficiencies. The potential probiotic strains capable of reducing anti-nutrients and thereby enhancing micronutrients (Fe and Zn) are the need of the hour.

Considering the attention to the reports mentioned above, we designed a hypothesis and identified gaps in knowledge. The present research included the isolation of lactobacilli from fermented food sources (*dahi, lassi,* and *raabadi*) in rural Haryana and their *in-vitro* screening for safety (antibiotic sensitivity and haemolytic activity) and probiotic candidacy (cell survival capability, auto-aggregation, hydrophobicity, biofilm formation, antioxidative potentials, bile salt hydrolase activity, and, most notably, phytate degradation ability) along with the assessment of fermentation/technological superiority (NaCl and ethanol tolerance) of selected lactobacilli isolates. Furthermore, the current study underscored the importance of the multidimensional ranking technique (TOPSIS) in identifying phytate-degrading isolates with probiotic properties.

Materials and Methods

Chemicals/reagents/kits and microbial strains

Crystal violet, xylene, and ethanol were procured from Fisher Scientific International (Mumbai, India) 0.2,2-Diphenyl-1-picrylhydrazyl (DPPH), taurodeoxycholate, 2,2'-Azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and phytic acid, were procured from Sigma-Aldrich (Bangalore, India). Phosphate buffer saline, pancreatin, bile, lysozyme, NaCl, MRS broth, MRS agar, nutrient agar, nutrient broth, glycerol, gram's staining kit, negative staining kit, HiLacto test kit, HiPurA Genomic DNA Purification Kit, antibiotic disc ring (IC006, Icosa Universal-2), chloroform, ethyl acetate, n-hexadecane and MOPS buffer were procured from Hi-media (Mumbai, India). Only ACS/analytical grade chemicals and solvents were used in this investigation.

The indicator bacterial strains (*E. coli* ATCC 11229, *Listeria monocytogenes*, *Bacillus cereus* NCDC250, and *Staphylococcus aureus* NCDC109) were kindly gifted by Prof. Vijendra Mishra, Food Microbiology lab, NIFTEM, India.

Collection and processing of samples

The 82 fermented food samples (*dahi, lassi,* and *raabadi*) were collected from native households of rural Haryana in sterile sample containers (100 mL, Tarsons, Kolkata, India) and kept at 4 °C until delivered to the laboratory. The recording of sample pH was followed by serial dilution (0.85% saline), pour plating on MRS (de Mann Rogosa Sharpe) agar, and incubation for the next 24–48 h in aerobiosis. LAB colonies with diverse morphologies on the MRS agar plate were carefully chosen and further purified by repeated streaking to obtain a pure colony. The pure LAB cultures were preserved in glycerol stock (35% v/v) at − 80 °C for further use (Dhewa et al. 2010).

Morphological and biochemical characterisation of isolates

Isolate's primary identification was performed through culture characteristics, microscopic observations (gram's staining and negative staining), and colony morphology. Out of 120 isolated colonies, Gram's staining reduced the number of isolates to 20, which were further subjected to the biochemical analysis and carbohydrate fermentation pattern (Catalase, Esculin hydrolysis, Xylose, Celliobiose, Arabinose, Maltose, Galactose, Mannose, Mellibiose, Raffinose, Sucrose, and Trehalose). The test was carried out using the surface inoculation method to inoculate 50 µL of the test inoculum into each HiLacto test biochemical test kit well, followed by a 24-h incubation period at 37 °C.

Molecular identification of isolates for genus confirmation

Isolates showing gram's positive, catalase-negative, and rod-shaped nature were further considered for the PCR-based molecular identification for genus confirmation. Genomic DNA was collected for PCR using HiPurA genomic DNA purification Kit. DNA primers (amplicon length 250 bp) were used for the PCR (forward: CTCAAAACTAAACAAAGTTTC and reverse: CTTGTACACACCGCCCGTCA) to target the 16S–23S rRNA intergenic spacer region for *Lactobacillus*

genus identification (Dubernet et al. 2002). Amplification of DNA was carried out in a thermal cycler (CFX96™, Bio-Rad, California, United States); PCR program was as follows: denaturation at 95 ℃ for 5 min, followed by 35 cycles consisting of denaturation at 95 ℃ for 30 s, annealing at 55 ℃ for 30 s, extension at 72 ℃ for 30 s, and a 7 min final extension step at 72 ℃. Gel electrophoresis was performed using aliquots (10 µL) of amplified products and a 50 bp marker on 1% agarose gel (Electrophoresis grade, Invitrogen, UK) in the Tris–Acetate EDTA (TAE) buffer. Gel was stained with ethidium bromide and examined using a gel-doc system (GelDoc Go, Bio-Rad, California, United States).

Strains identification of LAB isolates by 16S rRNA gene sequencing

The 16S rRNA sequencing technique was used for the strain's identification of LAB isolates. 16S rDNA gene PCR amplification was performed for all the seven LAB isolates using universal primers, the forward primer 63f (5'-CAG GCC TAA CAC ATG CAA GTC-3') and reverse primer1387r (5'-GGG CGG WGT GTA CAA GGC-3') (Marchesi et al. 1998). Aliquots (10 µL) of the amplified products and 1000 bp marker were used for gel electrophoresis in 1% agarose gel (Electrophoresis grade, Invitrogen) in TAE buffer. Gel was stained with ethidium bromide and visualised under gel-doc system (GelDoc Go, Bio-Rad, California, United States). After confirming the correct amplification, the amplified PCR product was outsourced to Eurofins Genomics India Pvt., Ltd (Bengaluru, India) for purification and sequencing of 16S rRNA. Further, to identify the strains of LAB isolates, obtained sequences were evaluated by comparing with bacterial references, which are already available in GenBank database of NCBI (National Centre for Biotechnological Information) using BLAST search program with > 98% of DNA homology threshold.

Assessment of probiotic attributes (in vitro) of isolates

For the in vitro screening of potential strains of probiotics, parameters recommended by the Indian Council of Medical Research and Department of Biotechnology (ICMR-DBT) guidelines for safety evaluation of hemolytic potential and antibiotic resistance patterns and other potential probiotic attributes (Ganguly et al. 2011), were utilized for assessment in the current study.

Safety assessment

Haemolytic activity

Haemolytic activity of LAB isolates was determined by using blood agar plates (Himedia), following Bhushan et al. (2017). All the blood agar plates were streaked using active LAB isolates, followed by incubation at 37 ℃ for 48 h in a BOD incubator. After incubation, streaked plates were observed for haemolysis activity. Results were observed as γ-hemolysis (no zones or change), β-hemolysis (zones are lightened –yellow or transparent), and α-hemolysis (zone are greenish and dark).

Antibiotic susceptibility

All LAB isolates were evaluated using the Bauer-Kirby disk diffusion protocol with antibiotic disks. The antibiotic susceptibility was performed using methods of Bhushan et al. (2021) and Ahire et al. (2021), with some modifications. The soft MRS agar (0.8%, w/v) was inoculated with overnight-grown active LAB isolates, followed by pouring soft agar on preformed MRS agar (1.6% w/v) plates (200 mm, Himedia) and kept for 1 h in the laminar cabinet for proper drying. The soft agar surface was then covered with antibiotic discs, which were then incubated for 24 h at 37 °C. Results were analysed using breakpoint guidelines of the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (Matuschek et al. 2014; CLSI 2023).

Cell survival in oral, gastric, and intestinal conditions in vitro

Tolerances to lysozyme, hydrochloric acid, bile and pancreatin

Tolerances of isolates to lysozyme (100–300 mg/L), HCl (pH 3.0), bile (0.3%, w/v), and pancreatin (0.5%, w/v) in MRS broth were tested as previously suggested protocols (Bhushan et al. 2017; Nath et al. 2021; Pradhan & Tamang 2021; Shivangi et al. 2020) with some modifications. For each of the tests, active LAB isolates (grown for 18 h in MRS broth) were harvested by centrifugation (8000 *g* for 5 min at 4℃), washed, and resuspended in phosphate buffer saline (PBS, pH 7.2). The control suspensions contained pH 6.8 to 7.0 and were not added to the test compounds. The incubation for all assays was done in shaking, after each incubation, the viability of the isolates was evaluated using MRS agar count plate method. The below-given formula was used to assess the survival percentages (%):

$$\text{Cells Survival \%} \frac{\text{Log value of cells survived in CFU/mL}}{\text{Log value of initial cells inoculated in CFU/mL}} \times 100$$

In-vitro tests for cell adhesion to intestinal walls

Auto-aggregation ability

Auto-aggregation ability of LAB isolates was determined following the method of Ahire et al. (2021) with some modifications. Overnight (16 h) grown active LAB cultures were harvested by centrifugation at 11,000 g for 10 min at 4 °C, followed by washing of cell pellet twice using PBS (pH 7.2), and OD_{600} was set as~ 0.8 by diluting. Three mL of culture suspensions were taken in test tubes, vortexed moderately for 30 s, and then incubated at 37 ℃. The upper aqueous phase of samples was taken carefully after 3 h, and after 24 h of incubation, OD_{600} readings were taken. The auto-aggregation ability of LAB cultures is presented in percentage using the below-given formula:

$$\text{Auto}-\text{aggregation } \% = \frac{OD_{600} \text{ of bacterial suspension} - OD_{600} \text{ of upper suspension}}{OD_{600} \text{ of bacterial suspension}} \times 100$$

Cell surface hydrophobicity

The isolates' ability to adhere to organic solvents was determined using the Microbial Adhesion to Hydrocarbon (MATH) assay using the method of Ahire et al. (2013) and Shangpliang et al. (2017) with some modifications. Xylene (non-polar solvent), ethyl acetate (polar solvent), n-Hexadecane (non-polar solvent), and chloroform (monopolar acidic solvent) were used as test solvents. Experiment was done as mentioned for auto-aggregation assay, except aqueous phase OD was taken instead of upper phase suspension. The percentage of hydrophobicity or microbial adhesion of LAB cultures was calculated using the below-given formula:

$$\text{Microbial adhesion } \% = \frac{OD_{600}\ (A) - OD_{600}\ (B)}{OD_{600}\ (A)} \times 100$$

Where, OD_{600}(A) and OD_{600}(B) are the aqueous phase absorbance (before and after addition with solvents).

Biofilm formation property

Biofilm formation ability of LAB isolates was determined according to the method of Bhardwaj et al. (2021) with some modifications. Overnight grown (18 h) active LAB isolates OD_{600} was adjusted to 0.1 (0.5 McFarland standard), and 100 µL cell suspension (MRS broth) was poured in 96-well of polystyrene plates (Greiner Bio-One, Kremsmünster, Austria). With a pipette, the planktonic cells were carefully removed from the wells after incubation, and non-adherent cells were eliminated by washing them with PBS (pH 7.2). After that, crystal violet (1% (w/v)) was added and incubated for 30 min at room temperature. The remaining color was removed from the wells following washing with PBS. At final step, 100 µL of pure ethanol was filled in all the wells and absorbance was measured on OD_{540} nm using a multimode microplate reader (Molecular Devices, USA) after 5 min. Moreover, all other plates were processed using the same protocol for the final absorbance. 100 µL of MRS broth was used as a control for this experiment.

Functional attributes

Tolerance to technological stresses

The tolerance of LAB isolates to ethanol (4, 6, 8, and 10%, v/v) and sodium chloride [(NaCl) 1, 2, 4, and 6%, w/v] concentrations were evaluated using the method of Gold et al. (1992) with slight modifications. The active MRS cell suspensions and controls were prepared as described previously. Each inoculated MRS test broth was incubated at 37 ℃ in a BOD incubator for 24 h, and then OD_{600} nm readings were recorded to assess the tolerance.

Enzymatic activity of isolates

Bile salt hydrolase (BSH) activity With a few adjustments, Ahire et al. (2021), methodology was used to determine the BSH capacity of isolated cultures. On MRS agar medium containing 0.37 g/L $CaCl_2$ and 0.5% (w/v) sodium taurodeoxycholate, overnight grown active cultures were spot inoculated (10 µL). The presence of hydrolysed salt precipitation surrounding the colonies directed BSH activity.

Phytate degradation ability The qualitative phytase activity test for LAB isolate's anti-nutrient degrading characteristics was assessed by plate screening assay according to Pradhan and Tamang (2021), with some modifications. Spot inoculations (10 µL) of the overnight-grown active cultures were made on modified MRS agar, and they were then incubated at 37 °C for 72 h. The appearance of clear zones around the spot-inoculated culture (caused by phytic acid breakdown) was recorded as a positive result.

Antioxidative properties

Preparation of intracellular cell-free extract (CFE) and intact cells (IC)

Overnight grown (18 h) active LAB isolates were harvested by centrifugation at 10000 *g* for 7 min at 4 ℃. For the Intracellular cell-free extract (CFE) preparation, cell pellets were washed with PBS (7.2) two times, and

cell absorbance values were adjusted at~ 0.8 at 600 nm. with the same PBS. Cell suspension was then subjected to ultrasonic extraction for 45 min using an ultrasonication unit (CPX3800H-E, Bransonic, USA). The CFE extracts were filtered using a sterile 0.22-µm syringe filter (Nupore Filtration Systems, India) and collected in sterile vials for further experiments. For intact cell (IC) preparation, cell pellets were washed with PBS (7.2) two times, cells OD_{600} was adjusted at~ 0.8 with the same PBS and used for further experiments.

DPPH and ABTS DPPH assay and ABTS assay were used to perform the antioxidative activity in cell-free extract (CFE) and intact cells (IC) of LAB isolates by following methods Samtiya et al. (2023) and Ayyash et al. (2018), respectively. IC samples were centrifuged (1 Min, 2000 g) before taking absorbance at 517 nm or 734 nm using a multimode plate reader (SpectraMax, M2e) with a cuvette port.

The DPPH free radical scavenging ability was estimated using the given formula:

$$\text{RSA } \% = \frac{OD_{517}\ (A) - OD_{517}\ (B)}{OD_{517}\ (A)} \times 100$$

Where, OD_{517}(A) and OD_{517}(B) are the control and sample absorbance after incubation, respectively.

The ABTS radical scavenging ability was estimated using the given formula:

$$\text{Scavenging } \% = \frac{OD_{734}\ (A) - OD_{734}\ (B)}{OD_{734}\ (A)} \times 100$$

Where, OD_{734}(A) and OD_{734}(B) are the control and sample absorbance after incubation, respectively.

Antimicrobial activity

Antibacterial activity of cell-free supernatant (CFS, pH 7.0) of isolates in LB was observed against the indicator using the agar-well diffusion method, according to Bhushan et al. (2021), with some modifications. Bacterial cells were centrifuged at 5000 *g* for 10 min at 4 ℃. Cell-free supernatants (CFSs) were separated, pH was corrected to 7.0 with 5 M NaOH, and filtered through a sterile 0.22-µm syringe filter (Nupore Filtration Systems, India). All the target pathogen OD_{600} was adjusted to 0.1 (0.5 McFarland standard) and used for inoculating Mueller Hinton (MH) Agar. Through sterile borer (6 mm), well were formed in the MH agar containing different targeted bacterial cultures. Following the addition of 100 µL of CFSs (pH 7.0) to the wells, the plates were incubated at 37 °C in a BOD incubator for 12 to 16 h. Acetic acid (5%) and MRS broth (filter sterilised with 0.45-µm syringe filter) were used for controls.

Morphological visualisation of selected cultures using scanning *electron* microscopy

To visualise the *L. fermentum* MS005 and *L. plantarum* MS007 morphology, Scanning Electron Microscopy (Model: EVO 18, Zeiss Pvt. Ltd., UK) was performed. Cultures were freeze-dried and outsourced (Central Research Facility, Indian Institute of Technology, Delhi) for scanning electron microscopy.

TOPSIS: Multiple attribute decision making (MADM) analysis

Multiple Attribute Decision Making (MADM) approach called as Technique for Order Preference by Similarity to Ideal Solution (TOPSIS) was employed in the study to rank the cultures in order of probable best probiotic candidate. TOPSIS is a multi-attribute decision-making technique that delivers a ranking based on the characteristics in terms of perceived weight and satisfaction. The ranking also determines the distances to the positive ideal solution and the distances to the negative ideal solution. The best cultures will be those that are closest to the positive-ideal solution and the furthest away from the negative-ideal solution (Zavadskas et al. 2016). TOPSIS is used in various fields, such as engineering, business, biotechnology, and environmental management. It allows decision-makers to estimate the ranks of alternatives based on multiple criteria or attributes. TOPSIS uses a geometric method that associates each alternative with an ideal solution. By evaluating the closeness of each alternative to the ideal solution, TOPSIS yields a comprehensive ranking that allows decision-makers to make informed choices that balance conflicting objectives. This method provides a structured and systematic way to handle the intricacies of multi-criteria decision-making, facilitating the selection of the most suitable alternative in a transparent and quantitative manner. The method is consisting of the following steps:

Step 1: Make a decision matrix of order $m \times n$ where m is the number of alternatives and n is the number of attributes (say $A = e_{ij}$ of size $m \times n$).
Step 2: Calculate the normalized decision matrix n_{ij} by normalize the column wise data of the decision matrix by using the following equation:

$$n_{ij} = \frac{e_{ij}}{\sum_{i=1}^{m} e_{ij}} \qquad i = 1, 2, 3, \ldots, m; \quad j = 1, 2, 3, \ldots, n$$

Step 3: Calculate the information entropy h_j by using the following equation:

$$h_j = \sum_{i=1}^{m} n_{ij} \times ln(1/n_{ij})$$

Step 4; Calculate the degree of divergence as $d_j = 1 - h_j, \quad j = 1, 2, 3, \ldots, n$, and the degree of importance by using the equation

$$w_j = \frac{d_j}{\sum_{j=1}^{n} d_j} \qquad j = 1, 2, 3, \ldots, n$$

Step 5: Calculate the comprehensive weight as

$$w_j{\prime} = \frac{b_j \times w_j}{\sum_{j=1}^{n} b_j \times w_j}$$

where b_j represents the value of weight associated with an attribute.

Step 6: Calculate the weighted normalize matrix V_{ij} as

$$V_{ij} = n_{ij} w_j{\prime} \qquad i = 1, 2, 3, \ldots, m; \quad j = 1, 2, 3, \ldots, n$$

Step 7: Evaluate the positive (V^+) and negative (V^-) ideal solutions from V_{ij} matrix obtained from Step 7.

$$V^+ = \left\{V_1^+, V_2^+, \ldots, V_n^+\right\} = (\max\{V_{11}, V_{21}, \ldots, V_{m1}\}, \max\{V_{11}, V_{21}, \ldots, V_{m2}\}, \ldots, \max\{V_{11}, V_{21}, \ldots, V_{mn}\})$$

$$V^- = \left\{V_1^-, V_2^-, \ldots, V_n^-\right\} = (\min\{V_{11}, V_{21}, \ldots, V_{m1}\}, \min\{V_{11}, V_{21}, \ldots, V_{m2}\}, \ldots, \min\{V_{11}, V_{21}, \ldots, V_{mn}\})$$

Step 8: Evaluate the distance between each alternative from positive and negative ideal solution as:

$$d_i^+ = \sqrt{\sum_{j=1}^{n} (V_{ij} - V_i^+)^2}, i = 1, 2, 3, \ldots, m; \quad j = 1, 2, 3, \ldots, n$$

$$d_i^- = \sqrt{\sum_{j=1}^{n} (V_{ij} - V_i^-)^2}, i = 1, 2, 3, \ldots, m; \quad j = 1, 2, 3, \ldots, n$$

Calculate the closeness coefficient (R_i) between the alternative and ideal solution by using the following equation:

$$R_i = d_i^- / (d_i^+ + d_i^-)$$

Statistical analysis

Microsoft Office (version 2019) was used for raw data tabulation. GraphPad Prism (version 5.01) was used for grouped and column statistics, and one-way analysis of variance (ANOVA) was used for statistical analysis of data, followed by Tukey post-hoc test to separate the mean ($p \leq 0.05$), which was considered statistically significant. The p-values < 0.05 were considered to be statistically significant. The data are expressed as mean ± standard deviation of replicates.

Results and discussion

The current work employed a subtractive screening strategy to find potential probiotic strains from traditional fermented food. Before functional and technological evaluation, crucial probiotic factors such as safety and tolerance to gastrointestinal conditions were taken into account.

Origin, colour appearance, and pH of collected samples

Eighty two dairy-fermented foods (*lassi, dahi,* and *raabadi*) were collected from the different districts of Haryana. Sample source, pH range, and appearance of color are presented in Table S1. Fig. S1 represents the districts of Haryana from where dairy-fermented samples were collected in this study.

Morphological, biochemical test (s) and molecular identification

In the present study, LAB colonies were selected based on their morphology (irregular and round shape), those devoid of pigmentation, white and creamy from MRS agar plates, and have shown rod-shaped appearance in gram-positive staining and negative staining. Results of the biochemical and carbohydrate fermentation test for the 20 selected isolates (based on the preliminary character) are represented in Table 1. Fifteen LAB isolates were found catalase-negative, which were further selected

Table 1 Biochemical test (s) of the selected lactic cultures

Lactic cultures	Biochemical test												*Lactobacillus* confirmation through PCR, along with their assigned ID
	Esculin hydrolysis	Catalase	Xylose	Celliobiose	Arabinose	Maltose	Galactose	Mannose	Mellibiose	Raffinose	Sucrose	Trehalose	
1	-	-	V	V	V	V	+	+	+	+	+	V	MS001
2	+	-	+	V	+	+	+	V	+	V	-	-	
3	+	-	+	V	+	+	+	-	+	-	-	-	
4	-	-	V	-	-	-	+	V	+	+	+	V	MS002
5	-	+	-	-	-	+	V	+	-	-	+	-	
6	+	-	+	V	+	+	+	V	+	V	V	+	
7	-	-	V	-	-	-	+	V	+	+	+	**V**	MS003
8	-	-	+	V	+	+	+	V	+	+	+	+	MS004
9	-	-	+	-	+	+	+	-	+	+	+	V	MS005
10	-	-	V	V	-	-	+	V	+	+	+	V	MS006
11	-	-	-	-	-	+	+	+	V	V	+	-	
12	+	-	+	+	V	+	-	+	+	+	-	+	
13	+	+	-	V	-	V	-	V	-	-	-	-	
14	-	+	-	V	-	+	+	+	+	-	-	-	
15	-	+	V	+	-	+	+	+	V	-	+	-	
16	-	-	V	V	-	V	V	V	V	V	V	V	
17	+	-	+	+	V	+	+	+	+	+	+	+	MS007
18	-	-	+	V	V	V	+	V	+	+	+	V	
19	+	+	-	-	-	-	-	-	-	-	-	-	
20	-	-	V	-	-	+	V	+	V	-	+	V	

'-' = Negative, '+' = Positive, V = Variable'

for molecular identification at the lactobacilli genus level. PCR confirmed that seven out of 15 LAB isolates were lactobacilli. The gel electrophoretic separation of genus-specific PCR products for confirmed lactobacilli isolates (Fig. S2). In this study, the morphological and biochemical characteristics of isolated cultures resembled other reported *Lactobacillus* cultures isolated from the fermented food samples (Nath et al. 2020; Yadav et al. 2016). Selected lactobacilli isolates were given specific code numbers, i.e., MS001, MS002, MS003, MS004, MS005, MS006, and MS007 (Table 1). Further, selected lactobacilli were evaluated for their potential probiotic's attributes. All the strains were molecularly identified by 16S rRNA gene similarities (>99%) with the existing sequence of NCBI GenBank, as presented in Table S2. A gel electrophoresis photograph of the 16S rRNA gene product of lactobacilli isolates with a marker (Fig. S3). Six isolates were identified as *Limosilactobacillus fermentum* (MS001, MS002, MS003, MS004, MS005 and MS006) and one isolate as *Lactiplantibacillus plantarum* (MS007).

Safety assessment of isolates

The safety of lactobacilli isolates was determined using a blood haemolysis assay and the antibiotic susceptibility test. Probiotic bacteria (lactobacilli) are commonly considered safe for consumption, so these strains should be non-hemolytic in nature (Peres et al. 2014). So, only those cultures should be selected for further research, which cannot lyse erythrocytes of hosts and must be confirmed for γ-haemolytic activity on a blood agar plate. Our isolates were assessed for haemolytic activity, and results confirmed that all isolates (MS001, MS002, MS003, MS004, MS005, MS006, and MS007) are safe, as they do not show any kind of haemolysis activity (γ-haemolytic) and growth on sheep blood agar plate, hence can be used for further food development. Similar observations (γ-haemolytic activity) for *Lactobacillus* strains were reported by several studies such as *L. plantarum* CS (Nwachukwu et al. 2019), *L. plantarum* BIF43, BBC32B, BBC32A, and BBC33 (Bhushan et al. 2021), LAB cultures (Wu et al. 2021), *L. plantarum* UBLP40 (isolated from fermented food) (Ahire et al. 2021), *Lactobacillus* isolates (Yadav et al. 2016), *Lactobacillus* strains (isolated from goat milk) (Saliba et al. 2021), *L. fermentum* (NMCC-27, NMCC-17, NMCC-14, and NMCC-2) (Abid et al. 2022). Antibiotic tests are primarily used to evaluate probiotic bacterial cultures; cultures must be antibiotic sensitive to avoid the spread of undesired antibiotic resistance. According to the breakpoint scale (15–19 = ZOI ≥ 20), all tested isolates demonstrated sensitivity (intermediate to highly sensitive) to the majority

Table 2 Antibiotics susceptibility pattern/assay of lactic cultures

Antibiotic class	Antibiotics	LAB isolate						
		MS001	MS002	MS003	MS004	MS005	MS006	MS007
β-lactams	Ampicillin (10 µg)	S	S	S	S	S	S	S
	Amoxicillin (10 µg)	S	S	S	S	S	S	S
	Penicillin (10 Unit)	S	S	S	S	S	S	S
	Cloxacillin (1 µg)	R	R	R	R	R	R	R
Cephalosporins	Ceftazidime (30 µg)	S	S	S	S	S	S	S
	Cefoperazone (75 µg)	S	S	S	S	S	S	S
	Cefadroxil (30 µg)	S	S	I	S	S	S	S
	Ceftriaxone (30 µg)	S	S	S	S	S	S	S
Quinolones	Norfloxacin (10 µg)	R	R	R	R	R	R	R
	Ciprofloxacin (5 µg)	S	S	S	I	S	I	R
	Nalidixic acid (10 µg)	R	R	R	R	R	R	R
Azolidiones	Nitrofurantoin (300 µg)	S	S	S	S	S	S	S
Glycopeptides	Vancomycin (30 µg)	R	I	I	I	R	I	R
Aminoglycosides	Tobramycin (10 µg)	R	I	I	I	I	R	R
	Gentamicin (10 µg)	S	S	S	S	S	S	S
	Netillin (10 µg)	S	S	S	S	S	S	S
	Amikacin (30 µg)	I	I	S	S	S	S	R
Macrolides	Erythromycin (15 µg)	S	S	S	S	S	S	S
Sulfa drug Others	Co-trimoxazole (25 µg)	R	R	R	R	R	R	R
	Chloramphenicol (30 µg)	S	S	S	S	S	S	S

R resistant (ZOI ≤ 14), *I* intermediate (ZOI 15–19), *S* sensitive (ZOI ≥ 20), Clinical and Laboratory Standards Institute (CLSI), 2023

of the tested antibiotics (Table 2). If probiotic culture contains resistance genes, then it is more possible to transfer these genes to other pathogenic strains (in the intestine) and make them resistant to antibiotics. Therefore, it is necessary to evaluate new probiotic isolates for antibiotic susceptibility before using them in foodstuffs. Results of our study exhibited that all isolates showed sensitivity (ZOI ≥ 20) or intermediate sensitivity (ZOI 15–19) for more than 14 antibiotics (β-lactams, Cephalosporins, Quinolones, Azolidiones, Glycopeptides, Aminoglycosides, Macrolides antibiotics classes), and could be considered safe for further use. Our results are supported by the findings of several previous research studies that evaluated *Lactobacillus* strains (Ahire et al. 2021; Bhushan et al. 2021; Wu et al. 2021). In agreement with the previous findings (Yadav et al. 2016; Zhou et al. 2005), our LAB isolates showed natural resistance against nalidixic acid (antibiotic). Moreover, our isolates confirmed natural resistivity against Cloxacillin, Norfloxacin, and Co-trimoxazole antibiotics, so the resistivity may benefit their insistent establishment in the gut over antibiotic treatment. Although resistivity to antibiotics (Aminoglycopeptides and Quinolones) is an inherent characteristic (Ammor et al. 2007; Hummel et al. 2007), though, we didn't notice any such resistance, hence using our strains as probiotics is safer in terms of antibiotic resistance transmission and emergence. Henceforward, there may not be any safety concerns for the consumption of lactobacilli isolated in the present study.

Cell survival in oral, gastric, and intestinal conditions in vitro

The lysozyme tolerance ability of probiotics depicts its stability in the oral cavity as lysozyme is present in human saliva. Before reaching the intestinal cavity, they must face a stressful environment of mouth (Singhal et al. 2021). In the current evaluation, isolated lactobacilli showed high survivability rates (98 to 99%, 91 to 98%, and 85 to 92%) at different lysozyme concentrations (100 mg/L, 200 mg/L, and 300 mg/L), respectively, even after 3 h of exposure (Table 3). The lowest viability was observed for MS004 isolate, i.e., 91.02% and 85.71% at 200 mg/L and 300 mg/L lysozyme, respectively. Further, results depicted that MS005 LAB isolate was shown the highest tolerance, i.e., 92.44% at 300 mg/L of lysozyme. In agreement with our observations, lysozyme tolerance at a different concentration by other lactobacilli cultures was quantified by previous studies (Bhushan et al. 2017; Bosch et al. 2012). Our strains are showing better stability at oral conditions, compared to the previous isolated lactobacilli strains from cereal-based fermented food (Yadav et al. 2016). As per the current characterisations, potential probiotics must persist in the stomach's acidic environment if they come to the small intestine and colonise the host GI tract, where they can perform their activity (Hsiung et al. 2021). Our results confirmed that the majority of lactobacilli isolates showed high acid tolerance (between 85 to 97%) at pH 3.0; out of seven lactobacilli, MS005 and MS007 showed 97% of survivability, which denotes that these strains have good tolerance against acidic conditions (Table 4). Results of acid tolerance found that MS001 isolate showed a significant ($p < 0.001$) reduction to acidic conditions compared to the other six isolates. Our results corroborate the previous study, which reported that 13 out of 15 *Lactobacillus* strains showed survivability (84% to 100%) at acidic pH, isolated from fermented foods (Simões et al. 2022). Another study by Zielińska et al. (2015) and Nath et al. (2020) reported *Lactobacillus* strain's survivability at pH 3 in the range of 30 to 100% and 90%, respectively, when incubated for 3 h. Compared to previous findings some of our strains are showing better sustainable capacity at acidic pH. Besides gastric acid tolerability, tolerance to bile salt is considered a requirement to utilise beneficial

Table 3 Survival capability (%) of lactobacilli cultures in various concentrations of lysozyme

LAB isolate	100 mg/L (%)	200 mg/L (%)	300 mg/L (%)
MS001	98.31 ± 0.73^{a}	96.30 ± 0.56^{ae}	91.45 ± 0.46^{ad}
MS002	99.03 ± 0.81^{a}	92.15 ± 0.21^{b}	85.74 ± 0.82^{b}
MS003	99.71 ± 0.16^{a}	97.46 ± 0.59^{acd}	90.15 ± 0.09^{ad}
MS004	99.10 ± 0.44^{a}	91.02 ± 0.33^{b}	85.71 ± 0.84^{cb}
MS005	99.02 ± 0.07^{a}	98.43 ± 0.039^{cd}	92.44 ± 0.66^{a}
MS006	98.79 ± 0.74^{a}	98.39 ± 0.57^{d}	91.72 ± 1.05^{ad}
MS007	98.45 ± 0.75^{a}	95.06 ± 0.34^{e}	89.59 ± 0.61^{d}

Data expressed as mean ± SD

a–e: different superscript lowercase letters (a, b, c…..) in the same column indicate significant difference ($P < 0.05$)

Table 4 Survival capability (%) of lactobacilli cultures in acid (pH 3.0), bile (0.3%) and pancreatin (0.5%)

LAB isolate	pH 3.0	0.3% Bile	0.5% Pancreatin
MS001	85.03 ± 0.19^{a}	86.03 ± 0.011^{a}	92.99 ± 0.69^{a}
MS002	94.63 ± 0.09^{b}	96.55 ± 0.15^{b}	84.07 ± 1.02^{bc}
MS003	95.12 ± 0.42^{bc}	77.77 ± 0.19^{c}	83.05 ± 1.03^{b}
MS004	91.73 ± 0.55^{d}	79.98 ± 0.18^{d}	86.42 ± 0.12^{c}
MS005	97.16 ± 0.95^{b}	77.70 ± 0.44^{c}	96.33 ± 0.17^{d}
MS006	94.94 ± 1.20^{b}	88.59 ± 0.53^{e}	91.61 ± 0.46^{a}
MS007	97.09 ± 0.54^{b}	86.56 ± 0.51^{a}	82.55 ± 0.25^{b}

Data expressed as mean ± SD

a–e: different superscript lowercase letters (a, b, c…..) in the same column indicate significant difference ($P < 0.05$)

health effects (Daoudou et al. 2011). Commonly, the amount of bile salts was in the small intestine's range of 0.2 to 0.3% (Terpou et al. 2019). The potential of isolated lactobacilli culture to bypass the bile has also been evaluated in this study; results confirmed that most cultures showed more survivability than 77% (Table 4); MS002 isolate showed the highest survival percentage (more than 96%). In the current study, a few LAB isolates showed a low survival rate (77%); it has been reported in the earlier study that food-originating lactobacilli culture has less resistivity against intestinal conditions compared to gastric juice (Tokatlı et al. 2015). Pancreatic enzymes, particularly protease, lipase, and amylase, are crucial for regular digestion of proteins, fats, and carbohydrates. Hence, the ability to tolerate these enzymes is vital for the potential probiotic selection (Rayavarapu & Tallapragada 2019). In the present investigation, all the selected lactobacilli recorded more than 82% survival rate even at 0.5% pancreatin incubation for up to 48 h (Table 4). Our results showed that MS005 LAB isolates exhibited the highest cell survival capacity (< 96%) even after incubation of 48 h, which is in agreement with the previous finding, reported excellent growth ability of *Lactobacillus* strains at 0.5% pancreatin when incubated for up to 48 h (Khagwal et al. 2014). Overall result of the cell survival ability of LAB isolates in oral, gastric, and intestinal conditions suggested that MS005 isolate showed good potential to tolerate gastrointestinal environment.

In-vitro tests for cell adhesion to intestinal walls

Auto-aggregation is important parameter to evaluate the probiotic culture's ability to colonise and maintain itself in the intestinal tract. Different ranges of auto-aggregation potential from low (16–35%), medium (35–50%), and high (< 50%) can be exhibited by bacterial strains (Montoro et al. 2016). All the LAB isolates showed aggregation ability (Fig. 1) from 10.33 to 19.82% and 52.70 to 92.94% at incubation for 3 h and 24 h, respectively. A recent study reported that lactobacilli strains isolated from fermented food samples showed auto-aggregation ability (upto 82%) (Meena et al. 2022), which is lower than our isolate. MS007 isolates showed significantly ($p < 0.05$) the highest cell aggregation potential among all LAB isolates at 3 h and 24 h of incubation.

Bacterial adhesion to different solvents/hydrocarbons is another measure to estimate the isolate's adherence ability to intestinal tract cells (Shangpliang et al. 2017). Bacterial adhesion ability % for the different solvents (hexadecane, xylene, chloroform, and ethyl acetate) were in the range (1.37 to 55.04, 3.22 to 50.05, 50.09 to 85.59 and 36.72 to 84.82), respectively (Fig. 2). However, significantly ($p < 0.001$) highest adhesion property was observed for MS007 LAB isolate for hexadecane, xylene, and chloroform, while MS005 isolate showed the maximum adhesion (84.82%) for ethyl acetate. Our results are in agreement with the study of Bhushan et al. (2017), who reported adhesion ability in the range of 17 to 26% and 19 to 27% for hexadecane and xylene, respectively. Another study reported a very low ability of adhesion for *L. plantarum* to chloroform (13%) and ethyl acetate (2%) (Ahire et al. 2021), compared to our isolates. Comparatively, for chloroform, and ethyl acetate, isolates in present study were shown excellent cell adhesion capacity. A number of

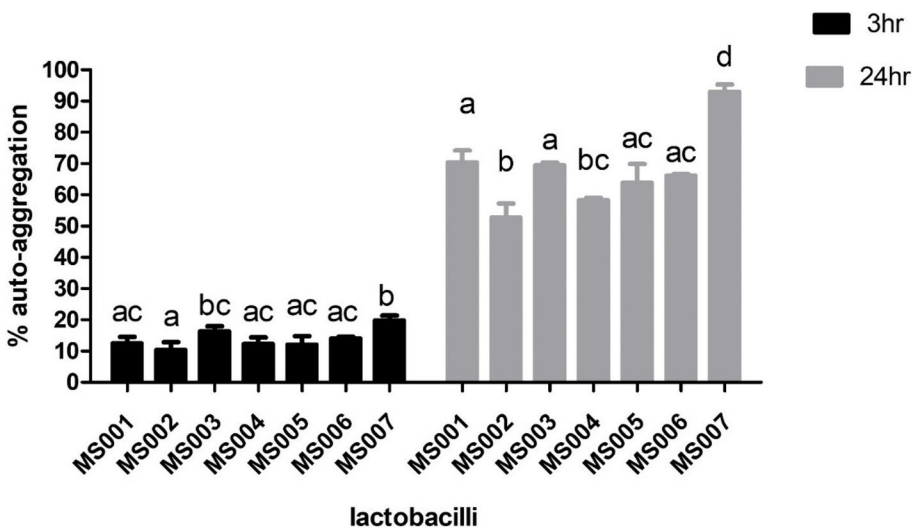


Fig. 1 Auto-aggregation potential of lactobacilli cultures at 3 h and 24 h incubation. Data expressed as mean ± SD, Different lowercase letters (a, b, c…..) above the bars denote statistically significant differences ($P < 0.05$)

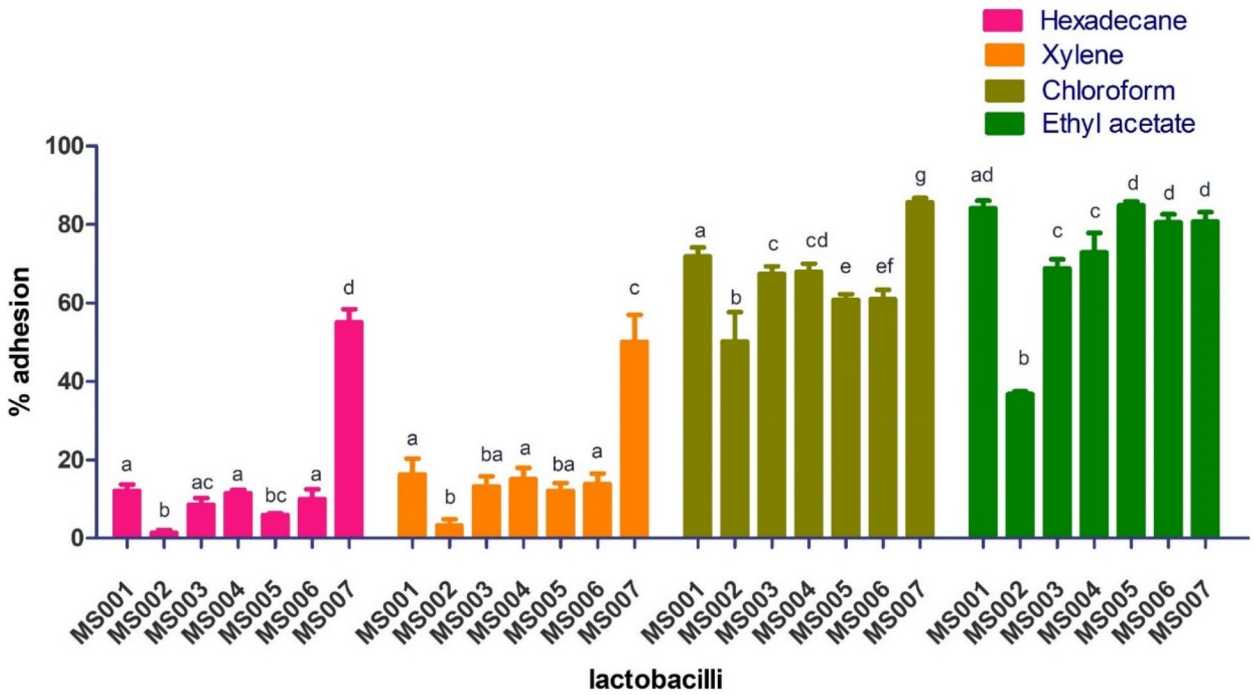


Fig. 2 Adhesion capability of lactobacilli cultures. Data expressed as mean ± SD, Different lowercase letters (a, b, c…..) above the bars denote statistically significant differences ($P < 0.05$). Data expressed as mean ± SD, Different lowercase letters (a, b, c…..) above the bars denote statistically significant differences ($P < 0.05$)

Table 5 Biofilm formation ability of lactobacilli cultures

LAB isolate	24 h	48 h	72 h	96 h
MS001	2.08 ± 0.23^{a}	2.27 ± 0.12^{a}	2.41 ± 0.20^{a}	2.25 ± 0.25^{ab}
MS002	0.47 ± 0.04^{b}	2.29 ± 0.50^{ac}	1.41 ± 0.23^{b}	1.29 ± 0.29^{a}
MS003	1.03 ± 0.14^{c}	2.75 ± 0.14^{ac}	2.25 ± 0.24^{a}	1.52 ± 0.58^{ac}
MS004	1.53 ± 0.17^{d}	2.79 ± 0.03^{bc}	2.59 ± 0.27^{a}	1.55 ± 0.20^{abc}
MS005	2.08 ± 0.23^{a}	2.23 ± 0.24^{ac}	2.50 ± 0.23^{a}	2.42 ± 0.26^{bc}
MS006	2.05 ± 0.10^{a}	2.29 ± 0.29^{ac}	2.47 ± 0.30^{a}	2.42 ± 0.18^{b}
MS007	1.35 ± 0.21^{cd}	2.79 ± 0.04^{c}	2.83 ± 0.07^{a}	2.45 ± 0.51^{bc}

Data expressed as mean ± SD

a–d: different superscript lowercase letters (a, b, c…..) in the same column indicate significant difference ($P < 0.05$)

variables, including the bacterial growth stage, the content of the surrounding medium, and the structure of the components of the cell surface, affect the hydrophobicity and auto-aggregating properties of bacteria, which are essential for the formation of biofilms and adhesion (Chaffanel et al. 2018). Different strains or genera of bacteria showed very different adhesion abilities against the same solvent, even though adhesion potential will be used to estimate the adhesiveness of LAB cultures. So, to further confirm the adhesion property, in vitro study (such as the Caco-2 cells model) should be used to assess these properties.

The capacity to form biofilm by beneficial bacteria (such as probiotics) could be useful to protect the host from diseases. This attribute permits the bacteria to maintain itself in the intestinal conditions, oppose pathogens for surface colonisation, and act as a defense purpose improving the microorganism survival rate (Probert & Gibson 2002). Table 5 shows the biofilm-forming potential of all LAB isolates for different incubation times. Significantly ($p < 0.05$), higher biofilm was formed by MS001, MS005, and MS006 isolates, at 24 h of incubation, while the lowest was formed by MS002 isolate at 24 h as well as 72 h of incubation. And as the time of incubation was increased, biofilm formation considerably increased, and maximum biofilm formation was achieved at 72 h of incubation for the MS007 isolate. The findings of our study are corroborated by the recent study of Rezaei et al. (2021), who also obtained similar results as incubation time was increased, and after 72 h of incubation, biofilm formation was decreased. Another recent study reported identical observations when LAB culture was incubated for 72 h on an abiotic polystyrene surface (Parolin et al. 2021).

Tolerance to technological stresses

The ability of probiotic cultures to withstand the intestinal environment with high salt concentration is one of the crucial characteristics for their selection. Results of

our study revealed that all the isolated lactobacilli showed maximum tolerance up to 4% of NaCl concentrations, which was supported by the previous finding, where lactobacilli isolated from curd tolerated 1–6% NaCl concentration (Prabhurajeshwar & Chandrakanth 2017). Since several fermented food's end products are alcohol, we here screened LAB isolates for ethanol tolerance ability. The present study showed that all the isolated LAB cultures recorded a maximum tolerance of up to 6% ethanol concentration, which is insisted by a recently reported study (Pradhan & Tamang 2021). Another study confirmed that the *Lactobacillus* strain could tolerate ethanol concentrations up to 16% (Gold et al. 1992), which is higher than our isolates. Tolerance to different concentrations of ethanol or NaCl mainly depends on the substrate and the strain of the cultures.

Enzymatic activity of isolates

Deconjugation of bile salts by the probiotic is the vital attribute of cultures assessed through bile salt hydrolase activity (Adebola et al. 2020; Horackova et al. 2020). In the present investigation, the qualitative assay for BSH activity indicated that all the strains, except MS005, MS006, and MS007, showed only slight growth and no ability to deconjugate TDC (bile salt) (Table 6). Furthermore, MS007 isolate were observed for slight precipitation zones also, which means MS007 strain possessed excellent BSH activity among all seven isolates. Our results are supported by the previous study, which reported diverse bile salt hydrolase activities for the LAB cultures isolated from *Raabadi* (a cereal-based fermented food) (Yadav et al. 2016). Previous studies supported our results of bile salt hydrolase activity for the probiotic bacteria isolated from fermented foods, such as *Lactobacillus* strains from fermented food samples of Rajasthan (Meena et al. 2022), *L. plantarum* subsp. *plantarum* NMB7 (Pradhan & Tamang 2021), *L. plantarum* UBLP40 isolated from traditional fermented food idli batter (Ahire et al. 2021).

Phytic acid is one of the key anti-nutrients that crucially affect micronutrient bioavailability by forming complexes with essential micronutrients such as iron and zinc (Jatuwong et al. 2020; Samtiya et al. 2020). The present study confirmed that all LAB cultures showed positive results for phytic acid degradation (Table 6). Moreover, MS001, MS002, MS004, and MS006 LAB isolates documented weak positive results, whereas the MS005 isolate was noted for excellent results for phytase degradation compared to all isolates. Previous several studies confirmed that LAB strains isolated from fermented foods produce phytase enzyme and phytic acid degradation properties such as LAB strains isolated from Ethnic Indian Fermented Foods (Sharma et al. 2019), *L. plantarum* (Uslu et al. 2016), and *Lactobacillus* strains (Saraniya & Jeevaratnam 2015). Culture possessing phytase production ability could be a subtle approach in selecting probiotic cultures, and this culture may further be used to develop micronutrient-rich plant-based foods.

Table 6 Bile salt hydrolase (BSH) activity and Phytate degradation potential of the lactic cultures

Lactic culture	BSH (Taurodeoxycholate)	Phytase activity
MS001	+	+ ve
MS002	+	+ ve
MS003	+	+ + ve
MS004	+	+ ve
MS005	+	+ + + ve
MS006	+	+ ve
MS007	+ +	+ + ve

For BSH: + growth only, + + slight precipitation

For Phytate degradation ability: + ve (weak); + + ve (good); + + + ve (excellent)

Antioxidative properties

Probiotic bacteria comprise antioxidants; when they are lysed in the intestinal cavity by the action of bile, their antioxidant components are released in the lumen. The antioxidative ability of intact cell and cell-free extract samples of all the LAB isolates is shown in Fig. 3 (a to d). Results showed a non-significant ($p > 0.05$) difference in the antioxidative potential of all LAB isolates cell-free extract using DPPH assay (Fig. 3a). MS002 and MS007 intact cell samples showed significantly ($p < 0.05$) lowest antioxidative % inhibition (Fig. 3b), i.e., 14% and 15%, respectively, for DPPH assay, whereas MS003 shows highest (34%) among all. For ABTS assay, % scavenging activity was found to be in the range 19.65 ± 0.34 to 21.53 ± 0.27% (Fig. 3c) and 18.52 ± 1.66 to 26.05 ± 1.44% (Fig. 3d) for cell-free extract and intact cell sample, respectively. The cell-free extract and intact cell suspension of isolate MS006 showed higher ABTS radical scavenging capacity of 26% and 21%, respectively. A study by Shokryazdan et al. (2017) showed similar result trends for ABTS antioxidative potential; intact cell samples of *L. acidipiscis* ITA44 and *L. pentosus* ITA23 were recorded for much higher antioxidant activity than cell-free extracts samples. Our results corroborated previous study findings that intact cell samples of 12 LAB strains (showed higher DPPH scavenging activity 6.69 to 37.74%) than cell-free extracts samples (Chen et al. 2014). Still, the percentage of inhibition was quite minimal, which could be attributed to lower concentrations of antioxidant chemicals such as antioxidant enzymes, iron chelators, and others, which will need to be validated using

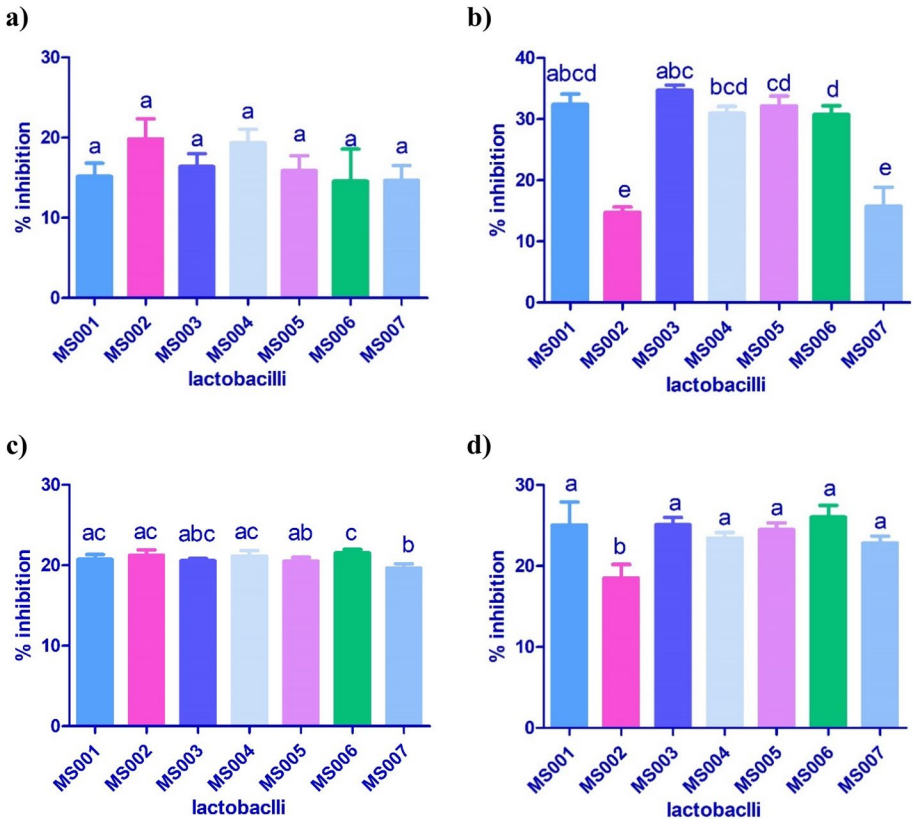


Fig. 3 Antioxidative potential of lactobacilli cultures. **a**. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) cell free extract (CFE). **b**. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Intact cell (IC). **c**. 2,2′-azino-bis 3-ethylbenzo-thiazoline-6-sulphonic acid (ABTS) cell free extract (CFE). **d**. 2,2′-azino-bis 3-ethylbenzo-thiazolin e-6-sulphonic acid Intact cell (IC). Data expressed as mean ± SD, Different lowercase letters (a, b, c…..) above the bars denote statistically significant differences ($P < 0.05$)

sophisticated methods such as chromatography. Overall results suggested that the intact cell sample of LAB isolates recorded considerably high antioxidative potential compared to cell-free extract for both ABTS and DPPH scavenging assays.

Antimicrobial activity

Antimicrobial compounds generally inhibit pathogens' growth by competitive exclusion in the intestinal lumen. Compounds like bacteriocins, surfactants, hydrogen peroxide, organic acids, and bacteriocin-like inhibitory substances are the components produced by lactic acid cultures to hinder the pathogen's growth (Ghanbari et al. 2018; Jung et al. 2019; Silva et al. 2020). The present study used CFSs of isolates to assess the antimicrobial potential of bacterial metabolites. None of the isolates CFSs showed any antimicrobial activity at adjusted pH of 7.0 (data not shown). Similar results for LAB cell-free supernatants (adjusted at 7 pH) were reported by several previous studies (De Keersmaecker et al. 2006; Gunyakti & Asan-Ozusaglam 2019; Layus et al. 2020; Tsai et al. 2005), our results were in agreements with these findings. Previous study results are with the agreements of our outcomes; all the LAB strains except strain C16 lost their antimicrobial potential supernatants were adjusted to pH 7 (Reuben et al. 2020). These findings recommended that inhibition of pathogenic strains at low pH supernatants is mainly due to the production of acidic substances (such as acetic acid, propionic, and lactic acid). Furthermore, more study on different pH needs to be evaluated to confirm isolated cultures' antimicrobial potential.

Scanning electron microscopy micrographs

Two strains (*Limosilactobacillus fermentum* MS005 and *Lactiplantibacillus plantarum* MS007) that were isolated for this study's objectives were observed by scanning electron microscopy technique. Figure 4 represents the scanning electron micrographs (Magnification: 15.00 K X) of *L. fermentum* MS005 (Fig. 4a) and *L. plantarum* MS007 (Fig. 4b).

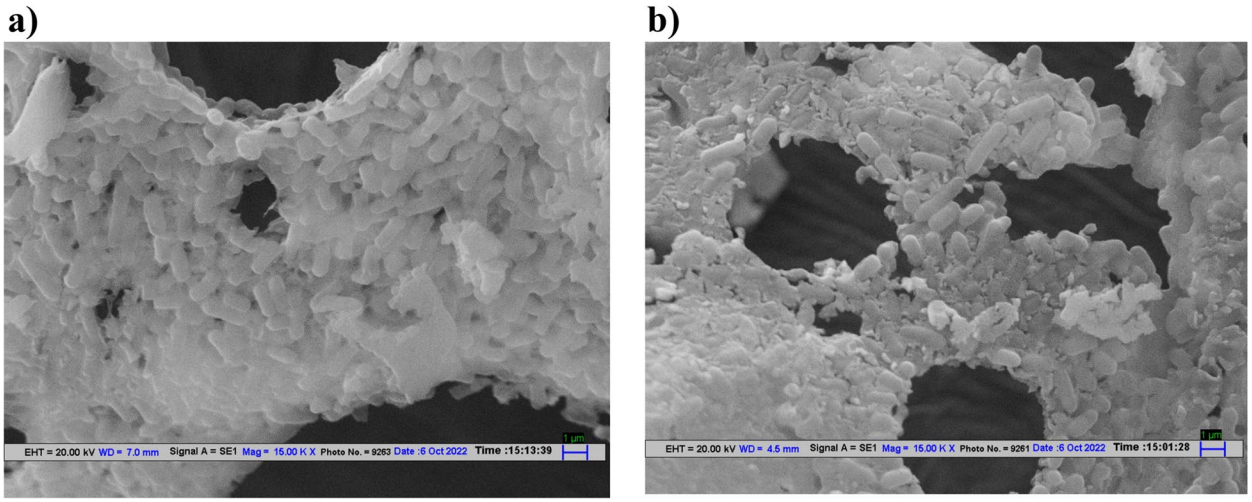


Fig. 4 Scanning electron micrographs (15.00 K X) of lactic cultures. **a** *Limosilactobacillus fermentum* MS005. **b** *Lactiplantibacillus plantarum* MS007

TOPSIS: Multiple attribute decision making (MADM) analysis

Multiple Attribute Decision Making (MADM) analysis, especially the Technique for Order of Preference by Similarity to Ideal Solution (TOPSIS), is a prevailing method for embark upon complex decision-making problems (Jaglan et al. 2023; Tzeng & Huang 2011). Herein, we work on seven alternatives possessing twenty-four attributes. All weights b_j associated to each attribute are considered as one except the phytate degradation, which is taken as 5. The normalized decision matrix, weighted normalize matrix, and closeness coefficients are calculated. Finally, corresponding to seven alternatives MS001 to MS007, we obtain the 4, 7,3,6,1,5,2 ranks, respectively. In a recent study by Jaglan et al. (2023), similar modelling was utilized to identify the potential gluten degrading probiotic candidate based on all screening criteria.

Phytate degradation assay is an important attribute in this study. We varied the weight associated with phytate degradation ability from 1 to 10. From Table 7, we can easily observe that MS007, which is initially at rank one changes to rank 2, whereas MS005 that is at rank 2 initially changes to rank 1 as we increase the weight of phytate degradation in this study. This is also established in the Fig. 5.

Conclusions

The present research was carried out to isolate and characterise the potential cultures from the traditional fermented foods (*Dahi, Raabadi,* and *Lassi*) of Haryana state, India. Seven LAB cultures were selected after being identified as lactobacilli through PCR using a genus-specific primer of *Lactobacillus*. For BSH activity, MS007 LAB isolate was detected for the slight precipitation zone. All the LAB isolates showed no haemolysis (γ-haemolytic activity) and were positive for the phytate degradation test, whereas MS005 recorded for an excellent degradation zone surrounding the colonies. The best isolate was chosen based on its attributes and degree of proximity to the chosen parameters, and this was explained logically by a straightforward mathematical equation generated with TOPSIS. Higher levels of flexibility in TOPSIS

Table 7 Effect of weight of phytate degradation potential on the ranking of the samples

Sample	Weight of phytate degradation test									
	1	2	3	4	5	6	7	8	9	10
MS001	3	4	4	4	4	4	4	4	4	4
MS002	7	7	7	7	7	7	7	7	7	7
MS003	6	3	3	3	3	3	3	3	3	3
MS004	5	6	6	6	6	6	6	6	6	6
MS005	2	2	2	1	1	1	1	1	1	1
MS006	4	5	5	5	5	5	5	5	5	5
MS007	1	1	1	2	2	2	2	2	2	2

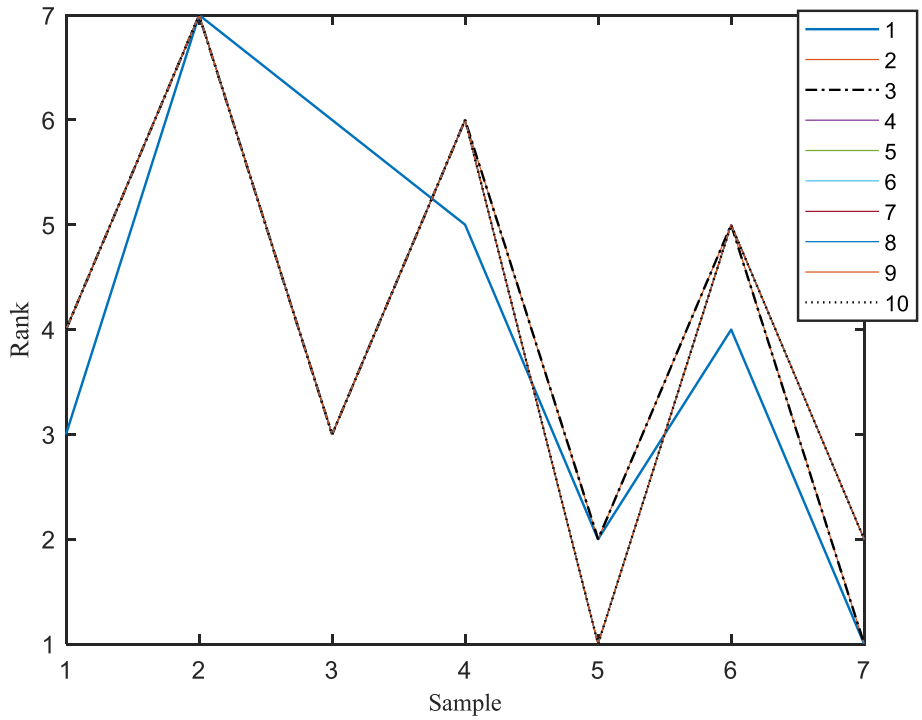


Fig. 5 Rank versus sample for different values of weight of the phytate degradation

application can aid in decision-making for investigations involving several analytic dimensions. Based on their potential probiotic qualities, strains *Limosilactobacillus fermentum* MS005 and *Lactiplantibacillus plantarum* MS007 were identified by the TOPSIS method as promising candidates for further use in the development of foods and other industrial applications. However, further in vivo trials are needed to validate their health-promoting attributes.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43014-024-00259-z.

Supplementary Material 1.

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Author's contributions
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Declarations

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