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CHARACTERIZATION OF PROBIOTIC BACTERIA ISOLATED FROM COMMERCIAL PROBIOTIC SACHET

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ABSTRACT: Background: Probiotics defined as live microorganism with beneficial effects on their host's health. In the present investigation commercial probiotic sachet was used as isolation source. The aim of this study was to evaluate the potential of probiotic bacteria and to assess the probiotic properties of the isolates. **Methods:** Probiotic bacteria were isolated from commercial sachet to assess their biochemical characteristics and probiotic properties like acid, bile, phenol, NaCl tolerance, antimicrobial activity and antibiotic susceptibility pattern with further identification at species level (16S rDNA sequencing). **Results:** The findings revealed that both the isolates showed moderate to high growth at pH 2, pH 3.5 and pH 5. Bile tolerance was highest at 0.05% bile concentration followed by 0.15% and 0.3% after 3 hrs of incubation. The isolates were able to survive at 0.4% phenol concentration. Moreover, they survived in saline concentrations of (1-4 % NaCl), showed minimal growth in 5% and 8% NaCl and exhibited no growth at 6%, 7% and 9 % NaCl concentrations. Both isolates demonstrated inhibitory activity against *Staphylococcus aureus and Escherichia coli.* Isolate1 showed resistance to Augmentin, Amikacin and Cefixime whereas Isolate2 exhibited resistance to Vancomycin, Chloramphenicol, Rifampicin, Clindamycin, Cefpodoxime Augmentin, Amikacin and Cefixime with intermediate resistance or sensitivity to some other antibiotics. **Conclusion:** This study concludes that the isolated strains identified as *Lactobacillus acidophilus* and *Lacticaseibacillus paracasei,* exhibit promising probiotic characteristics.

INTRODUCTION: Probiotic bacteria are live microorganisms that, when consumed in sufficient quantities, provide health benefits to the host [1]. Probiotics such as *Lactobacillus* and *Bifidobacterium* are generally recognized as safe (GRAS) [2] and their ingestion reduces the number of viable pathogens while enhancing the body's immune system [3]. The probiotic microorganism must be tolerant to acidic condition and bile-rich environments of the upper digestive tract [4].

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Prior to their incorporation into food products, probiotic strains should undergo safety evaluations, including antibiotic susceptibility testing [5], as many are naturally resistant to various antibiotics [6, 7]. Probiotics offer numerous health benefits, such as reducing chronic inflammation, preventing infectious diseases, and improving conditions like high cholesterol and lactose intolerance [8].

They are used in both human and animal health, available in forms like capsules, fermented foods, and products containing enzymes and vitamins, primarily featuring *Lactobacillus* and *Bacillus* species [9-11]. The beneficial effects of probiotics consumption orally promote the health of gut flora, enhancing the immune system and have demonstrated therapeutic benefits for acute diarrhea, antibiotic-associated diarrhea, and

traveler's diarrhea [12]. Most of the probiotic microorganisms can be consumed as pharmaceutical preparations which are available in lyophilized forms, such as sachets or capsules, pills with the use of cryoprotectants to maintain bacterial membrane integrity during the lyophilization process [13, 14].

These commercial products may contain one or mixed species of probiotic microorganism mainly from the genera *Lactobacillus* and *Bacillus* [15]. The objective of this study was to isolate bacteria from a commercial probiotic sachet and evaluate their biochemical characteristics and potential probiotic properties, including acid tolerance, bile tolerance, phenol tolerance, NaCl tolerance, antimicrobial activity, and antibiotic susceptibility patterns.

MATERIALS AND METHODOLOGY:

Collection of Probiotic Samples: For this experiment probiotic bacteria was isolated from commercially available probiotic sachet Orodispersible Probiotic Sachets (*Darolac*- Aristo Pharmaceutical Pvt Ltd., India, Batch no-MPH202771, Mfg. Date-Aug, 2020, Exp. Date-Jan, 2022) available in the local retailer medical shop of Guwahati City, Assam, India. This sachet contains *Lactobacillus acidophilus, Lactobacillus rhamnosus*, *Bifidobacterium longum* and *Saccharomyces boulardii.* This probiotic sachet was stored at 4°C before working.

Bacterial Isolation from Sachet: One gram of the sample was aseptically measured and homogenized in 99 ml of sterile saline solution. The mixture was solubilized for approximately 5-6 minutes, followed by tenfold serial dilution up to 10^{-6}. One milliliter aliquot of the diluted sample was plated onto MRS (Man, Rogosa, and Sharpe) agar (pH 6.2) to isolate Lactobacillus species. The plates were incubated anaerobically at 37°C for 24–48 hours in an anaerobic jar. Following incubation, individual colonies were picked and transferred to sterile broth medium. Using the streak plate technique the isolates were further purified.

Identification:

Characterization of the Isolated Colony: All isolates were spreaded on MRS agar and incubated at 37°C for 24 hours. Individual colonies were observed for distinct variations in color, shape, elevation, margin, and opacity to aid in identifying different microbial groups. Morphological characteristics, including shape, arrangement, and Gram staining properties, were analyzed using the Gram staining technique.

Biochemical Analysis: Different biochemical test was conducted such as carbohydrate fermentation, Catalase, Oxidase, Voges Proskauer, Methyl red, Citrate Utilization Test and the results were interpreted according to Bergey's Manual of Determinative Bacteriology [16].

Probiotic Characterization

Acid Tolerance: The isolated strain was subjected to acid tolerance and was assessed by the following method outlined by Hassanzadazar *et al*.[17]. Isolates were cultured overnight in MRS broth at 37 °C; 0.1 ml aliquots were adjusted to pH 2.0, 3.5, and 5.0 using 5 N HCl, incubated for 3 hr at 37 °C and sampled hourly. Viable counts were determined via 10-fold serial dilutions in 0.1% peptone water by pour plate methods; bacterial growth was monitored spectrophotometrically at 600 nm.

Resistance to Bile: The bile salt resistance was assessed by the method outlined by Hoque *et al*.[18]. The isolated strains were cultured overnight in MRS broth, and 1% (v/v) of each culture was inoculated into 10 ml of fresh MRS broth supplemented with bile (sodium taurocholate) at 0.05%, 0.15%, or 0.3% (w/v) concentration and incubated at 37 °C for 3 hours and culture turbidity measured hourly at 620 nm spectrophotometrically; also bacterial viable count were monitored by pour plate method.

Tolerance to Phenol: The phenol tolerance of isolated strains was assessed following the method described by Yadav *et al*.[19]. Sterilized MRS broth was prepared in test tubes and supplemented with 0.4% (v/v) phenol and inoculated with activated overnight cultures and incubated at 37 °C for 24 hours. At 0 and 24-hour intervals, cultures were spread onto MRS agar plates using the serial dilution technique, and cell viability was determined through plate counting.

NaCl Tolerance: The salt tolerance of isolated strains was evaluated using a modified method from Hoque *et al*.[18].

Nine test tubes containing MRS broth were prepared with varying NaCl concentrations (1–9%). Following sterilization, 1% (v/v) fresh overnight culture of the isolated strains was inoculated in each tube and incubated for 24 hours at 37°C. Growth was visually assessed by measuring turbidity after the incubation period. Growth levels were denoted as follows: maximum growth with a triple positive sign (+++), normal growth with a double positive sign (++), minimal growth with a single positive sign (+), and no growth with a negative sign (-).

Antimicrobial Assay: The antimicrobial activity was evaluated using the Agar well diffusion assay method Ridwan *et al.* [20]. Isolates were grown overnight in MRS broth and tested pathogens in Nutrient broth. The pathogen cultures were evenly spread on Mueller-Hinton agar plates with 6 mm wells cut into the agar. Cell-free supernatant (CFS; 0.5 ml), obtained by centrifugation at 8000 rpm for 15 minutes at 4°C, adjusted to pH 7.0 by using 1 M NaOH which was then added to each well. The plates were incubated at 37°C for 24–48 hours, and the zone of inhibition (ZOI) was measured. The tested pathogens included *Staphylococcus aureus* (ATCC 25293) and *Escherichia coli* (ATCC 10536).

Antibiotic Sensitivity: The antibiotic susceptibility was evaluated using the disc diffusion method on Mueller-Hinton agar (MHA) plates, as described by Singh *et al.* [21].

MHA plates were prepared, inoculated with 100 µl of fresh culture and allowed to solidify at room temperature. Antibiotic discs (Hi-media Laboratories Pvt. Ltd. Mumbai) were placed on the plates, followed by incubation at 37°C for 24 hours. The diameter of the inhibition zones was measured using an antibiotic zone scale.

The antibiotics tested included Vancomycin (VA, 30 mcg), Chloramphenicol (C, 30 mcg), Rifampicin (RIF, 5 mcg), Streptomycin (S, 10 mcg), Tetracycline (TE, 30 mcg), Clindamycin (CD, 2 mcg), Cefpodoxime (CPD, 10 mcg), Levofloxacin (LE, 5 mcg), Ceftriaxone (CTR, 30 mcg), Augmentin (AMC, 30 mcg), Amikacin (AK, 30 mcg), and Cefixime (CFM, 5 mcg).

Genotypic Analysis: The isolated bacteria were phylogenetically described using 16S rDNA sequencing. Bacterial genomic DNA isolation was obtained following a standard method using DNA isolation kit. Agarose gel electrophoresis was used to assess the quality of the DNA and the 16S rDNA gene was amplified with primer **(**8F-5'AGAGTTTGATCCTGGCTCAG3', 806R-5' GGACTACHVGGGTWTCTAAT 3') (Turner *et al.,* 1999) [22]. The condition for 16S gene PCR was 95 °C for 10 minutes, 94 °C for 1 minutes, 54 °C for 1 min, 72 °C for 1 min 30 s and 72 °C for 10 minutes. Amplicon obtained was purified and sequenced and the resulting sequence was subjected to nucleotide BLAST at the NCBI database for species-level bacterial identification through phylogenetic analysis based on partial 16S rRNA gene sequences.

Statistical Analysis: The experiments were conducted in triplicate and data were represented as mean ± SD. The analysis of variance (ANOVA) was performed and the results were analyzed using SPSS, Version 20.0

RESULTS AND DISCUSSION:

Pure Culture Isolations: Purest culture were obtained by multiple quadrants streaking on MRS Agar (Himedia M369) followed by morphological analysis. **Table 1** represents the morphological analysis of pure isolates.

TABLE 1: MORPHOLOGICAL ANALYSIS OF THE PURE ISOLATES

Colony no.	Color	Shape	Elevation	Margin	Opacity
Isolate 1	White	Round	Flat	Undulate	Opaque
Isolate 2	Pale white	Round	Flat	Regular	Opaque

Gram Staining: The isolates were subjected to gram staining and the results were observed to be gram positive and rod in shape.

Biochemical Test for Bacterial Confirmation: The isolates were conducted for biochemical characterization which was found to be negative for catalase, Oxidase, Voges Proskauer, Methyl red, Citrate Utilization Test. Biochemically both the isolates were homogenous and produced only acid and observed no gas production.

TABLE 2: BIOCHEMICAL TEST FOR BACTERIAL CONFIRMATION

Biochemical Test	Isolate 1	Isolate 2
Catalase test	Negative	Negative
Oxidase test	Negative	Negative
Indole test	Negative	Negative
Voges Proskauer Test	Negative	Negative
Methyl red test	Negative	Negative
Citrate utilization Test	Negative	Negative
Carbohydrate test		
Glucose	Positive	Positive
Sucrose	Positive	Positive
Fructose	Positive	Positive
Lactose	Positive	Positive
Dextrose	Positive	Positive
Gas production	No gas production	No gas production

Assessing Probiotic Properties Test:

Acidic pH Tolerance Test: In the present study the isolates were subjected to pH 2, 3.5, and 5 for 3 hrs, and the observed results were represented with viable counts (log CFU/ml) in **Fig. 1,** the results showed that there are significant differences in the viable counts between the pH concentrations as both isolates gave the highest number at the pH 5 concentration followed by pH 3.5 and 2 respectively. The reason is due to low pH environment which inhibit probiotic metabolism, decreasing growth and viability. Similarly, studies by Prasad *et al.* [23] and Chan *et al.* [24] established pH 2 and 3 as thresholds for acid resistance after 3 hr of incubation, simulating bacterial survival in the stomach. Similarly, Liong and Shah [25] identified pH 3 as the acid tolerance threshold for probiotic cultures. In this study, **Fig. 1** shows reduced viable bacterial counts at pH 2, with growth declining as exposure time increased, while growth remained stable at pH 5. Probiotic strains exhibited varied survival across a pH range of 1.5 to 6.5, with *L. acidophilus* strains B and V-74 demonstrating greater tolerance to acidic conditions [26]. Additionally, Hoque *et al.* [18] observed Lactobacillus growth across a pH range of 2.5 to 8.5, confirming the strains' ability to survive in both acidic and alkaline environments.

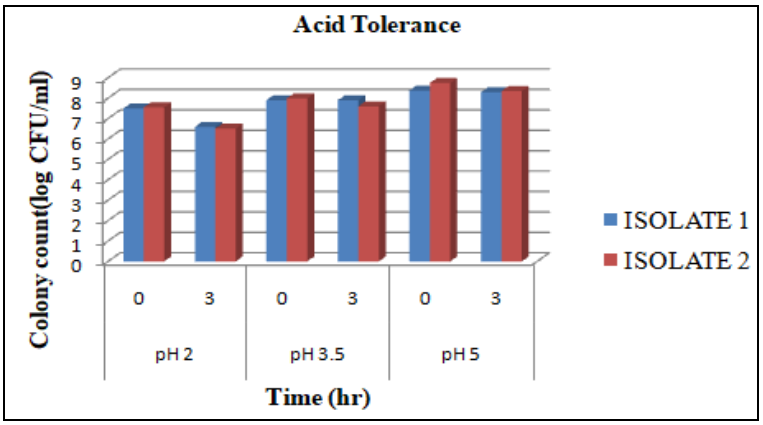

FIG. 1: VIABLE COUNT (LOG CFU/ML) AT DIFFERENT PH (pH 2, pH 3.5 AND pH 5)

Bile Tolerance: In the present study, bile concentrations of 0.05 %, 0.15 %, and 0.3 % were tested, reflecting levels found in the human intestinal tract, with 0.3% being the maximum concentration in healthy individuals [27]. Gilliland *et al.* [28] noted that probiotic organisms intended for human consumption should withstand at 0.3 % bile concentration. In the present study, cell viability counts were depicted in the graph in **Fig. 2** and observed that robust bacterial growth in all bile salt concentration at 0 hr and moderate growth observed after 3 hrs incubation period. Both the isolates showed survivability at lower concentrations (0.05 %, 0.15 %). However at 0.3 % bile salt concentration the isolates showed moderate growth the decrease in bacterial growth after bile salts exposure is due to the changes in cellular homeostasis, causing the dissociation of

lipid bilayers and integral proteins of the cell membranes, resulting in bacterial content leakage and eventually the death of cells [29]. Similar study observed by Noor Nawaz *et al.* [30] who reported good survivability after 3 hrs incubation of bile salt tolerance at different concentration (0.3 %, 0.4 %, 0.75 % and 1.0 %).

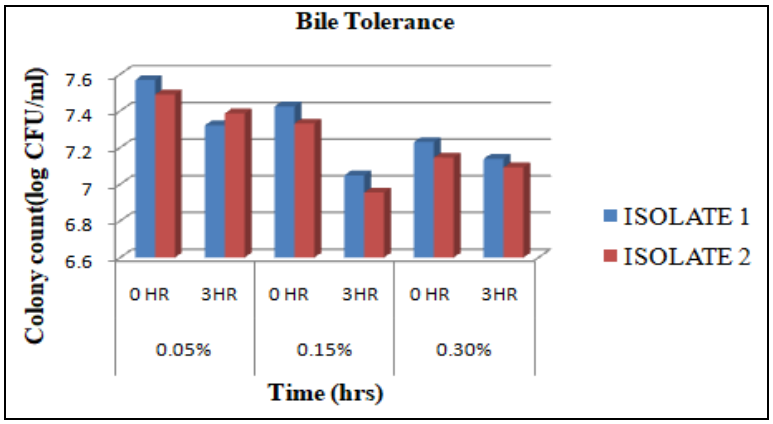

FIG. 2: VIABLE COUNT (LOG CFU/ML) AT 0.05 %, 0.15 % AND 0.30 % BILE SALT

Phenol Tolerance: In this study, both the isolates were exposed to a 0.4% phenol concentration, and their cell viability is shown in **Fig. 3.** The results indicated that both the isolates successfully survived at 0.4 % phenol concentration and were significantly ($p < 0.05$) decreased during 24 hrs incubation. Similarly, Hoque *et al.*[18] reported that most Lactobacillus strains could withstand up to 0.3% phenol concentration. Another study by Yadav *et al.* (2016) [19] reported that *L. plantarum* RYPR1 and RYPR were able to withstand 0.4 % phenol after 24 hrs incubation period.

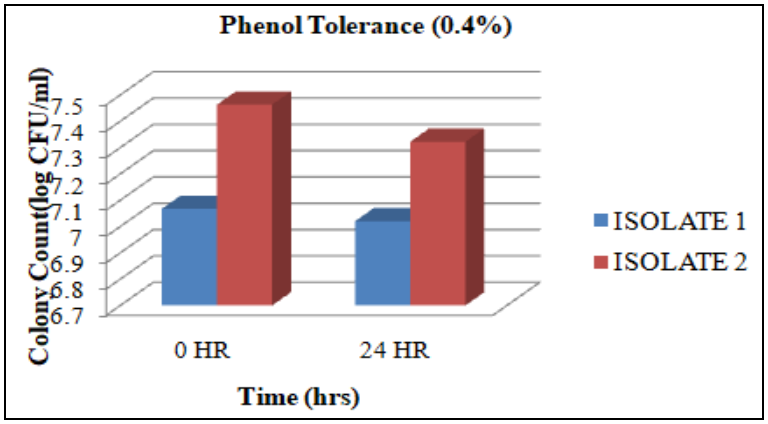

FIG. 3: VIABLE COUNT (LOG CFU/ML) AT 0.4% PHENOL

NaCl Tolerance of the Bacterial Isolates: NaCl acts as an inhibitory compound that reduces the survival of certain bacteria [18]. In this study, bacterial isolates were evaluated for NaCl tolerance across concentrations of 1–9 %. Results showed robust growth at 1%, 2%, and 3% NaCl, moderate growth at 4%, and reduced growth at 5%. No growth was observed at 6%, 7%, or 9% NaCl, as detailed in Table 4. From this study it has been observed that the growth decreased with the increasing NaCl concentration in the medium and revealed that the isolated bacteria can withstand osmotic challenges at 1-4% NaCl concentration and remains active in the gut, enhancing their potential as probiotics. These findings align with Mannan *et al.* [31], who reported that isolates from yogurt and cheese grow well at 2% and 4% NaCl but exhibited minimal or no growth at 8%. Another study by Aktas and Cetin [32] (2022) investigated pickle derived 5 lactic acid bacteria and reported that LAB growth was good at 4% NaCl concentration than 6.5% NaCl and also observed no growth at 10% NaCl concentration. Similarly, Forhad *et al.* [33] found that high NaCl concentrations suppressed the growth of Lactobacillus species. In the study conducted by Karasu *et al.* [34] (2010) 12 pickles-

derived *L.plantarum* isolates can withstand 8 % NaCl content which is in conformity with the present study. This happens due to high saline concentration which causes the bacterial cell to lose its bloating pressure, affecting their physiological, enzymatic, water activity and metabolic functioning of the cell [35].

TABLE 3: NACL TOLERANCE OF THE BACTERIAL ISOLATES AT (1-9 % NACL)

Colony no.	Isolate 1	Isolate 2
Control	+++	+++
1%	+++	+++
2%	++	++
3%	++	++
4%	++	++
5%	+	+
6%	-	-
7%	-	-
8%	++	++
9%	-	-

+ less growth/ ++moderate growth/ +++ high growth/- no growth

Antimicrobial Activity: The antimicrobial effects of the cell-free supernatant (CFS) from the isolates were evaluated against *E. coli* and *S. aureus* using the agar well diffusion assay. **Table 5** shows the zone of inhibition (ZOI) in millimeters after 24 hrs of incubation. In this study, Isolate 1 exhibited a ZOI of 16.33±1.24 mm against *E. coli* and 14.00±0.81 mm against *S. aureus*, while Isolate 2 showed a ZOI of 14.33±1.24 mm against *E. coli* and 16.66±0.94 mm against *S. aureus*. In the present study both the isolates observed no significant differences ($p>0.05$) against the test pathogen. Mishra and Lambert [36] suggest that probiotic bacteria enhance resistance to intestinal infections through antimicrobial mechanisms such as bacteriocin production, competitive colonization, and the synthesis of organic acids like lactic and acetic acid, as well as hydrogen peroxide. Ozbas and Aytac [37] reported that *Lactobacillus acidophilus* exerts an antagonistic effect on pathogens including *Staphylococcus aureus, Salmonella typhimurium, Yersinia enterocolitica, and Clostridium perfringens*. In contrast, Shokryazdan *et al*. [38] found that *L. acidophilus* HM1 displayed moderate inhibition against *E. coli* (11 mm) and low inhibition against *S. aureus* (6.7 mm). Shahverdi *et al*. [39] documented that *Lactobacillus paracasei* exhibits antibacterial activity against various pathogens, with the highest inhibition against *S. aureus* (33.0 mm), followed by *E. coli* (29.0 mm), K. pneumoniae (8.3 mm), and *L. monocytogenes* (5.3 mm).

TABLE 4: INHIBITORY EFFECTS AGAINST PATHOGENIC MICROORGANISM

Isolates	*E. coli* (ATCC 10536)	*S. aureus* (ATCC 25293)
Isolate1	16.33±1.24	14.00±0.81
Isolate 2	14.33±1.24	16.66±0.94

Values are expressed as Mean ± SD (n=3)

Antibiotic Susceptibility Test: The antibiotic susceptibility test evaluated 12 antibiotics with distinct mechanisms of action, including inhibition of cell wall, nucleic acid, and protein synthesis. Results, observed after 24 hrs of incubation, are presented in **Table 6.** Isolate 1 exhibited the highest sensitivity to LE (38 mm), followed by TE (32 mm), C and CD (30 mm), CTR (27 mm), and VA (24 mm), with intermediate sensitivity to RIF (20 mm), S (14 mm), and CPD (12 mm). Conversely, Isolate 2 showed maximum sensitivity to LE (33 mm) and intermediate sensitivity to S (20 mm), CPD (18 mm), TE (14 mm), and CTR (12 mm). Isolate 1 was resistant to AMC, AK, and CFM, while Isolate 2 displayed resistance to VA, C, AMC, RIF, CD, AK, and CFM. According to FAO/WHO[1] assessing antibiotic resistance in probiotic isolates is recommended, as they may contribute to antibiotic therapy efficacy. In the present study, Isolate 1 exhibits vancomycin susceptibility which may be due to its peptidoglycan precursor which terminates in D-alanyl-D-alanine (D-Ala-D-Ala) and vancomycin exerts its antibacterial effect by binding with high affinity to D-Ala-D-Ala terminus, thereby inhibiting cell wall synthesis in gram positive bacteria [40]. This in contrast with other Lactobacillus species like *L. plantarum*, *L. rhamnosus, L. casei* and *L. fermentum* which are intrinsically resistant because their peptidoglycan precursors terminate in D-alanyl-D-lactate(D-Ala-D-Lac) that have low affinity for vancomycin, preventing effective binding [41]. Also another study reveals that *Lactobacillus lactis* exclusively produces D-Ala-ended peptidoglycan precursors through D-Ala-D-Ala ligase (Ddl_{Lc}) action, resulting in natural sensitivity to high level of vancomycin [42]. Anal *et al*. [43] found that *L. acidophilus, L. rhamnosus, and B. bifidum* from pharmaceutical probiotic sachets exhibited strong antibiotic resistance. However,

Temmerman *et al.* [44] reported that Lactobacillus strains from commercial products were sensitive to penicillin G, chloramphenicol, ampicillin, sulphatriad, streptomycin, and tetracycline. In this study, Isolate 2's resistance to vancomycin aligns with findings by Klein *et al.* [45]. Ocana *et al.* [46] noted that tested lactobacilli could grow at low vancomycin concentrations (1 µg/ml). Similarly, James *et al.* [47] reported resistance to cell wall-inhibiting antibiotics (ampicillin, cefalexin, and cefixime) in Lactobacillus isolates from carrot, idli batter and curd. Shahverdi *et al.* [39] also found *Lactobacillus paracasei* resistant to vancomycin, consistent with the present study. Previous studies reported that *L. acidophilus, L. helveticus, L.bulgaricus, L.lactis* and *Bifidobacterium* proved to be susceptible to vancomycin [48, 49].

TABLE 5: DISTRIBUTION OF INHIBITION ZONE DIAMETER (MM) USING DISK DIFFUSION METHOD

Antibiotic Dosage (mcg)	Isolate 1	Isolate 2
Tetracycline	32.00±0.814*	15.00±0.816*
Ceftriaxone	27.00±0.816*	13.00±0.816*
Levofloxacin	37.00±0.816	32.00±0.816
Vancomycin	23.00±0.816	R
Streptomycin	15.00±0.816*	21.00±0.816*
Chloramphenicol	30.33±0.471	R
Augmentin	R	R
Rifampicin	21.00±0.816	R
Clindamycin	31.00±0.816	R
Cefpodoxime	12.33±0.471*	18.00±0.816*
Amikacin	R	R
Cefixime	R	R

Zone of clearance Key- R/Resistant (≤ 11 mm); I/Intermediate (12-20 mm); S/Sensitive (≥ 21 mm). The data is represented as Mean ± SD (n=3), statistical difference is indicated as * ($p<0.05$).

Genotypic Analysis: In the present study phylogenetic tree constructed based on neighbor-joining analysis of 16S rRNA gene revealed that *Lactobacilli* strains were placed in the evolutionary clade of corresponding *Lactobacillus* sequences from the GenBank database. The two isolates were identified as *Lactobacillus acidophilus* (Isolate1) and *Lacticaseibacillus paracasei* (Isolate 2).

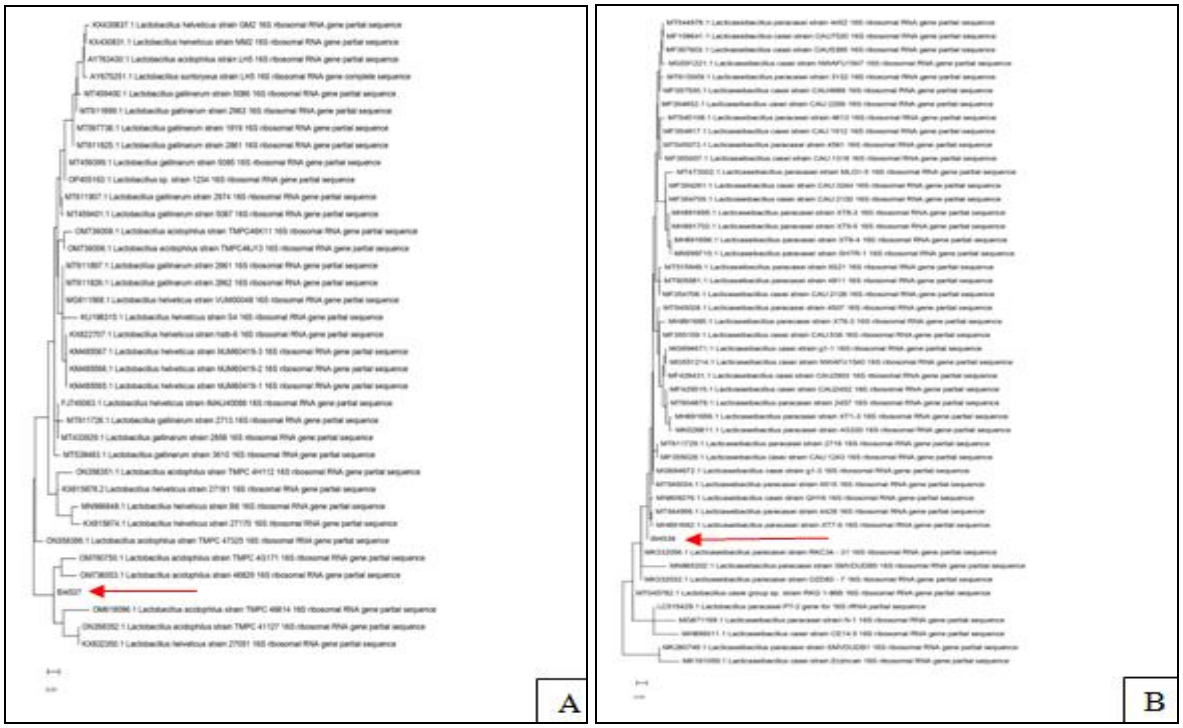

FIG. 4: NEIGHBOR-JOINING PHYLOGENETIC TREE OF *LACTOBACILLUS ACIDOPHILUS* (A), *LACTICASEIBACILLUS PARACASEI* (B) USING MEGA 11.0 SOFTWARE. THE RED ARROW INDICATES THE SAMPLE CORRESPONDS WITH THE *LACTOBACILLUS* NEIGHBORING SPECIES

CONCLUSION: The conclusion of this investigation reveals that multiple Lactobacillus species were present in the commercially available pharmaceutical probiotic sachet. The isolated strains were *Lactobacillus acidophilus* and *Lacticaseibacillus paracasei* exhibited strong probiotic potential satisfying key selection criteria, such as tolerance to acidic pH, bile salts, NaCl, and phenol, along with the production of extracellular antibacterial substances that inhibit pathogenic organisms while demonstrating resistance to several test antibiotics. Thus, the study suggests that *Lactobacillus acidophilus* and *Lacticaseibacillus paracasei* are promising candidates for use as safe, health-promoting probiotics and can be further incorporated in the development of functional food products in future application.

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CONFLICT OF INTEREST: The authors have no conflict of interest.

REFERENCES:

1. FAO/WHO: Guidelines for the evaluation of probiotics in food. Report of a Joint FAO/WHO Working Group on Drafting Guidelines for Evaluation of Probiotics in Food, London, Ontario, Canada, 2002.
2. Kleerebezem M and Vaughan EE: Probiotic and gut lactobacilli and bifidobacteria: Molecular approaches to study diversity and activity. Annual Rev Microbiol 2009; 63: 269–290.
3. Bertazzoni-Minelli E and Benini A: Relationship between number of bacteria and their probiotic effects. Microbiol Ecol Health Dis 2008; 20(4): 180-183.
4. Tuomola E, Crittenden R, Playne M and Isolauri E: Quality assurance criteria for probiotic bacteria. Am J Clin Nutr 2001; 73: 393–398.
5. Parvez S, Malik KM, Kang SA and Kim HY: Probiotics and their role in gut health. JAM 2006; 100: 1171–1185.
6. Argyri AA, Zoumpopoulou G, Karatzas KA, Tsakalidou E, Nychas GJ, Panagou EZ and Tassou CC: Selection of potential probiotic lactic acid bacteria from fermented foods. Food Microbiol 2013; 33(2): 282–291.
7. Saarela M, Mogensen G, Fonden R, Matto J and Mattila-Sandholm T: Probiotic bacteria: Safety, functional and technological properties. J Biotechnol 2000; 84: 197–215.
8. Marco ML, Pavan S and Kleerebezem M: Towards understanding the molecular basis for probiotic functionality. Curr Opin Biotechnol 2006, 17(2): 204–210.
9. McFarland LV and Elmer GW: Properties of probiotic bacteria in anaerobic conditions. Anaerobe 1997; 3: 73–78.
10. Parvez S, Malik KA, Kang SA and Kim HY: Probiotics and their fermented food products are beneficial for health. J Appl Microbiol 2005; 100: 1171–1185.
11. Hong HA, Huang JM, Khaneja R, Hiep LV, Urdaci MC and Cutting SM: The safety of Bacillus subtilis and Bacillus indicus as food probiotics. J Appl Microbiol 2008; 105: 510–520.
12. Anal AKD, Singh SN, Agarwal S, Kumar R and Lawrence R: Probiotic potential of lactic acid bacteria isolated from fermented dairy products. Int J Life Sci Res 2015; 1(2): 94–99.
13. Forssten DS, Sindelar CW and Ouwehand AC: Probiotics from an industrial perspective. Anaerobe 2011; 17: 410–413.
14. Nader-Macías MEF, Otero MC, Espeche MC and Maldonado NC: Advances in the design of probiotic products for the prevention of major diseases in dairy cattle. J Indus Microbiol Biotechnol 2008; 35(11): 1387-1395.
15. Nagpal R, Kumar A, Kumar M, Behare PV, Jain S and Yadav H: Probiotics, their health benefits and applications for developing healthier foods: a review. FEMS Microbiol Lett 2012; 334(1): 1-15.
16. Holt JG, Krieg NR, Sneath PHA: Bergey's Manual of Determinative Bacteriology. The Williams and Wilkins Co.; Baltimore, MD, USA, 1994.
17. Hassanzadazar H, Eshani A, Mardani K and Hesari J: Investigation of antibacterial, acid and bile tolerance properties of lactobacilli isolated from Koozeh cheese. Vet Res Forum 2012; 3(3): 181-185.
18. Hoque MZ, Akter F, Hossain KM, Rahman MSM, Billah MM and Islam KMD: Isolation, identification and analysis of probiotic properties of Lactobacillus spp. from selective regional yoghurts. World J Dairy Food Sci 2010; 5: 39–46.
19. Yadav R, Puniya AK and Shukla P: Probiotic properties of *Lactobacillus plantarum* RYPR1 from an indigenous fermented beverage Raabadi. Front Microbiol 2016; 7: 1683.
20. Ridwan BU, Besselink M, Brouwer EC, Gooszen H, Koning CJM, Timmerman HM, Verhoef J and Akkermans LMA: Antimicrobial activity of a multispecies probiotic (Ecologic 641) against pathogens isolated from infected pancreatic necrosis. Lett Appl Microbiol 2008; 46(1): 61-67.
21. Singh TP, Kaur G, Malik RK, Schillinger U, Guigas C and Kapila S: Characterization of intestinal *Lactobacillus reuteri* strains as potential probiotics. Probiotics Antimicrob Proteins 2012; 4: 47–58.
22. Turner S, Pryer KM, Miao VP and palmer JD: Investigating deep phylogenetic relationship among cyanobacteria and plastids by small subunit rRNA sequence analysis. J Eukaryot Microbiol 1999; 46:327-338.
23. Prasad J, Gill H and Smart J: Selection and characterisation of Lactobacillus and Bifidobacterium strains for use as probiotics. Int Dairy J 1998; 8: 993–1002.

24. Chan HK, Sahadev RPK and Leong SF: Antioxidant activity of fermented milk containing probiotic bacteria. Int Food Res J 2011; 18(4): 1515–1522.
25. Liong MT and Shah NP: Optimization of cholesterol removal by probiotics in the presence of prebiotics. J Dairy Sci 2005; 88: 55–66.
26. Soliman AHS, Sharoba AM, Bahlol HEM, Soliman AS and Radi OMM: Evaluation of *Lactobacillus acidophilus, Lactobacillus casei and Lactobacillus plantarum* for probiotic characteristics, Middle East. J Appl Sci 2015; 5(1): 10-18.
27. Graciela FV and María PT: Probiotic properties of lactic acid bacteria. In: *Food Microbiology Protocols*, Spencer, J.F.T., Spencer, A.L.R. Humana Press Inc., Totowa, NJ, Second Edition 2001:173–181.
28. Gilliland SE, Staey TE and Bush LJ: Importance of bile tolerance of Lactobacillus acidophilus used as a dietary adjunct. J Dairy Sci 1998; 67: 30–45.
29. Mandal S, Puniya AK and Singh K: Effect of alginate concentration on survival of encapsulated *Lactobacillus casei* NCDC-298. Int Dairy J 2006; 16:1190-1195.
30. Noor Nawaz AS, Jagadeesh KS and Krishnaraj PU: Isolation and screening of Lactic acid bacteria for acidic pH and bile tolerance. Int J Curr Microbiol App Sci 2017; 6(7): 3975-3980.
31. Mannan SJ, Rezwan R, Rahman MS and Begum K: Probiotic properties of lactic acid bacteria isolated from traditional fermented milk. Bangladesh Pharm J 2017; 20(1): 27–33.
32. Aktas H and Cetin B: Investigation of starter culture properties and antifungal activities of pickle-derived lactic acid bacteria. GIDA The J Food 2022; 47(3):387-398.
33. Forhad MH, Rahman SMK, Rahman MS, Saikot FK and Biswas KC: Probiotic properties of Lactobacillus spp. isolated from fermented dairy products. Arch Clin Microbiol 2015; 7(1): 5.
34. Karasu N, Simsek O and Con AH: Technological and probiotic characteristics of *Lactobacillus plantarum* strains isolated from traditionally produced fermented vegetables. Annals Microbiol 2010; 60: 227-234.
35. Liu W, Pang H, Zhang H and Cai Y: Biodiversity of lactic acid bacteria. In Lactic Acid Bacteria; Zhang H, Cai Y, Eds. Springer: Dordrecht, The Netherlands 103-203.
36. Mishra P and Lambert B: Probiotic potential of Lactobacillus species in clinical settings. J Clin Sci 1996; 5: 20–24.
37. Ozbas ZY and Aytac SA: Antimicrobial activity of lactic acid bacteria in dairy products. Milk Sci Int 1998;50: 626–629.
38. Shokryazdan P, Sieo CC, Kalavathy R, Liang JB, Alitheen NB, Faseleh Jahromi M and Ho YW: Probiotic potential of Lactobacillus strains with antimicrobial activity against pathogens. Bio Med Res Int 2014; 1–16.
39. Shahverdi S, Barzegari AA, Bakhshayesh RV and Nami Y: In-vitro and in-vivo antibacterial activity of potential probiotic *Lactobacillus paracasei* against *Staphylococcus aureus* and Escherichia coli. Heliyon. 2023; 9: e14641.
40. Song X, Fengjiao L, Mingshu Z, Yongjun X, Lianzhong A and Guangqiang W: Effect of D-Ala-ended Peptidoglycan precursors on the immune regulation of *Lactobacillus plantarum* strains. Front Immunol 2022; 12:825825.
41. Deghorain M, Goffin P, Fontaine L, Mainardi JL, Daniel R, Errington J et al: Selectivity for D-lactate incorporation into the peptidoglycan precursor of Lactobacillus plantarum: Role of Aad, a VanX-Like D-Alanyl-D-Alanine Dipeptidase. J Bacteriol 2007; 189(11):4332-4337.
42. Grangette C, Nutten S, Palumbo E, Morath S and Mercenier A: From the Cover: Enhance anti-inflammatory capacity of a *Lactobacillus plantarum* mutant synthesizing modified techoic acids. Proc Natl Acad Sci 2005; 102 (29): 10321-10326.
43. Anal AKD, Singh SN, Agarwal S, Kumar R and Lawrence R: Probiotic potential of lactic acid bacteria isolated from fermented dairy products. Int J Life Sci Res 2015; 1(2): 94–99.
44. Temmerman R, Pot B, Huys G and Swings J. Identification and antibiotic susceptibility of Lactobacillus isolates from food. Inte J Food Microbiol 2003; 81(1): 1–10.
45. Klein G, Zill E, Schindler R and Louwers J: Molecular identification of probiotic Lactobacillus strains. J Clin Microbiol 1998; 36(6): 1781–1783.
46. Ocana V, Silva C and Nader-Macias ME: Antibiotic susceptibility of potentially probiotic vaginal lactobacilli. Infec Dis Obstet Gynecol 2006; 18182:1–6.
47. James L, Beena AK, Anupa A and Sreeshma N: Probiotic characterization of lactic acid bacteria from fermented foods. J Microbiol Microb Technol 2016; 1(1): 4.
48. Klare I, Konstabel C, Werner G, Huys G, Vankerckhoven V, Kahlmeter G, Hildebrandt B, Muller-Berting S, Witte W and Goossens H: Antimicrobial susceptibilities of Lactobacillus, Pediococcus and Lactococcus human isolates and cultures intended for probiotic or nutritional use. J Antimicrob Chemother 2007; 59: 900-912.
49. Ammor MS, Florez AB, Van Hoek AH, de Los Reyes-Gavilan CG, AArts HJ, Margolles A and Mayo B: Molecular characterization of intrinsic and acquired antibiotic resistance in lactic acid bacteria and bifodobacteria. J Mol Microbiol Biotechnol 2008; 14(1-3): 6-15.

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