European Journal of Nutrition & Food Safety

14(8): 1-14, 2022; Article no.EJNFS.89875
ISSN: 2347-5641

Isolation, Characterization and Screening of Lactic Acid Bacteria from Non-dairy Foods: An Attempt to Unveil their Probiotic Potential

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJNFS/2022/v14i830516

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/89875

Original Research Article

Received 12 May 2022
Accepted 21 July 2022
Published 25 July 2022

ABSTRACT

The growing need for probiotics has emerged due to the imbalances in the gut microbiota. Changes in the microflora of the gut lead to various disorders. Hence, the consumption of probiotics is imperative and rewarding. They provide various benefits when consumed, including antagonistic activities against pathogens by lowering pH to inhibit the growth of other microorganisms, disease treatment, and prevention, as well as health restoration and maintenance. This study aimed to isolate, identify, and characterize various Lactic acid bacteria from non-dairy items to determine their probiotic potential. Five isolates were chosen and identified further using 16S rRNA gene sequencing. The chosen isolates were then tested in vitro for probiotic properties by employing various tests including tolerance to Bile salt, Sodium Chloride, Phenol, and pH. The culture's supernatant of these isolates were also tested for their antibacterial efficacy against various pathogens. Five LAB isolates showed resistance to varying concentrations of Bile acid, NaCl, Phenol, and simulated gastric juice. Gelatinase and Hemolytic activities were absent in the isolates.

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They were resistant to several of the antibiotics examined. They also showed effective antibacterial activity against test pathogens. The isolated strains meet the criteria for being probiotic and safe for human consumption thus conferring various health benefits.

Keywords: Microbiota; probiotics; lactic acid bacteria; 16S rRNA; tolerance; bile; gastric; antibiotics; antibacterial.

ABBREVIATIONS

LAB : Lactic acid bacteria;
PBS : Phosphate buffer saline;
LSB : Lactobacillus Selection base;
MIC : Minimum Inhibitory Concentration;
LB : Luria Bertani;
BLAST : Basic Local Alignment Search Tool;
NCBI : National Center for Biotechnology Information;
GIT : Gastrointestinal tract;
AGE : Agarose gel Electrophoresis;
MEGA : Molecular Evolutionary Genetic Analysis;
CTAB : Cetyl trimethyl ammonium bromide;
PCR : Polymerase Chain Reaction;
MTCC : Microbial Type Culture Collection, and Gene Bank

1. INTRODUCTION

The symbiotic interactions between resident microorganisms and the gastrointestinal tract (GIT) influence gut homeostasis. The GIT microbiota is crucial for host health because it participates in nutritional, immunologic, and physiological activities. Infantile diarrhoea, necrotizing enterocolitis, antibiotic-associated diarrhoea, relapsing *Clostridium difficile* colitis, *Helicobacter pylori* infections, inflammatory bowel disease, and cancer have all been linked to microbial imbalances in the gastrointestinal tract [1]. Changes in nutrition or lifestyle can disturb the microbiome's symbiotic relationship with the gastrointestinal system, leading to illnesses such as inflammatory bowel disease and cancer [2]. GIT imbalance has paved the way for probiotics, which are bacteria that have been demonstrated to benefit human health when ingested as food. Ingestion of probiotics, alters the structure of this microflora in the digestive system, providing a range of benefits to the host [3]. Probiotics are described as live microorganisms that give health advantages to the host when supplied in adequate amounts [4].

Lactic Acid Bacteria (LAB), which are naturally prevalent in the gastrointestinal system, are the most often used probiotics. Lactic acid bacteria are probiotics that play a vital role in the GI tract of the host. *Lactobacilli* commonly accomplish antagonistic activities against pathogens by producing organic acids, which lowers pH and therefore creates an unfavorable environment for the development of other bacteria [5]. It is not a new notion to use them for disease treatment and prevention, as well as health restoration and maintenance [6]. The critical characteristics to influence the immune system make them appealing for health applications such as antibacterial activity, anti-inflammatory, ACE-inhibitory, antioxidant, antidiarrhoeal, antiviral, immunomodulatory, hypocholesterolemic, anti-diabetic and making them preferred starter cultures in the food industry [7]. They are naturally present in raw milk and dairy products such as cheese, yogurts, and fermented milk, with yogurt being one of the most well-known probiotic foods. Consumers have spurred a renaissance of interest in using probiotics instead of antibiotics in recent years.

The study aimed to identify and characterize LAB from dietary sources such as non-dairy fermented foods and determine their probiotic potential by performing several tests such as Anti-microbial activity, Antibiotic susceptibility, Hemolytic activity, Gelatinase liquefaction, and Bile and Acid tolerance.

2. MATERIALS AND METHODS

2.1 Fermentation, Isolation and Maintenance of Bacterial Isolates

The Lactic acid bacteria strains were isolated from non-dairy fermented food products such as White peas, Green peas, Chickpeas, Dragon fruit, and Sweet lime. The food products were crushed and a paste was formed after they were allowed to ferment for 12 hours and were added to sterile Phosphate Buffer Saline (PBS) by performing the required dilutions. Then, 1 mL aliquots of the samples' suitable dilutions were plated onto the Lactobacillus selection medium (HI Media, Mumbai, India) plate. Later, incubated at 37°C under aerobic and anaerobic conditions for 72 hours. Post incubation five prominent

colonies were streaked on Lactobacillus selection media plates for further purification and glycerol stocks of the isolates were stored at 4°C.

2.2 Isolates Characterization

All five isolates were exposed to biochemical examination such as catalase, oxidase, indole, and H_2S production. Gram staining was employed on the isolates to determine the Gram nature and colony characteristics [8].

2.3 Identification using 16S rRNA Sequencing

16S rRNA gene was amplified from extracted DNA using 8F (5′-GGATCCAGACTTTGATYMT GGCTCAG-3') and 907R (5′-CCGTCAATTCMT TTGAGTTT-3') universal primer as described by Jha et al. [9]. The PCR products were analyzed by Agarose Gel electrophoresis (AGE) for purity and amplification and further taken for Sanger sequencing reaction. The phylogenetic tree was built using Molecular Evolutionary Genetic Analysis (MEGA 11) software's Neighbor-joining method. The gene sequences were submitted to GenBank. The isolates accession numbers are listed in Table 1.

2.4 Determination of Probiotic Potential

Tolerance to NaCl: Sodium chloride (NaCl) tolerance was determined by growing LAB isolates in Lactobacillus Selection base (LSB) (Himedia, Mumbai, India) broth adjusted with various NaCl concentrations. For each LAB isolate, the lowest inhibitory concentration of Sodium chloride salt was evaluated using a 96-well microtiter plate assay [9]. Isolates were cultured for 48 hours in LSB broth at 37°C. Separately, a sterilized saturated NaCl salt solution was prepared. 1% culture of each isolate was inoculated in 10 mL of fresh LSB broth and adjusted to concentrations of 0.3%,0.6%,0.8%, and 10%. The growth at an incubation temperature of 37°C for 7 hours was hourly monitored against control (1 % inoculum in LSB broth). The plate count technique was used to determine cell viability. Plating 100µl of cultures onto LSB agar plates, viable cell colonies were counted, and the findings were represented as log CFU/mL.

Tolerance to pH: The pH of the oesophagus is 6.8 to 7.2, while the pH of the stomach is 1.3 due to Hydrochloric acid secretion, and the pH of the small intestine is alkaline at 7 to 8. As a result, the selected isolate must be pH-tolerant to qualify as a probiotic. A 96- well microtiter plate was used to assess pH tolerance [10]. 1% culture of each isolate was introduced into fresh LSB broth adjusted to varied pH using varying concentrations of HCl and NaOH to determine their survival. The plate count methodology was used to assess cell viability. Viable cell colonies were counted by plating 100µL of cultures onto LSB agar plates and the results were reported as log CFU/mL. A 1 % inoculum in LSB broth was used as the control.

Tolerance to Simulated gastric fluid: Effective probiotic bacteria must acclimate themselves to the high acidity of pH 1 to 3. Test for adaptation and resistance to gastric juice was done by mimicking the environment of the stomach. As Li et al suggested, gastric fluid was prepared by dissolving 2.0g sodium chloride, 3.2g pepsin, and 7.0mL HCl in one liter of distilled water, resulting in a pH of 1.2 of the prepared solution [11]. 1% culture of each isolate grown for 48 hours was inoculated in 10 mL of prepared Simulated gastric fluid. The growth at an incubation temperature of 37°C was monitored for 0, 1, 2, 3, and 6 hours against control. To investigate cell survival, the plate count approach was applied. Viable cell colonies were counted by spreading 100µL of cultures onto LSB agar plates.

Table 1. Isolation source and sequence analysis

SR No	Isolate	Scientific Name	Isolation Source	Accession-ID
1	LAB01	*Weissella paramesenteroides*	*Lathyrus sativus* (White Peas)	ON754071
2	LAB02	*Weissella confusa*	*Pisum sativum* (Green Peas)	ON754072
3	LAB03	*Weissella cibaria*	*Cicer arietinum* (Chickpeas)	ON754073
4	LAB04	*Lactiplantibacillus plantarum*	*Hylocereus undatus* (Dragon Fruit)	ON754074
5	LAB05	*Secundilactobacillus mixtipabuli*	*Citrus limetta* (Sweet lime)	ON754075

Tolerance to Bile salts: The intestinal bile concentration is around 0.3% (w/v), and food residing time in the small intestine is to be around 4 hours. Bile salts cause effects such as changing cytoplasmic pH to acidic, protein misfolding, and alterations in lipid packing in microbes [12]. The required isolates must endure these conditions. In this study, the survival of LAB was tested by growing cultures with varied concentrations of Bile salts. Bile salt solution was prepared and sterilized by the 0.4 micron filter. The experiment referred to Shehata et al. [13] with slight changes. A 96-well microtiter plate assay determined the minimum inhibitory concentration of bile salt for each selected isolate. The 1% culture of each isolate grown for 48 hours was added into 10 mL of fresh LSB broth containing 0.6%, 1.2%, 2.5%, 5%, 10%, 20% (w/v) bile salt concentration. To determine viable cells, aliquots of 0.1 mL at varying intervals of 0, 1, 2, 3, 4, 5, and 6 hours were removed and spread onto the LSB agar plate. For control, 1% inoculum in LSB broth was used.

Tolerance to Phenol: Intestinal flora can deaminate aromatic amino acids obtained from dietary proteins, resulting in the formation of phenols [14]. These phenolic compounds inhibit Lactic Acid Bacterial growth. As a result, probiotics' phenol tolerance is critical for their survival in the gastrointestinal tract [15]. The minimal inhibitory concentration of phenol for each isolate was evaluated using a 96-well microtiter plate. Isolates were cultured for 48 hours at 37°C in Lactobacillus selection base (LSB) broth. Utilizing sterile concentrated Phenol solution, 1 % culture of each isolate was added to 10 mL of fresh LSB broth and adjusted to concentrations of 0.1 %, 0.3 %, 0.6 %, 1.2 %, 2.5 %, and 5.0 %, respectively [16]. At 37°C incubation temperature, growth was measured at varied intervals against control by plate count method.

Growth at different temperatures: Lactic acid bacteria isolates were grown in LSB broth and incubated at 37°C for 48 hours. Then 100 µL inoculum from the culture was transferred to LSB agar plates using the spread plate technique and incubated as suggested by Ayo-Omogie et al. [17], growth was observed by the formation of colonies at 25°C, 30°C, 37°C, and 47°C for 48-72 hours.

Gelatinase activity: The strains' capacity to make gelatinase was examined as described by Mureşan [18] with a few modifications. Plates were incubated at 37°C and 42°C for 48 hours and 25°C for 72 hours after being plated on Gelatine containing LSB agar (3 % gelatine). The formation of a clear zone around the gelatinase-producing colonies was recorded.

Hemolytic activity: Another key property to qualify as good probiotic bacteria is that they must not cause lysis of red blood cells [19]. Isolated strains were screened for hemolysis activity on blood agar plates containing 5% (v/v) sheep blood. The isolates were grown at 37°C for 48 hours in LSB medium streaked onto blood agar and incubated at 30°C for 24–48 hours [20]. A clear zone around the colony indicated hemolytic activity. The hemolysis reaction was determined by observation of a clear zone of hemolysis around the colonies (β-hemolysis), a partial hydrolysis and greening zone (α-hemolysis), or no reaction (γ-hemolysis).

Resistance to antibiotics: The antibiotic susceptibility of selected isolates was tested using the disc diffusion method following the modified standard Kirby–Bauer procedure as suggested by Sharma et al. [21]. The plates were prepared by pouring 24 hours old 1% inoculum. The antibiotic discs were placed on the agar surface, and the plates were incubated for 48 hours at 37°C. Antibiotic discs containing Amikacin (30µg), Ciprofloxacin (5µg), Trimethoprim (5µg), Levofloxacin (5µg), Vancomycin (30µg), Gentamicin (10µg), Chloramphenicol (30µg), Ofloxacin (5µg), Tetracycline (30µg), and Erythromycin (15µg) were used to assess antibiotic resistance and susceptibility patterns.

Antimicrobial susceptibility test: The disc diffusion method is frequently used in antibacterial susceptibility tests [22]. Using this method, the inhibitory effects of the LAB strains on the indicator pathogens were investigated [23]. Selected LAB isolates were inoculated in LSB broth for 48-60 hours at 37°C. The broth was recovered by centrifugation for 10 minutes at 4°C and 7500 rpm and subsequently filtered through a 0.22 µm filter membrane [24]. The cell-free supernatant was collected and stored at -80°C. The antimicrobial activity of the liquid was assessed against three Gram-negative *(Escherichia coli, Acinetobacter baumannii, Proteus mirabilis*) and three Gram-positive (*Staphylococcus epidermidis, Streptococcus pyogenes, Staphylococcus aureus*) bacteria, and one drug-resistant strain Methicillin-resistant

Staphylococcus aureus which were obtained from MTCC Chandigarh. On Luria–Bertani agar plates, approximately 100 µL of indicator pathogenic strains adjusted to 0.5 McFarland standard were plated. Filter paper discs (6 mm) were soaked in aliquots of liquid before being placed on agar seeded with bacterial strain [25]. After incubation, antibacterial activity was assessed by measuring the diameter of the inhibitory zone surrounding the discs.

3. RESULTS AND DISCUSSION

3.1 Isolation, Microscopic Evaluation and Biochemical Evaluation of Lactic Acid-Producing Isolates

This study isolated Lactic acid bacteria from non-dairy fermented food products (White peas, Green peas, Chickpeas, Dragon fruit, and Sweet lime). A total of five LAB isolates were obtained from samples. Table 1 and Table 2 represent the sources of isolation and biochemical characterization of the five isolated Lactic acid bacteria. The biochemical traits of bacterial isolates were found to be positive in Gram nature and the presence of bile salt. On the other hand, Lactic acid-producing isolates tested negative for oxidase, catalase, indole, H_2S production, and gelatin liquefaction.

3.2 Molecular Identification of Selected Lactic Acid Bacteria

Genomic DNA was extracted for the designated LAB isolates and 16S rRNA sequencing was conducted. All of the isolated bacteria' 16S rRNA genes were amplified. The amplicons were then purified using a column and sequenced. The Basic Local Alignment Search Tool (BLAST) was used to evaluate the resulting nucleotide sequences to known sequences in the National Center for Biotechnology Information (NCBI) database. These isolates were screened as *Weissella paramesenteroides* (LAB01), *Weissella confusa* (LAB02), *Weissella cibaria (*LAB03) and *Lactiplantibacillus plantarum* (LAB04*)* and *Secundilactobacillus mixtipabuli* (LAB05).

Tolerance to NaCl: Sodium chloride tolerance is crucial for the survival of probiotic organisms in the gastrointestinal tract. The five isolates were also evaluated for their varying levels of sodium chloride tolerance. It was observed that LAB01, LAB02, LAB03, and LAB04 showed tolerance to concentrations of sodium chloride from 0.31% to 10% concentrations as the time of incubation increased. The isolates initially showed a decrease in log CFU/mL for 10% NaCl, however, they developed tolerance further. LAB05 showed a decrease in tolerance for most concentrations of NaCl. Studies showed that *Lactobacillus* strains isolated from traditional Iranian dairy products could tolerate NaCl concentrations up to 5% [26]. Lower concentrations of NaCl (1 to 2.5%) stimulate the growth of Lactic acid bacteria, producing acid which further inhibits the growth of other microorganisms in the GI tract [27].

Tolerance to pH: To ensure the survival and functionality of potential probiotic isolates, tolerance to acidic and alkali environments is a crucial factor to consider. Since they are acidophilic, Lactic acid bacteria can tolerate low Ph [28]. LAB01, LAB02, and LAB03 were tolerant to NaOH from 0.06 to 1N concentrations. LAB04 showed an escalation in tolerance for 0.03 to 0.25N concentrations of NaOH as the incubation time increased. However, it was observed that the isolate was not tolerant to 0.5N and 1N concentrations as indicated by the steady decrease. LAB05 showed tolerance towards all concentrations of NaOH with an increasing incubation period. LAB01, LAB02, and LAB03 were shown to have varying tolerances to all concentrations of HCl. The core metabolic pathways, proton pump, changes in cell density and membrane composition, DNA and protein damage repair, as well as neutralization, are some of the mechanisms that control the acid resistance of LAB [29]. The bacterial isolates were found to be tolerant to acidic and alkali conditions, strengthening their suitability as probiotic organisms.

Tolerance to simulated gastric fluid: Lactic acid bacteria (LAB) are characterized by the production of lactic acid and metabolites such as antioxidants, organic acids, and antibacterial compounds, which modulate and enhance gut microbial homeostasis [30]. The acidic pH of the stomach is thought to suppress LAB survival in a host's gastrointestinal system [31]. The growth of five distinct LAB isolates was influenced primarily by the acidic pH conditions. It was observed that the log CFU/mL of LAB01 initially decreased within 1 hour and then gradually increased over time. When cells are exposed to acidic conditions, they strive to maintain pH homeostasis by discharging H+ from the cell via

H^+-ATPase [32]. Within 4-5 hours, the survival rate of isolates LAB02, LAB03, LAB04, and LAB05 significantly increased. After 6 hours of incubation, the survival rate decreased as the duration of time increased. It has previously been demonstrated that when LAB are incubated under acidic conditions, their H^+-ATPase activity increases, whereas that of non-acid-tolerant organisms declines, resulting in a general reduction or loss of viability [33]. Hence, it is well known that bacteria enter the noxious environment of the upper intestinal system after passing through the stomach, where gastric juices are secreted into the gut [34]. Likewise, potential probiotic strains must be able to withstand acid for at least 90 minutes before offering any therapeutic benefits [35]. As documented, one of the determining factors for the strains' ability to maintain vitality through GIT is their ability to tolerate low stomach pH [36]. As a result, the LAB isolates could tolerate high concentrations of gastric acid, making them promising probiotic candidates.

Table 2. Biochemical characterization of the isolated strains

Biochemical tests	LAB01	LAB02	LAB03	LAB04	LAB05
Gram nature	+	+	+	+	+
Catalase	-	-	-	-	-
Oxidase	-	-	-	-	-
Gelatinase Liquefaction	-	-	-	-	-
Indole	-	-	-	-	-
Bile salts	+	+	+	+	+
H_2S production	-	-	-	-	-

Key (+): Positive (-): Negative

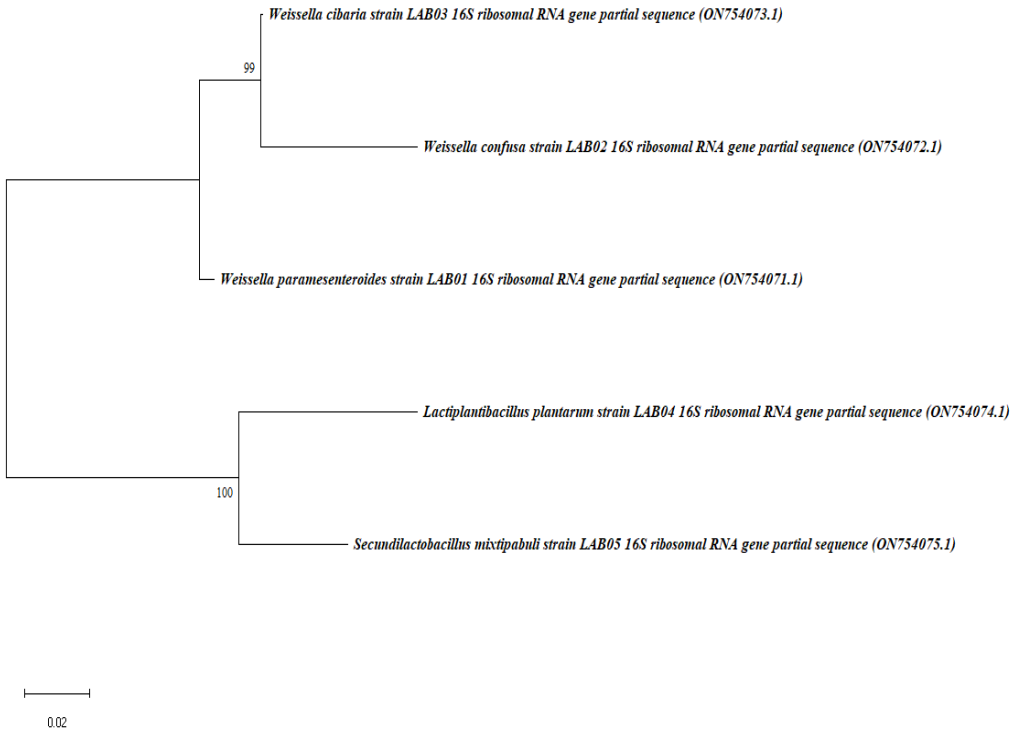

Fig. 1. Phylogenetic tree of isolated LAB strains obtained by Neighbour-joining (NJ) method using MEGA 11 software. The branch node number shows percent bootstrap support. The accession numbers of the organisms are included in parentheses and the bar scale value 0.02 indicate the nucleotide substitutions per site

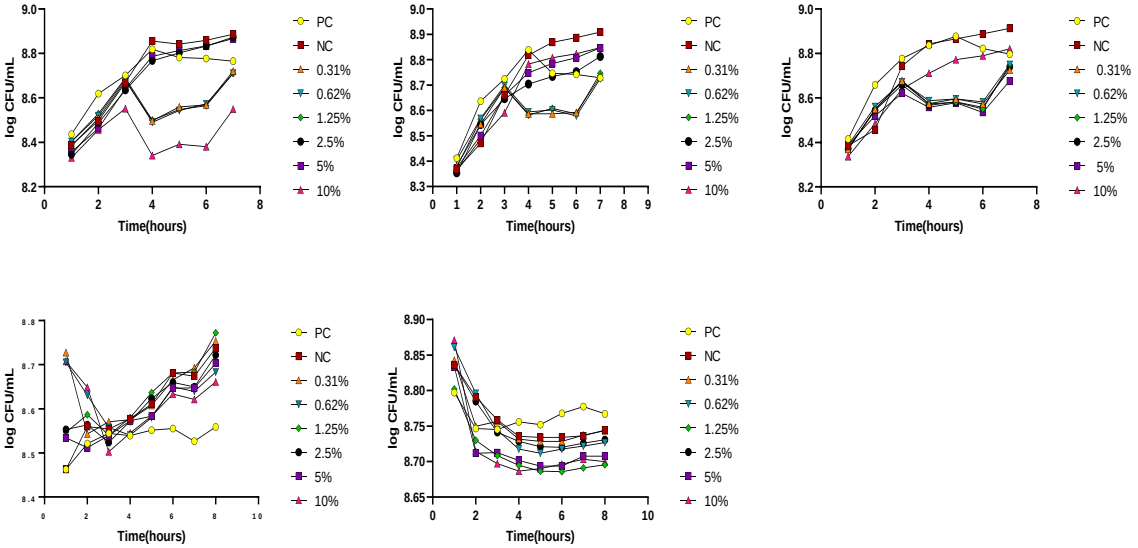

Fig. 2. Probiotic potential determination of LAB isolates after treatment with varying NaCl concentrations ranging from 0.31% to 10%

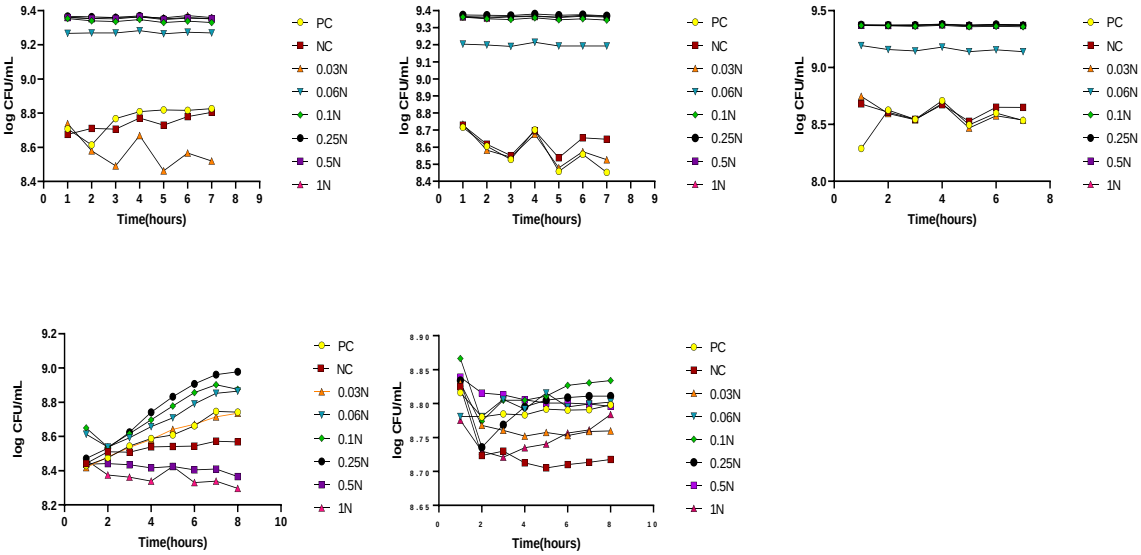

Fig. 3. Probiotic potential determination of all strains after treatment with varying NaOH concentrations ranging from 0.03N to 1N

Tolerance to Bile Salts: Bile tolerance is one of the most crucial attributes of probiotic bacteria since it impacts its potential to function as a probiotic by determining its ability to survive in the small intestine [37]. Five isolates from this investigation were evaluated for their varying levels of Bile salt resistance. It was observed that LAB01, LAB02, LAB03, and LAB04 showed increased tolerance to Bile salt as the concentration of Bile salt increased. LAB05 showed a decrease in tolerance to Bile salt concentration with an increase in Bile salt concentration.LAB01, LAB02, LAB03, and LAB04 showed an increase in CFU/mL for the first few hours for most concentrations. LAB01, LAB02, and LAB03 isolates showed an elevation

in CFU/mL in the later period of incubation. LAB04 showed a gradual rise in CFU/mL as the incubation time increased. A steady stabilization and increment in CFU/mL were observed from the 4th hour of incubation. When bacteria are exposed to Bile salts, cellular homeostasis was disrupted, which led to the separation of the lipid bilayer and integral protein of the cell membrane, triggering bacterial content leakage and ultimately cell death [38]. An efficient bacterial defense against bile toxicity is the active extrusion of the bile acids and salts that build up in the cytoplasm through bile efflux pumps. Such mechanisms have been observed in several *Lactobacilli* and *Bifidobacteria* [39]. Bile salt hydrolases (BSHs) belong to the chologlycine hydrolase family of enzymes [40]. It has been hypothesized that Bile salt hydrolases confer protection by enabling the deconjugation of glycine and taurine from Bile salts such that the corresponding unconjugated acids can be metabolized further by other gut bacteria enabling probiotic organisms to adapt to a bile environment [41]. The selected LAB isolates could therefore resist high concentrations of Bile salts, making them qualified candidates for a probiotic.

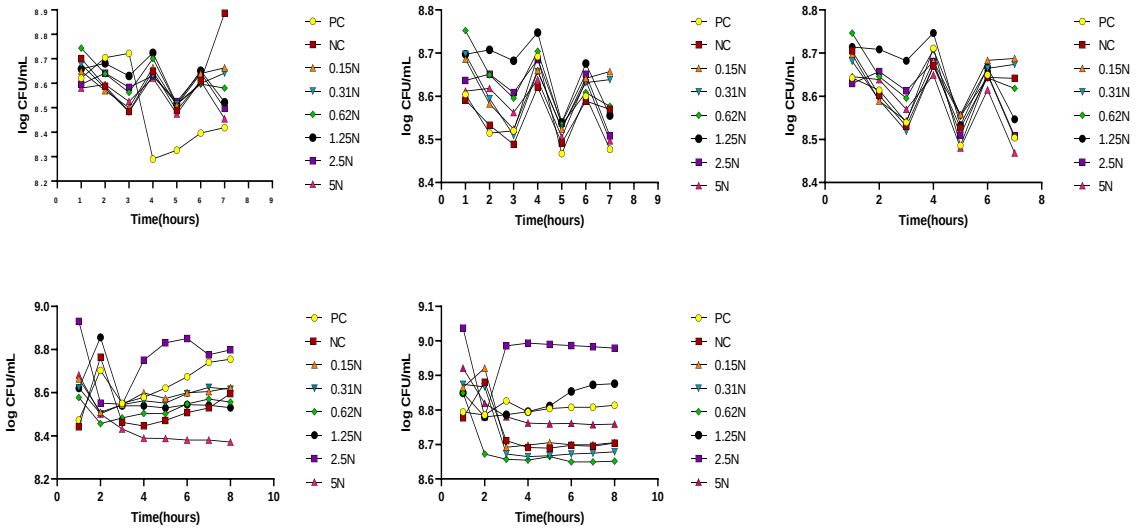

Fig. 4. Probiotic potential determination of all strains after treatment with varying HCl concentrations ranging from 0.15N to 5N

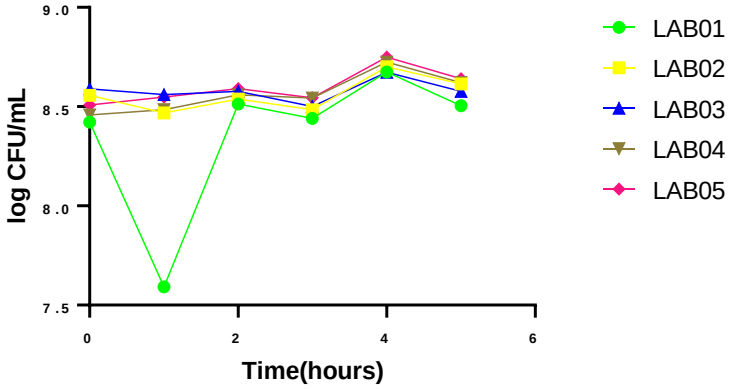

Fig. 5. Probiotic potential determination of all strains after treatment with Simulated gastric fluid

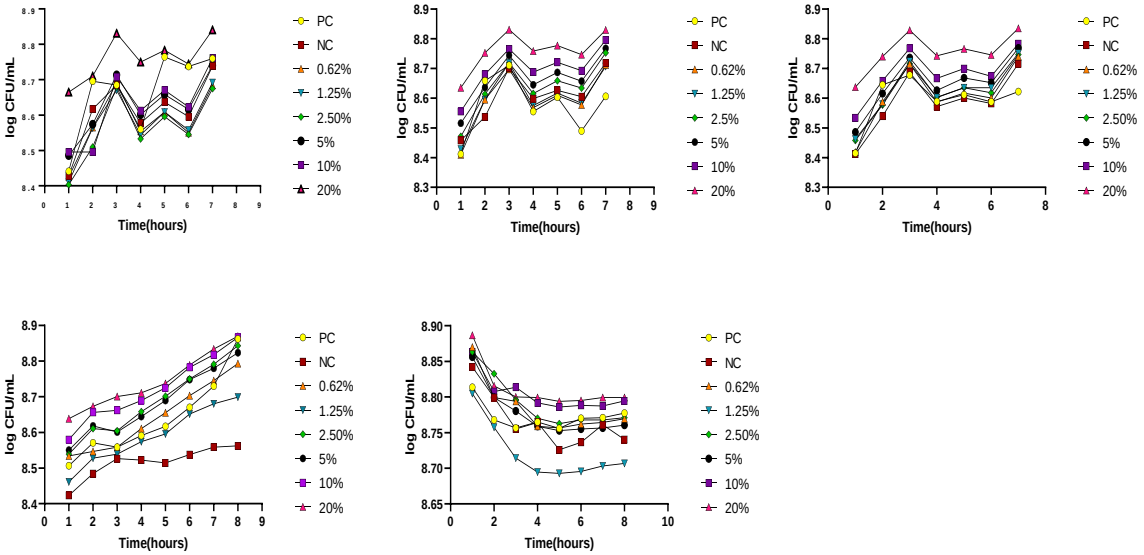

Fig. 6. Probiotic potential determination of LAB isolates after treatment with varying Bile salts concentrations ranging from 0.62% to 20%

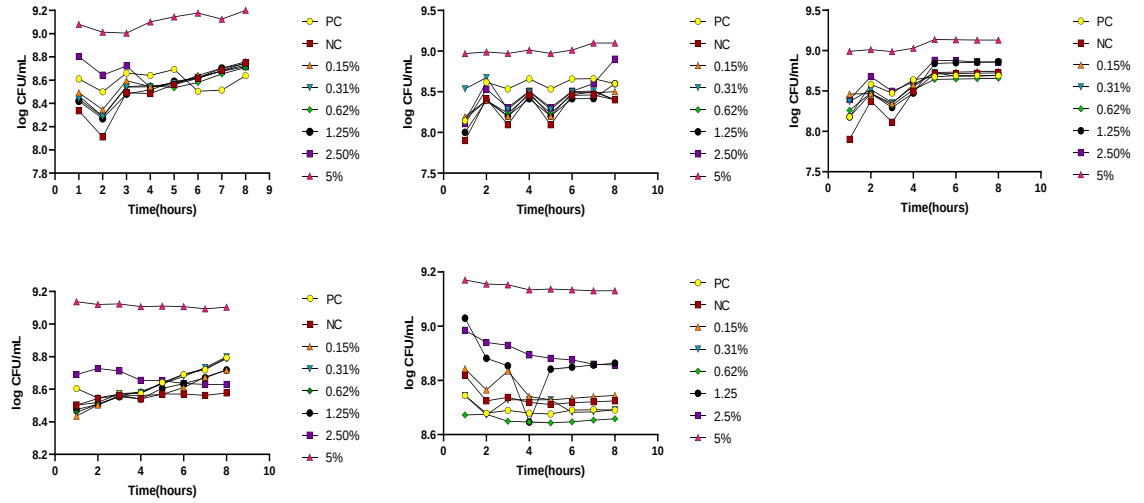

Fig. 7. Probiotic potential determination of all strains after treatment with varying phenol concentrations ranging from 0.15% to 5%

Tolerance to Phenol: Phenol tolerance is a necessary criterion to qualify as a probiotic because it is a dangerous microbial metabolite generated in the GIT because of amino acid deamination [42]. At 5 % phenol, all LAB isolates demonstrated maximum survival. LAB01, LAB02, LAB03, and LAB04 survived at approximately consistent rates at all concentrations over the entire observation period. LAB01 growth was lowest at 2 hours and then growth was observed linearly for almost all concentrations. Before reaching a consistent growth rate, LAB02 and LAB03 displayed varying survival across all concentrations (except 5%). LAB05's survivability varied according to time and concentration. It exhibited results ranging from 8.8 log CFU/mL to 9.0 log CFU/mL at 1.25 % phenol concentration, progressively decreasing with time. However, by the 4th hour, colony-forming units had

plummeted to 8.6 log CFU/mL. There are several instances in LAB of phenol tolerance isolated from naturally fermented food sources [43]. The findings show that the isolates tested in this investigation can survive in human gastrointestinal settings.

3.3 Growth at Different Temperatures, Hemolytic Activity and Gelatinase Liquefaction

Selected LAB isolates were grown at 25°C, 30°C, 37°C, and 47°C to determine viability. All The isolates' growth flourished at all the above-mentioned temperatures. Hemolytic activity is considered a safety factor in selecting probiotic strains [44]. Hemolysis was not observed in all LAB strains when grown on sheep blood agar plates. The paucity of gelatinase in LAB is noted in safety evaluation studies on potential probiotics [45]. The tested LAB isolate in the current study had no gelatinase activity and the outcomes were equivalent to Rajput et al. [46].

3.4 Resistance to Antibiotics

The emergence of advanced antibiotics for use in treating microorganisms that have developed antibiotic resistance is the most challenging endeavour in medical biology. LAB isolates were subjected to ten different antibiotics to determine resistance. According to the extent of the growth inhibition zones, LAB isolates were classified into sensitive and resistant [47]. The Table below demonstrates each LAB strain's antibiotic susceptibility and resistance profile. All isolates were resistant to Amikacin, Ciprofloxacin, Trimethoprim, Levofloxacin, Vancomycin, and Ofloxacin. LAB01, LAB02, and LAB03 strains were more susceptible to Erythromycin and Chloramphenicol whereas Gentamicin and Tetracycline were moderately susceptible to the strains [48]. An increased level of resistance was also found in LAB, mostly in isolates from chicken and fermented dairy products [49]. LAB04 and LAB05 were resistant to almost antibiotics. High susceptibility to chloramphenicol has also been observed in tested LAB strains. The presence of the cat gene is often linked to the genotypic resistance to this antibiotic class, and it has been seen in various LAB strains [50].

3.5 Antimicrobial Susceptibility Test

The most elemental property of probiotic bacteria points to their potential to eliminate any likely pathogens that can enter the body on ingestion or environmental contact. The antimicrobial properties of probiotics play an important role in defense against pathogens Six test pathogens namely *Acinetobacter aumannii, Escherichia coli,* Methicillin-Resistant *Staphylococcus aureus, Proteus mirabilis, Streptococcus pyogenes, and Staphylococcus epidermidis* were used to evaluate the antibacterial potential. Amongst the various test organisms used, antimicrobial activity against *Streptococcus pyogenes* and *Proteus mirabilis* by LAB02 and LAB01 respectively was found to be maximum owing to a zone of clearance of 12mm in both. LAB03 exhibited significant zones against all test organisms except Methicillin-Resistant *Staphylococcus aureus* LAB05 showed a zone of 10mm against *Acinetobacter baumannii* and MRSA. LAB manifests antimicrobial activity by producing inhibitory substances like organic acids, bacteriocins, H_2O_2, and free fatty acids. *Weissella cibaria* exhibits a broad spectrum of antibiotic activity against *Escherichia coli*, as per a prior study conducted by Yu et al. [51]. According to previous studies, *Lactobacillus* spp exhibits resistance to *Escherichia coli* and *Proteus mirabilis* [52,53].

Table 3. Exhibiting the gelatinase & Haemolytic activity of isolated LAB strains at different temperatures

Isolates	Temperature				Gelatinase activity	Hemolytic activity
	25°C	30°C	37°C	47°C		
LAB01	+	+	+	+	-	-
LAB02	+	+	+	+	-	-
LAB03	+	+	+	+	-	-
LAB04	+	+	+	+	-	-
LAB05	+	+	+	+	-	-

Key (+): Positive (-): Negative

Table 4. Antibiotic susceptibility and resistance profile of LAB isolates

Antibiotics			Lab Isolates Zone of inhibition in mm				
Sr. No.	**Name**	**Conc.in µg**	**LAB 01**	**LAB 02**	**LAB 03**	**LAB 04**	**LAB 05**
1	Amikacin	30	R	R	R	R	R
2	Ciprofloxacin	5	R	R	R	R	R
3	Trimethoprim	5	R	R	R	R	R
4	Levofloxacin	5	R	R	R	R	R
5	Vancomycin	30	R	R	R	R	R
6	Gentamicin	10	13	R	R	R	R
7	Chloramphenicol	30	25	31	26	R	R
8	Ofloxacin	5	R	R	R	R	R
9	Tetracycline	30	16	R	13	R	R
10	Erythromycin	15	29	31	28	29	R

Key (R)- Resistance

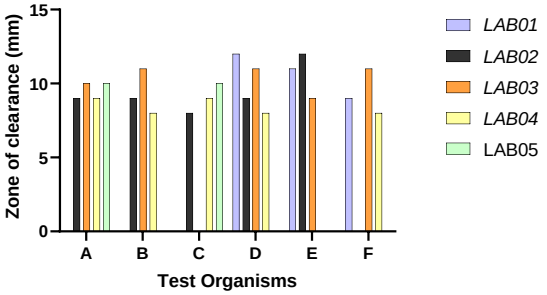

Fig. 8. Antimicrobial activity of LAB isolates against test pathogens (A) *Acinetobacter baumannii*,(B) *Escherichia coli*,(C) Methicillin-Resistant *Staphylococcus aureus*,(D) *Proteus mirabilis*,(E) *Streptococcus pyogenes*,(F) *Staphylococcus epidermidis*

Table 5. Antimicrobial activity of LAB isolates

Test Organisms	Zone of Clearance (in mm)				
	LAB01	**LAB02**	**LAB03**	**LAB04**	**LAB05**
Acinetobacter baumannii	-	09	10	09	10
Escherichia coli	-	09	11	08	-
Methicillin-Resistant *Staphylococcus aureus*	-	08	-	09	10
Proteus mirabilis	12	09	11	08	-
Streptococcus pyogenes	11	12	09	-	-
Staphylococcus epidermidis	09	-	11	08	-

4. CONCLUSION

Probiotics provide beneficial health effects when administered in adequate amounts. It must, nevertheless, endure and survive the hostile circumstances of the GIT while also protecting itself from pathogens by producing antimicrobial compounds. To assess its probiotic potential, the LAB were isolated from non-dairy products such as white peas, green peas, chickpeas, dragon fruit, and sweet lime and subjected to a series of in vitro tests such as Tolerance to Bile, Acid, pH, and Gelatinase Liquefaction and Hemolysis, as well as Resistance to Antibiotics and Antimicrobial activity. Our results are quite promising, implying that the LAB strains we evaluated are potential candidates for probiotics. It is reasonable to conclude that LAB strains isolated from non-dairy food items possess a broad spectrum of antibacterial activity and can be employed as food preservatives. All the five isolates were resistant to gastrointestinal conditions and had high antibacterial activity. LAB01, LAB03, and LAB04 demonstrated to be the best of the five isolates chosen for screening, surviving the low pH and bile conditions in the

stomach, as well as the severe intestinal environments, making them an appealing probiotic. Finally, the LAB revealed probiotic properties with strong antimicrobial activities, indicating the possibility of using them as probiotics in food. However, more studies are required to validate the potential.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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