

Research Paper

Isolation, Phenotypic Characterization and Functional Analysis of Probiotic Lactic Acid Bacteria from Indigenous Food Products

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Abstract

The objective of this research work was to screen some useful probiotic Lactic Acid Bacteria (LAB; n=15) from various indigenous food sources. Preliminary identification based on Gram reaction and catalase test, revealed 60% cocci and 40% bacilli. Antagonistic activity was assessed by agar well diffusion against *Bacillus subtilis*, *Salmonella typhi*, *Escherichia coli*, *P. aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*, showed that the cell-free culture supernatant of LBM1 produced inhibition zones of 37mm (*E. coli*), 25mm (*P. aeruginosa*), and 30mm (*S. aureus*). LCT2 showed 30mm (*S. aureus*) and 34mm (*E. coli*), while LCR8 exhibited 40mm (*S. typhi*) and 38mm (*C. albicans*). All three strains survived at pH 3 for 3h with counts of 2.1×10^5 , 2.2×10^5 , and 1.8×10^5 CFU/mL and survival rates of 94%, 96%, and 93%, respectively. Biofilm formation, confirmed by Congo red agar, and microtiter assays, showed OD₆₃₀ values of 0.368 ± 0.05 (LBM1), 0.342 ± 0.17 (LCT2), and 0.233 ± 0.04 (LCR8). Heavy-metal resistance testing revealed that all three strains tolerated nickel nitrate up to 10 ppm, indicating potential resilience under stress conditions. Moreover, production of exopolysaccharides (EPS) was also observed by LBM1, LCT2 and LCR8. The antimicrobial activity of LAB EPS was also assessed for *S. aureus*, *E. coli*, *S. typhi*, and *C. albicans*. Notably, all these strains exhibited significant antimicrobial activity. These findings highlight the adaptability and potential applications of LAB strains, particularly those with antimicrobial activity, pH resistance, bile salt tolerance and robust EPS production.

Introduction

Lactic acid bacteria (LAB) represent a taxonomically and functionally diverse group of microorganisms (Akpogheli et al., 2025). Being facultative anaerobe, LAB utilizes fermented carbohydrates to generate lactic acid. These microorganisms exhibit Gram-positive characteristics, typically lacking peroxidase activity, displaying mild aerobic nature and manifesting resilience to acidic environments. Morphologically, LAB does not belong to a category of homogenous class of bacteria; they encompass rod-shaped organisms found in chains, such as those within the genera *Lactocaseibacillus*, *Carnobacterium* and *Weissella*; cocci organized in chains, exemplified by species of *Lactococcus*, *Enterococcus* and *Vagococcus*; and those arranged in tetrads, as observed in the genus *Tetragenococcus* (Kieliszek et al., 2021). These microbes are renowned for their capacity to metabolize anaerobically, yielding lactic acid as their key end-product and for synthesizing an array of metabolites that positively influence the nutritional, sensory and technological attributes of fermented foods (Anumudu et al., 2024). The beneficial impacts exerted by LAB on human health have long been recognized, leading to their widespread industrial application for enhancing the flavor, aroma along with long term storage of various foods (Fraberger et al., 2020).

They inhabit a variety of environments, including foods, the natural surroundings and the gastrointestinal tract of animals, with fermented foods (FFs) being recognized as their principal habitat of activity (Taye et al., 2021). Historically, LAB have been utilized in fermentation processes and continue to hold prominence in the food industry due to their functional attributes as probiotics (Manan, 2025).

LAB have potential in regulating the balance of gut microflora by effectively controlling undesirable microbial populations and inhibiting the proliferation of pathogenic bacteria through the synthesis of bioactive metabolites (Anjana and Tiwari., 2022), encompassing organic acids, antimicrobial peptides, short-chain fatty acids, extracellular enzymes and exopolysaccharides (Bhat and Bajaj, 2018).

LAB have garnered extensive attention as promising probiotics (Gupta and Bajaj, 2018). Essential characteristics for prospective probiotics include the ability to tolerate the severe circumstances seen in the gastrointestinal system, characterized by acidic pH, high salt concentrations and bile concentrations, along with the capacity to colonize the GIT in substantial numbers (Meena et al., 2025). The significant functionalities of probiotics encompass antimicrobial and immunomodulatory activities, modulation of gut microbiota function and structure (Plessas et al., 2020). Cellular attachment emerges as a crucial aspect for facilitating probiotic action, particularly in the competitive omission of enteropathogens, as evidenced through in vivo and in vitro experiments (Tuo et al., 2018).

Metals like copper, zinc, and iron are vital for cellular functions, acting as cofactors in numerous processes crucial for cell survival. Conversely, metals like cadmium and arsenic lack known cellular roles and are intrinsically toxic, disrupting cellular functions and inducing cell death beyond specific thresholds (Parsons and Kathariou, 2020).

Multiple classical probiotic species, notably *Lactobacillus* species, exhibit high-affinity to heavy metals and possess various advantageous properties favorable for heavy metals detoxification and bioremediation (Arun et al., 2021). Numerous studies reported that *L. plantarum* can be used for detoxification of heavy metals in the soil and food products, while also improving the nutritional value of food (Ameen et al., 2020). Oral ingestion of LAB can potentially inhibit the absorption of heavy metals into the body and efficiently eliminate them from the body (Darbandi et al., 2025).

Hence, study objectives of this research work were the isolation of useful probiotic LAB from indigenous food sources and assessment of their phenotypic (morphological and biochemical characteristics), probiotic (resistance to gastric, ileum and bile acid, bile salt hydrolysis) and technological (antagonism with pathogens, heavy metal tolerance and adherence to intestinal epithelial cells) characterization.

Materials and Methods

Isolation and identification

All food samples (n=15) including dairy products (milk, yogurt), vegetables (onion, cucumber, rice) and fruits (tomato) were collected from local markets in Karachi. The samples were diluted 100 folds in PBS and then 0.1mL of each diluted sample was transferred and spread over de Man Rogosa and Sharpe (MRS) agar by using a sterile glass rod followed by incubation at 37°C for 24 to 48 hours (Bartkiene et al., 2019). Preliminary identification of the colonies was done by Gram staining and catalase test. Catalase test was performed by adding a drop of 3% hydrogen peroxide solution on a clean glass slide and bacterial colony was emulsified and observed for bubble formation. All Gram-positive cocci or bacilli and catalase negative (which produce no bubbles) were initially identified as LAB (Goa et al., 2022). Pure cultures were also preserved in 15% glycerol broth and kept in a refrigerator at -20°C until further used (Sunarno et al., 2021).

Preparation of indicator bacterial inoculum

Overnight culture of indicator strain was centrifuged at 3000rpm for 5min and supernatant was discarded and pellets were re-suspended in PBS buffer and turbidity was compared with 0.5 McFarland index (Bohora et al., 2019).

Assessment of antimicrobial activity and preparations of cell free (CFCS) and neutralized cell free culture supernatant (NCFCS)

Antagonistic activity of all LAB isolates was assessed by agar well diffusion assay. Overnight culture of LAB isolates was centrifuged at 3000rpm or 10 minutes. The supernatant was separated in microcentrifuge tubes. Supernatant was divided in two aliquots, one with unaltered pH, labeled as cell free culture supernatant (CFCS) while second with pH 7 labelled it as neutralized cell free supernatant (NCFCS) (Garzon et al., 2017).

Agar overlay assay

Pathogenic strains such as *S. aureus*, *B. subtilis*, *E. coli*, *S. typhi*, *P. aeruginosa* and *C. albicans* were transferred (100µL) in nutrient soft agar (0.75%) and were overlaid on nutrient agar plates. Borer (11mm) was used to cut the wells in each plate then 200µL of CFCS and NCFCS of each test strain was added in separate wells. Sterile MRS broth was added as control. Petri plates were put into the refrigerator at 4°C for 45 minutes to allow diffusion of supernatant. This is followed by 37°C incubation for 24 hours. Results were observed and size of inhibition zone was measured in mm (Bohora et al., 2019). LAB strains LBR1, LBO4 and LCM7 that inhibited pathogenic organisms were selected for further characterization at low pH (pH3), 0.3% bile salts concentration and presence of exopolysaccharide attribute.

Characterization of selected LAB strains

Preparation of producer bacterial inoculum

A loop of culture of producer strains was inoculated into 3mL of MRS broth and kept in incubator set at 37°C for 24 hours. The 1% of this bacterial culture was poured into 5mL of fresh MRS broth and again incubated at 37°C for 24 hours. On the third day, culture was centrifuge at 3000rpm for 10min. Supernatant was discarded and pellet was collected and resuspended in 2mL PBS (pH 7.2) and washed twice. 1% of washed culture was transferred to 15mL of MRS broth for further evaluation and referred as activated culture (Meena et al., 2022). Pure cultures were also preserved in MRS agar slants and kept in a refrigerator at -5°C.

Tolerance to acidic pH

Activated culture (20 µL) of producer strains was transferred into 100 mL of MRS broth (pH 3) to create gastric like environment. MRS broth having pH to 6.5 served as a control sample. Broths were kept in incubator set at 37°C for 3 h. After every 1 hour, sample from control and test culture bottles were drawn for 3 hours and 10^{-1} to 10^{-4} dilution prepared in sterile PBS. 0.1 mL of each diluent was spread onto MRS agar plates and incubated at 37 °C for 24 hours. Total number of colonies were counted and calculated in $\log_{10}$ CFU/mL by given formula (Gajjar et al., 2019; Meena et al., 2022; Simões et al., 2022)

Survival at pH 3 (%) or bile salt (%) = Final viable count/Initial viable count $\times$ 100

Tolerance to Bile Salts

Activated LAB cultures were suspended in 100mL MRS broth containing 0.5% (w/w) bile salts. Bile salts deficient sample is added as a control. Culture bottles were kept in incubator set at 37°C for 3 hours. MRS agar plates surface covered with 100µl of serially diluted sample. The viable counts recorded and reported as $\log_{10}$ CFU/mL (Kusada et al., 2021).

Biofilm Formation

Screening for biofilm producing LAB was performed by two different methods mentioned as follows. Each assay was performed in triplicates. Sterile TSB was added in all tests. International reference strain (*S. aureus* ATCC 29213) was added as positive controls while *S. aureus* ATCC 33591 was included as negative control.

Microtitre Plate Assay (96 wells plate)

For microtitre plate assay, 150µL of TSB and 50µL of freshly cultivated overnight LAB cultures were mixed in each well of sterilized 96-wells micro titer plate, and incubated at 37°C for 48 h. After incubation, the wells were carefully washed thrice with sterile distilled water (Mekky et al., 2022), drained and left to dry at room temperature following a 15-minute fixation step with 99% methanol. Wells were stained with 1% crystal violet solution for 30 minutes followed by gentle wash with tap water to eliminate any stain that was not taken up by adhering bacteria. The plates were then left to air dry. Absorbance of wells was determined by ELISA microtitre plate reader at 630nm (OD_{630}). Results were interpreted as strongly adherent (>0.222), weakly adherent (>0.111 or ≤ 0.222) or non-adherent (≤ 0.111) (Martins et al., 2019).

Congo Red Agar Assay (Qualitative Assay)

Freshly grown culture of each LAB isolate was spread on Congo red agar plates followed by incubation for 24 hours at 37°C. Biofilm positive isolates formed black colonies, moderate or weak biofilm producers formed blackish-red colonies whereas non-biofilm formers were appeared as red colonies (Mekky et al., 2022).

Screening for Heavy Metal Resistance

Metal solutions of Ni(NO₃)₂, Cd(NO₃)₂ and Cr(NO₃)₂, were added to MRS agar at concentration 1ppm, 5ppm and 10ppm. Each LAB isolate was then streaked over metal amended MRS agar (Ameen et al., 2020).

Extraction of Exopolysaccharides

Lactic acid bacteria i.e. LBR1, LB04 and LCM7 grew at 37°C for 18-24 hours in MRS broth. In order to denature the proteins, 14% of trichloroacetic acid was added to the MRS broth containing producer strains and incubated at 37°C for 30-40 min in a shaking incubator at 90rpm. Subsequently, the culture was centrifuged at 5,000×g for 20 minutes to settle down the cells. Supernatant was carefully collected in a sterilized test tube then chilled absolute ethanol in a ratio of 1:2 was added in supernatant and incubated at 4°C for 24-48 hours to facilitate precipitation. Furthermore, after incubation supernatant was discarded and pellet was collected. In order to remove traces of media components, the precipitate was centrifuged at 5,000×g for 20 minutes. The resulting pellet was collected and filtered using a filter membrane then dissolved in distilled water (Kamaliya et al., 2023; Zaghloul et al., 2023).

Antimicrobial activity of LAB exopolysaccharides

Antimicrobial activity of the extracted exopolysaccharides (EPS) was assessed for the producing strains against *S. aureus*, *S. typhi*, *E. coli* and *C. albicans* by using agar well diffusion assay. Firstly, inoculum was prepared from test strains and then cotton swab was dipped into the inoculum and spread over the entire surface of nutrient agar (NA). Using borer (11mm) wells were cut in the agar and 100µL of extracted EPS was added in these wells while in one well only PBS was used as a control. All plates were refrigerated for 45 minutes for proper diffusion of extracted EPS and then, incubated at 37°C for 24 hours. Next day, inhibitory zone diameter was measured and recorded in mm (Tarannum et al., 2023).

Results

A total of 15 Gram positive and catalase negative organisms were identified as Lactic acid bacteria among which 60% were cocci and 40% were bacillus based on colony morphology and microscopic characteristics (Figure 1; Table 1).

Antagonistic activity of lactic acid bacteria

Among 15 isolates, three strains (LBM1, LCT2, and LCR8) exhibited inhibitory effects against the pathogenic indicator organisms. The CFCS of LBM1 produced inhibition zones of 37 mm (*E. coli*), 25 mm (*P. aeruginosa*), and 30 mm (*S. aureus*). LCT2 showed 30mm and 34mm zones of inhibition against *S. aureus* and *E. coli*, respectively, and LCR8 exhibited 40 mm and 38 mm zones of inhibition against *S. typhi* and *C. albicans*, respectively (Figure 2; Table 2). The neutralized CFCS (pH 7) displayed negligible activity against all pathogens, indicating that organic acids are primarily responsible for the observed antagonism. Based on the antagonistic activity, 3 efficient isolates (LBM1, LCT2, and LCR8) were chosen for acidic pH and bile salts tolerance.

Table 1: Colony Morphology and Microscopic characteristics and Catalase test of isolated LAB strains

S. No.	Source	Isolate	Colony Morphology	Microscopic characteristics and Gram Staining	Catalase Test
1	Soil	LBS	Small, punctiform, white, opaque, brittle	G+ve, rods in chain	N
2	Yoghurt	LBY	Small, punctiform, opaque, brittle	white G+ve, short rods, scattered	N
3	Tomato	LCT1	Large, round, off white translucent, raised	mucoïd, G+ve, cocci, scattered	N
4	Tomato	LCT2	Small, circular, opaque, flat	white, G+ve cocci, arranged as tetrads or in clusters.	N
5	Milk	LBM1	Medium, circular, opaque, brittle	white, G+ve medium rods, scattered	N
6	Rice	LBR1	Medium, off white, mucoïd, flat	G+ve short rods, scattered	N
7	Rice	LBR2	Large, white, circular	G+ve short rods in chain	N
8	Rice	LBR3	Small, opaque, flat	G+ve, rods, scattered	N
9	Rice	LCR8	Small, punctiform, white, opaque, brittle.	G+ve, cocci in clusters	N
10	Rice	LCR2	Small, circular, opaque, flat	white, G+ve, cocci, scattered	N
11	Rice	LCR3	Large, round, mucoïd	G+ve, diplococci	N
12	Rice	LCR6	Small, circular, opaque, flat	white, G+ve, round shaped cells arrange in pairs and clusters	N
13	Onion	LCO2	Medium, white, mucoïd	G+ve, cocci, scattered	N
14	Onion	LCO3	Medium, off white, dry	G+ve, cocci arranged in clusters	N
15	Mango	LCM	Large, round, off white translucent, raised	mucoïd, G+ve, cocci arranged in pairs and clusters	N

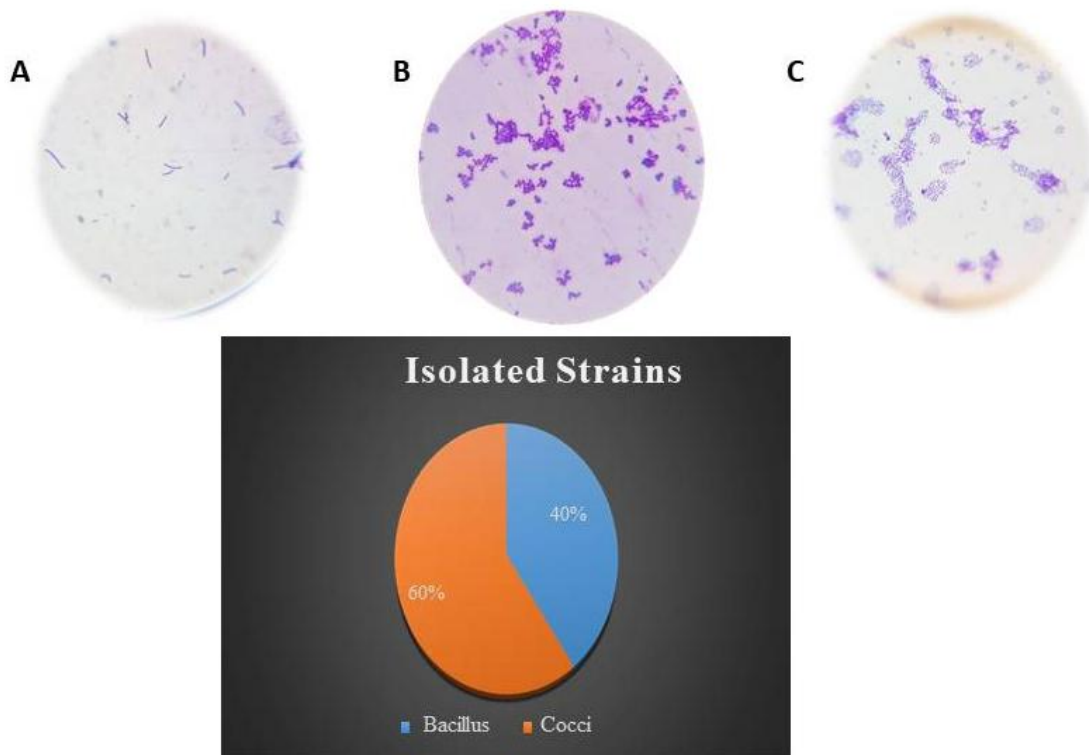


Figure 1: Percent prevalence of isolated strains from different sources. *Insert:* Colony morphology of isolates selected for further evaluation (A) LBM1 (milk), (B) LCT2 (tomato), and (C) LCR8 (rice).

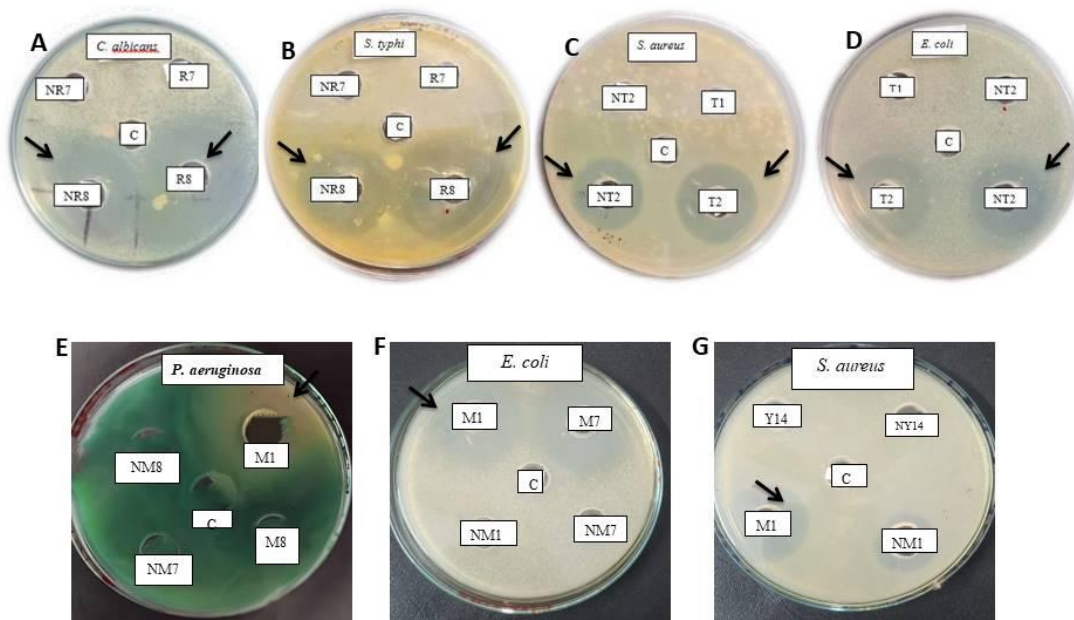


Figure 2: Zones of inhibition produced by CFCS and NCFCS of LCR8 against (A) *C. albicans*, (B) *S. typhi*. LCT2 against (C) *S. aureus*, and (D) *E. coli*. LBM1 against (E) *P. aeruginosa*, (F) *E. coli*, (G) *S. aureus*.

Table 2: Antagonistic Activity of isolated LAB strains with inhibition zone diameter

S. No.	LAB Strains	Cell free culture supernatants	Gram Positive organisms (Zone diameter in mm)		Gram Negative organisms (Zone diameter in mm)			Fungi (Zone diameter in mm)
			<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. typhi</i>	<i>C. albicans</i>
1	LBM1	CFCS	30	N	37	25	N	N
		NCFCFS	17	N	12	10	N	
2	LCT2	CFCS	30	N	34	N	N	
		NCFCFS	25	N	27	N	N	N
3	LCR8	CFCS	N	N	N	N	40	38
		NCFCFS	N	N	N	N	32	30
4	LCR6	CFCS	22	N	N	N	24	15
		NCFCFS	12	N	N	N	N	N
5	LBY	CFCS	N	N	N	N	N	
		NCFCFS	N	N	N	N	N	N
6	LCT1	CFCS	N	N	N	N	N	N
		NCFCFS	N	N	N	N	N	N
7	LBR1	CFCS	N	N	N	N	N	
		NCFCFS	N	N	N	N	N	
8	LBR2	CFCS	N	N	N	N	N	
		NCFCFS	N	N	N	N	N	N
9	LBR3	CFCS	N	N	N	N	N	N
		NCFCFS	N	N	N	N	N	
10	LCR3	CFCS	N	N	N	N	N	
		NCFCFS	N	N	N	N	N	
11	LCR6	CFCS	N	N	N	N	N	
		NCFCFS	N	N	N	N	N	
12	LCO2	CFCS	N	N	N	N	N	
		NCFCFS	N	N	N	N	N	N
13	LCO3	CFCS	N	N	N	N	N	N
		NCFCFS	N	N	N	N	N	
14	LCM	CFCS	N	N	N	N	N	N
		NCFCFS	N	N	N	N	N	N

Key; N= negative results

Tolerance to acidic pH 3

All 3 candidate strains were able to survive at low pH when kept at 37°C for 3 hours. The tested strain LBM1 (2.1×10^5 CFU/mL), LCT2 (2.2×10^5 CFU/mL) and LCR8 (1.8×10^5 CFU/mL) survived at pH 3 with survival rates of 94%, 96% and 93% respectively as shown in Figure 3(A-C).

Tolerance to bile salt at 0.3% concentration

When subjected to 0.3% bile salts solution, LBM1 (3.8×10^6 CFU/mL), LCT2 (2.11×10^7 CFU/mL) and LCR8 (1.25×10^7 CFU/mL) survived for three hours resembling digestion process in human. (Figure 3 D-F).

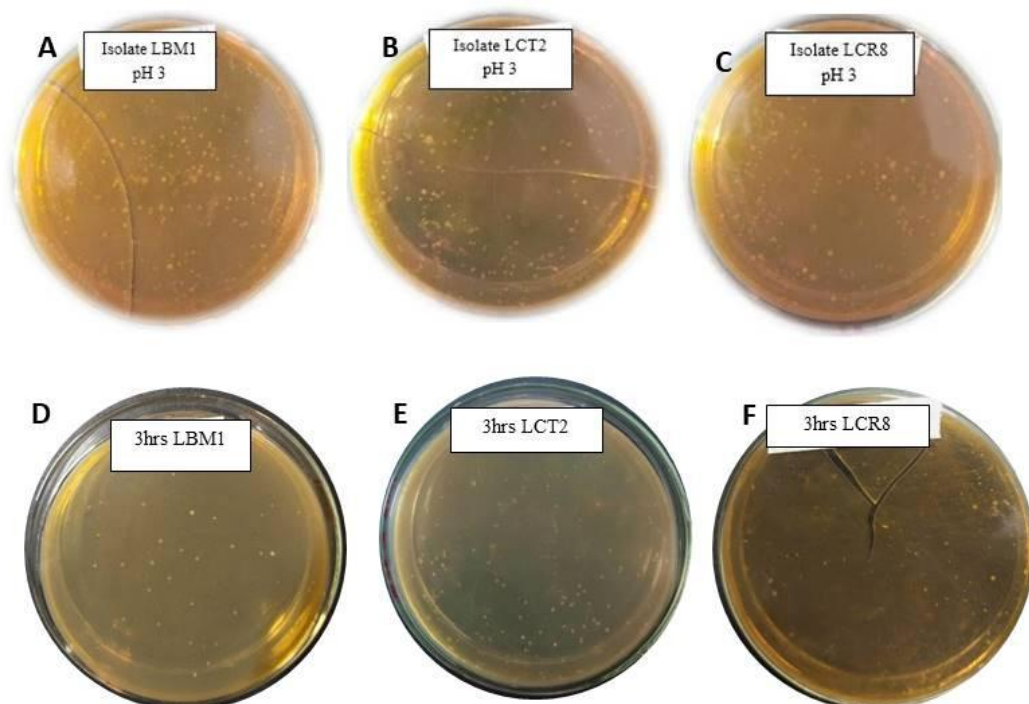


Figure 3: Upper panel: Acid Tolerance test of LAB isolate (A) LBM1 with 210 colonies, (B) LCT2 with 220 colonies, and (C) LCR8 with 180 colonies at pH 3. Lower panel: Bile tolerance test of LAB isolates for 3 hours (D) LBM1, (E) LCT2 and (F) LCR8 in 0.3% bile solution.

Biofilm Formation

Microtitre Plate Assay (96-wells plate)

A total of fifteen LAB isolates were assessed for biofilm production. Of these, three isolates were classified as biofilm producers. The ELISA microplate reader displayed absorbance values of 0.368 ± 0.05 for LBM1, 0.342 ± 0.17 for LCT2, and 0.233 ± 0.04 for LCR8, respectively. Among the three, LBM1 exhibited the highest biofilm-forming ability, followed by LCT2 and LCR8, indicating variation in the biofilm production potential among the isolates (Figure 4A).

Congo Red Agar Assay

On Congo red agar plates the tested producer strains LBM1, LCT2 and LCR8 produced black colonies which indicate that the tested strains exhibit the ability of biofilm production (Figure 4 B-D).

Heavy Metal Resistance

The ability of producer strains to resist heavy metals was assessed by introducing them in the media amended with heavy metals including nickel II nitrate, cadmium nitrate and chromium nitrate with concentrations 1ppm, 5ppm and 10ppm. According to our findings, LAB strains LBM1, LCT2 and LCR8, were able to tolerate nickel II nitrate (Figure 4E-G). It was unable to grow in the presence of other two heavy metals.

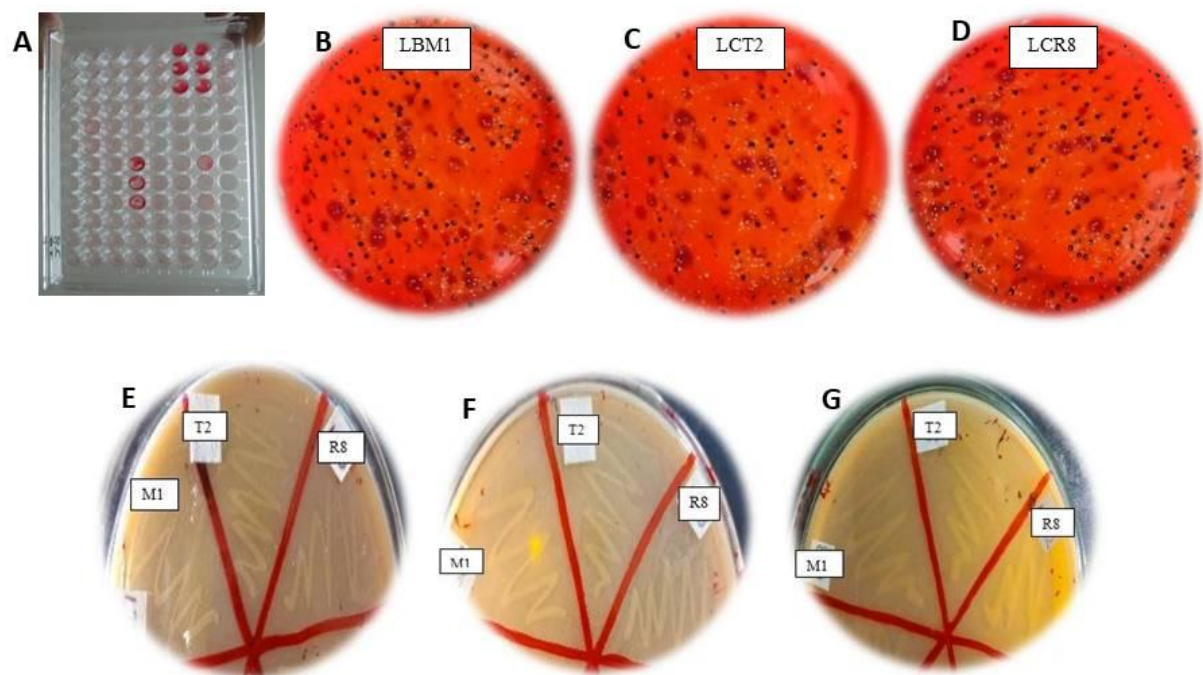


Figure 4: Upper lane: Representative picture of biofilm formation on (A) micro titer plate and (B-D) Congo Red agar by LBM1, LCT2, and LCR8.

Lower lane: Growth of isolates LBM1, LCT2 and LCR8 against nickel(II)nitrate at concentration (E) 1ppm, (F) 5ppm, and (G) 10ppm of $\text{Ni}(\text{NO}_3)_2$ amended in MRS agar plate.

Antimicrobial activity of Exopolysaccharides

In this study we have observed production of exopolysaccharides (EPS) by LCR8, LCT2 and LBM1 (Figure 5A). The antimicrobial activity of LAB's EPS was also assessed against *S. aureus*, *S. typhi*, *E. coli* and *C. albicans*. Notably, all these strains exhibited significant antimicrobial activity.

LCR8, LCT2, and LBM1 exhibited pronounced antagonistic activity against *S. aureus*, with inhibition zone diameters of 30mm, 23mm, and 25mm, respectively (Figure 5B). Furthermore, these isolates (LCR8, LCT2, and LBM1) also inhibited the growth of *Escherichia coli*, with

zone diameters of 28mm, 24mm, and 26mm, respectively (Figure 5C). In contrast, isolates LCR8, LCT2, and LBM1 demonstrated inhibitory effects against *Salmonella typhi*, producing zones of 30mm, 22mm, and 23mm, respectively (Figure 5D). In addition, LCR8, LCT2, and LBM1 showed antagonistic activity against *Candida albicans*, exhibiting inhibition zones of 24mm, 20mm, and 30mm, respectively (Figure 5E).

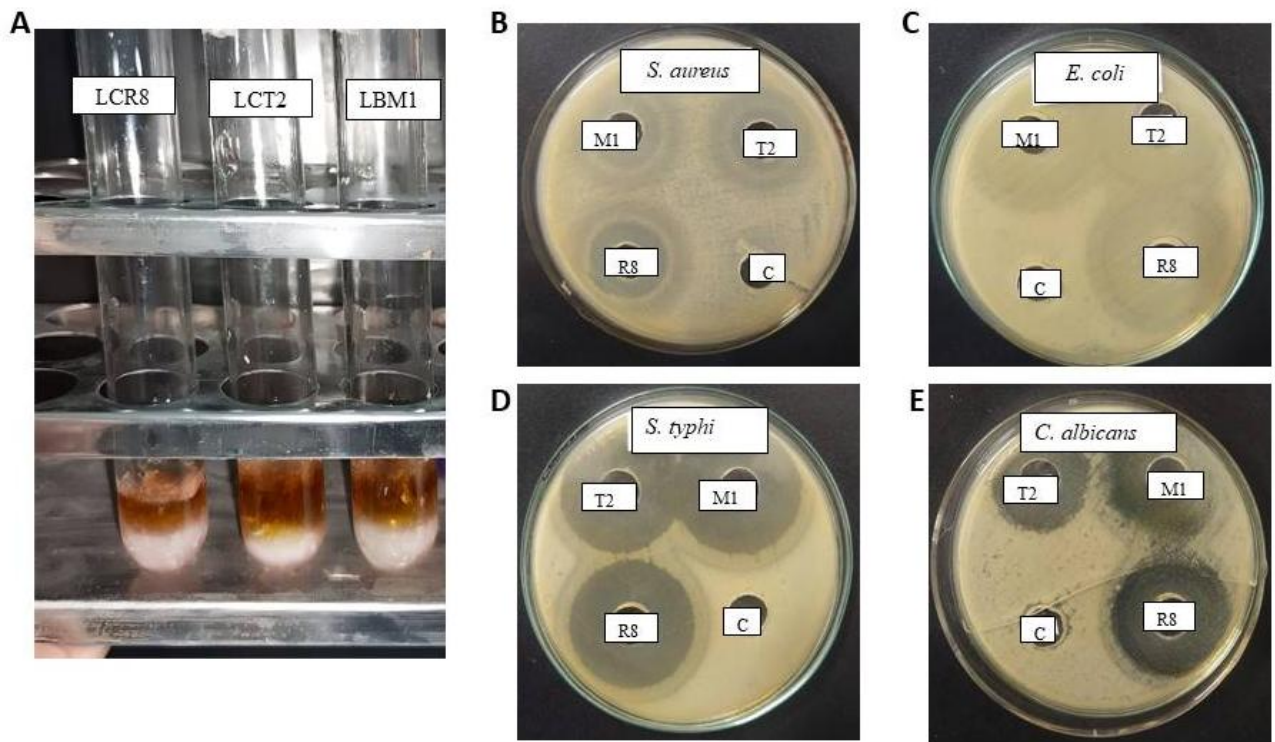


Figure 5: (A) Extraction of exopolysaccharides (EPS) from producer LAB strains. Antagonistic activity of extracted EPS of LCR8, LCT2 and LBM1 isolates against (B) *S. aureus*, (C) *E. coli*, (D) *S. typhi*, and (E) *C. albicans* by agar well diffusion assay.

Discussion

Lactic acid bacteria (LAB) constitute of prokaryotes characterized by distinctive morphological, metabolic, and physiological traits. Typically, they are facultative anaerobes and non-sporulating, catalase-negative status and fastidious nature, existing as cocci or rods. While mostly non-motile, they exhibit adaptability to high salt concentrations and low pH environments (Miranda et al., 2021).

Over time, LAB has played a pivotal role in various fermentation processes within the food industry. Their functional characteristics vary significantly, enabling them to ferment diverse substrates and produce a wide range of products and industrial raw materials during fermentation. This versatility underscores the importance of LAB in food production and highlights their potential for various applications in industrial processes (Wang et al., 2021).

This study provides a comprehensive review of recent research findings regarding the isolation, characterization, and functional properties of LAB strains, highlighting their significance in food science, nutrition, and health. Recent studies have focused on isolating LAB strains from diverse food sources, with promising results. Through the research, we have identified 15 LAB strains, including LBM1, LCT2, and LCR8, showcasing probiotic potential.

Isolated LAB strains exhibit significant antagonistic activity against both Gram-positive and Gram-negative bacteria, as well as yeast. Previous studies reported the potent antimicrobial effects of LAB strains resourced from variety of foods including fruits including fermented foods highlighting their potential for bio-control strategies and food preservation. Moreover, all three producer strains LBM1, LCT2, and LCR8 survived at low pH (pH 3). Our results are in line with the studies conducted by (Feng et al., 2017; Megur et al., 2023; Liu et al., 2024) highlighting the suitability of LAB strains for probiotic applications.

Our findings regarding the biofilm formation showed that out of 15, 3 isolates i.e. LBM1, LCT2 and LCR8 produced black colonies on Congo red agar that indicates the 3 strains exhibit the potential of biofilm formation. Moreover, 96 wells plates adhered with LAB cells were also assessed by using ELISA microplate reader.

Recent investigations have explored the resistance of LAB strains to heavy metals, indicating their potential in environments with metal exposure. According to findings, LAB strains LBM1, LCT2 and LCR8, were able to resist nickel nitrate. Previous studies showed the bioquenching/biosorption potential of Lab isolates to be employed as natural treatment to decrease metal toxicity from different food sources including drinking water and dairy products (Patel et al., 2017). This study results support the findings of Ameen et al. (2020) which focused on functionality of metal-resistant LAB in metal remediation. Their findings concluded that *L. plantarum* isolated from seacoast in Egypt, showed high level of tolerance towards nickel and chromium at concentration 500 ppm. They also reported about the biosorption potential of cadmium and lead by *L. plantarum*. They further suggested that this specie of LAB can be employed as a potent biosorbent to eliminate heavy metals from factory sewage.

Additionally, bile juice is present in digestive fluid of humans that aids in the solubilization and emulsification of lipids and lipid-soluble vitamins, thereby ensuring efficient digestion and nutrient absorption. The Food and Drug Administration (FDA) has declared bile components as generally recognized as safe (GRAS) and regulated their use for both human and animal consumption (Talib et al., 2019). In the present study, the acid tolerance (pH 3) and bile salt tolerance (0.3%) of probiotic candidates were evaluated to assess their ability to survive under simulated gastric conditions. LAB strains isolated from rice, tomato, and milk demonstrated tolerance to pH 3 and 0.3% bile salt concentration for three hours at 37°C, indicating their potential resilience during gastrointestinal transit. Similarly, Zhang et al. (2020) reported that LAB strains exhibited resistance to pH 3 and 0.3% bile salts, while Gajjar et al. (2019) observed that LAB isolates exposed to 0.3% bile salts showed a reasonable survival rate, supporting the findings of this study.

Moreover, the synthesis of exopolysaccharides (EPS) plays a critical role in bacterial defense, colonization, and host-microbe interactions (Kamaliya et al., 2023; Yasmin et al., 2020). EPS contributes to the protection of gut microbiota from phagocytosis and bacteriophage invasion while simultaneously acting as a growth-promoting substrate (Kamaliya et al., 2023; Bengoa et al., 2018). In the present investigation, all EPS-producing strains—LCT2, LBM1, and LCR8—were able to tolerate pH 3 for three hours, highlighting their acid resistance and adaptability. Morales et al. (2020) similarly reported EPS-producing LAB strains isolated from vegetables, while Kamaliya et al. (2023) demonstrated that LAB isolates from dairy products were also capable of EPS production, emphasizing the widespread occurrence of this trait among LAB from different sources. Furthermore, Tarannum et al. (2023) reported that antimicrobial activity was more pronounced against Gram-positive bacteria such as *S. aureus* and *B. cereus* compared to Gram-negative bacteria including *Salmonella typhi*, *E. coli*, and *P. aeruginosa*. Consistent with these findings, the current study revealed that all EPS-producing strains exhibited antagonistic activity against *C. albicans*, *S.*

typhi, *E. coli*, and *S. aureus*. This demonstrates the broad-spectrum antimicrobial potential of LAB-derived exopolysaccharides, suggesting their promising application in both food preservation and therapeutic formulations.

Conclusion

LAB offer numerous health benefits, including immune modulation, antimicrobial activity, and potential anticancer effects. Different studies underscored role of LAB strains in promoting gut health and enhancing the nutritional and health-promoting aspects of fermented foods. Current findings contribute to our understanding of LAB diversity and their applications in food science, nutrition, and health. To further understand the therapeutic potential of LAB strains and how they contribute to human health and wellbeing, more investigation is required.

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Conflict of interest: The authors declare that they have no conflict of interest.

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