Sircar and Mandal
Future Journal of Pharmaceutical Sciences (2023) 9:54
https://doi.org/10.1186/s43094-023-00506-z

Future Journal of Pharmaceutical Sciences

RESEARCH Open Access

Exploring the probiotic potentiality and antibacterial activity of idli batter isolates of lactic acid bacteria from West Bengal, India

Bijayanta Sircar[1] and Shyamapada Mandal[1*]

Abstract

Background The lactic acid bacteria (LAB), from diverse sources, are of great importance as probiotics, and several authors from around the globe have reported LAB, isolated from various fermented foods, as potential antimicrobial agents. The current study explored the antibacterial activity and probiotic property of idli batter isolates of LAB, for the first time from Malda (West Bengal, India).

Results The LAB procured from fresh and fermented idli batter samples had antibacterial activity against pathogenic as well as food-borne bacteria with zone diameter of inhibition of 16, 18 and 23 mm with concentrations 25, 50 and 75 µl/well, respectively, as determined by agar-well diffusion method. The identification of isolated LAB was executed through biochemical tests, 16S rRNA gene sequencing and phylogenetic analysis. The LAB isolates from fresh idli batter: LMEM1001 and LMEM1002, showed maximum (96.81% and 95.20%, respectively) similarities with *Lactiplantibacillus pentosus* and *Lactiplantibacillus plantarum,* respectively, whereas the fermented idli batter isolates, LMEM1006 and LMEM1008, showed maximum (96.11% and 98.40%, respectively) similarities with *Lactiplantibacillus plantarum* and *Limosilactobacillus fermentum,* respectively. Safety profiling of isolated LAB was executed using antibiogram, DNase and gelatinase tests.

Conclusions The idli batter-derived lactobacilli have been demonstrated as good probiotics, which displayed excellent antibacterial activity against clinical and food-borne bacteria. Overall, the idli batter isolates of LAB might be useful as probiotics for human consumption and as biotherapeutics in combating bacterial antibiotic resistance.

Keywords Probiotic bacteria, Antibacterial activity, LAB, Idli batter, 16S rRNA gene sequencing, Phylogenetic analysis

Background

Multiple antibiotic-resistant (MAR) bacteria are life threatening including the global food-borne infections [1, 2], and most of them are related to fresh-cut fruits, which is caused by *Salmonella*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Yersinia* sp., *Shigella sonnei* and *Escherichia coli* [3, 4]. Antimicrobial resistance (AMR) was one of the top ten worldwide public health issues for human in 2019, and total 143 countries (approximately 90% of the global population) together with 11 member States of the WHO South East Asia region have settled a national action strategy 2019–2023, which identifies containment of AMR as precedence [5]. In the current scenario, nosocomial infections are involved various pathogens, including *Enterococcus* species, coagulase-negative *Staphylococci, S. aureus*, *Proteus* species, *Enterobacter* sp., *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *E. coli,* whereas multidrug-resistant (MDR) isolates include extended-spectrum cephalosporin-resistant *Enterobacter* species, vancomycin-resistant *E. faecium*, and

*Correspondence:
Shyamapada Mandal
samtropmed@gmail.com
[1] Laboratory of Microbiology and Experimental Medicine, Department of Zoology, University of Gour Banga, Malda, WB 732103, India

methicillin-resistant *S. aureus* (MRSA), carbapenem-resistant *A. baumannii*, *Enterobacter* sp., *E. coli*, *P. aeruginosa*, *K. oxytoca*, *K. pneumoniae* [6]. Due to lack of new active compounds in the existing worldwide, treatment regimen has directed to a major upsurge in antibiotic resistance [7]. To counter the global AMR situation, selection of alternative treatment including probiotic therapy is warranted.

Currently the bio-preservation technique is an integrated biotechnology using lactic acid fermentation for humanizing properties of food and vegetables. Two genera such as *lactobacilli* and *lactococcus* can show probiotic potentials with large range of bioactivity [8]; by producing antimicrobial peptides (AMP) like bacteriocins, acidic pH, hydrogen peroxide, carbon dioxide or diacetyl [9–11], and has strong antagonistic activity on the development and toxin fabrication of other bacteria. Lactic acid bacteria (LAB) are useful beneficial microorganisms, with different well-known probiotic strains, promote good health and generally accepted as safe for human intake [12]. Several researches indicate that fermentation products of probiotics can enhance the synthesis of some bioactive component with beneficial effects that advances the functional value and acceptability of food products [13, 14].

Probiotics isolated from dairy and non-dairy sources can exhibit broad spectrum of antibacterial activity [15–17]. As per previous studies, potent LAB can be isolated from cereal dough fermentation [18], traditionally fermented legume products [19], mulkimchi fermentation [20] as well as idli batter [21, 22]. So, probiotic characterization in terms of stress (NaCl, bile salt, low pH, wide range of temperature, etc.) tolerances and antibacterial capacity testing are necessary task to know their beneficial effects on human.

The current investigation was set in order to search the alternative therapeutics against bacterial infection and in combating their antibiotic resistances. In this part of the globe, there is scanty report in terms of isolation, characterization and bioactivity of non-dairy-based probiotics. Thus, isolation and characterization of lactobacilli from non-dairy product, such as idli batter samples (both fermented and non-fermented), were chosen to be explored in order to assess their (lactobacilli) potentiality and inhibition efficacy against infectious bacteria.

Methods

Idli batter sample and lactic acid bacteria

Two idli batter samples (one freshly prepared, and the other was fermented for 24 h) collected from local vendor from Malda district, West Bengal, India, were utilized in the current study. The making of idli batter involved four steps including soaking the rice, urad dal/black gram and a few numbers of fenugreek (methi) seeds, mixing them, and lastly fermenting the batter.

In order to isolate the lactic acid bacteria (LAB), de Man, Rogosa and Sharpe (MRS) broth (Hi-Media, Mumbai, India) was inoculated with freshly collected as well as 24-h fermented idli batter samples separately, and after incubation for 24–48 h at 37 °C, single isolated colonies were procured on MRS agar (Hi-Media, India) plate, from each of the idli batter samples, by streak dilution of the broth culture as described earlier [23].

Phenotypic identity

The size, shape, margin, opacity and colour of the colonies of isolated LAB were documented. Phenotypic and biochemical characterization of the isolates was done following the standard protocol [24, 25]. To study the biochemical properties, catalase, citrate utilization, nitrate reduction, indole production, methyl-red (MR), Voges–Proskauer (VP), urease, oxidase tests including different sugar ($n=20$, Hi-Media, India): Adonitol (Ad), Arabinose (Ar), Dextrose (De), Dulcitol (Du), Fructose (Fc), Galactose (Ga), Inositol (Is), Inulin (In), Lactose (La), Maltose (Ma), Mannitol (Mn), Mannose (Mo), Melibiose (Mb), Raffinose (Rf), Rhamnose (Rh), Salicin (Sa), Sorbitol (Sb), Sucrose (Su), Trehalose (Tr), and Xylose (Xy), fermentation was performed following Bergey's manual [25], as described earlier [23].

Molecular identity

The identity validation of the selected idli LAB isolates was done by 16S rRNA gene sequencing and phylogenetic analyses.

The identification of isolated LAB (LMEM1001, LMEM1002, LMEM1006 and LMEM1008) was confirmed based on 16S rRNA gene sequence analysis. The ~ 1.3 kb/1.5 kb, 16 s-rDNA fragment was amplified using high-fidelity PCR polymerase and sequenced bi-directionally using 16S rRNA specific primers (forward primer: 5′-GGATGAGCCCGCGGCCTA–3′ and reverse primer: 5′-CGGTGTGTACAAGGCCCGG–3′) from Biokart Pvt Ltd, India.

The sequence data were aligned using the software "MEGA X" and analysed by ClustalW [26]. The nearest-known relatives of tests sequences obtained using nucleotides homology search through NCBI website with BLAST (Basic Local Alignment Search Tool) technique. Evolutionary distances have been calculated using the method of Nei and Kumar [27], and the phylogenetic trees with the sequences were prepared following the neighbour-joining method using bootstrap with 1000 replicates [28].

Probiotic property

The sodium chloride, bile salt, low-pH and temperature (for 17, 45 and 60 °C) tolerance were determined as probiotic properties of the isolated lactobacilli. The bile salt and low-pH (acid) tolerance were tested at an interval of 24, 48 and 72 h, respectively, following the protocol of Liong and Shah [29], and to sodium chloride (NaCl), by using the protocol of Chowdhury et al. [30], with slight modifications as mentioned elsewhere [23]. Briefly, the isolated lactobacilli were grown (for 24 h at 37 °C), in sodium chloride containing (of 2, 4, 6% and 8%) MRS broth, and then, the growth of lactobacilli, following subculture of the MRS broth cultures, on MRS agar (for 24 h at 37 °C), showed their tolerance to sodium chloride.

Antibacterial activity

The antibacterial activity of eight LAB isolated from fresh and fermented idli batter was determined by agar-well diffusion method, against the indicator strains of pathogenic bacteria (procured from clinical samples including urine, threat swab and pus), both Gram negative: *Pseudomonas aeruginosa* ($n=3$; strain code PA1, PA2 and PA3), *Escherichia coli* ($n=2$; strain code EC1, EC2) and Gram positive: *Staphylococcus aureus* strain SA1, and food borne bacteria, Gram positive: *Bacillus cereus* ($n=2$; strain code: BC1, BC2) along with two standard strains of Gram negative: *E. coli* ATCC 25922 and Gram positive: *Listeria monocytogenes* MTCC 657. The bacterial strains are maintained in cystine tryptone agar (Hi-Media, India) stabs in room temperature.

Agar-well diffusion method

On the surface of nutrient agar plate swabbed with indicator bacterial broth culture, wells (of 6 mm diameter) were prepared, and isolated LAB culture filtrates of increasing concentrations (25, 50 and 75 µL/well) were loaded in the wells followed by the protocol of Tagg and McGiven [31]. After 24-h incubation at 37 °C (in occurrence of atmospheric CO_2), ZDI (zone diameter of inhibition) values (nearest whole) were recorded, and interpreted as less active, moderately active and highly active with ZDIs ≤ 10 mm, 11–14 mm and ≥ 15 mm, respectively [15].

The antibacterial activity of the test LAB (at minimum concentration in which inhibition observed) in arbitrary unit per mL (AU/mL) was determined as a measure of production of bioactive components using the formula mentioned elsewhere [32]. The "*R*" (width of clear zone) values of all the isolates were also calculated (at maximum concentration of culture filtrate: 75 µl/well) as per the formula specified previously [33]. The scores of antagonism of indicator bacteria were measured as zero inhibition capacity when "*R*" was < 2 mm; low or intermediate inhibition capacity with "*R*" values of 2–5 mm, and high inhibition capacity with "*R*" values ≥ 5.5 mm [34, 35].

Safety profiling

The safety profile of the idli isolates (LMEM1001, LMEM1002, LMEM1003, LMEM1004, LMEM1005, LMEM1006, LMEM1007 and LMEM1008) was determined by their gelatine liquefaction test, DNase test and antibiotic susceptibility.

Gelatine liquefaction test

Gelatine liquefaction test was performed using nutrient gelatin media (following 24-h incubation at 37 °C then freezing at 4 °C in alternative manner up to 7 days and checking the liquefaction of gelatin media) followed by the protocol of Dela Cruz and Torres [36] with slight modifications, to confirm the capacity of isolated LAB to hydrolyse gelatine by producing gelatinase.

Deoxyribonuclease (DNase) test

The DNase test was done using DNase agar (Hi-Media, India) for all the isolated LAB in order to determine the ability of an organism to produce the DNase enzyme followed by protocol of Bergey's manual [25].

Antibiotic susceptibility test

All the LAB isolates were tested against antibiotics (Hi-Media, India): amikacin (Ak: 30-µg/disc), ampicillin (Am: 10-µg/disc), cefoxitin (Cx: 30-µg/disc), chloramphenicol (C: 30-µg/disc), ciprofloxacin (Cp: 5-µg/disc), gentamycin (Gn: 10-µg/disc), nalidixic acid (Na: 10-µg/disc), piperacillin (Pi; 100-µg/disc), tetracyclines (Te: 30-µg/disc) and vancomycin (V: 30-µg/disc), using the disc diffusion method [37], as per the Clinical and Laboratory Standards Institute (CLSI) criteria [38], as described earlier [39].

Cumulative probiotic potential (CPP)

The probiotic potential of the LAB isolates was evaluated using 8-point scores, and the CPP was calculated as per the formula mentioned elsewhere [15, 40] and slightly modified as per Wadoum et al. [41]. We had considered probiotic characters ($n=4$), safety aspects ($n=3$) and antagonistic activity ($n=1$) in one scale and that was the main modification in terms of CPP value determination.

Results

Identification of lactic acid bacteria

Among eight lactic acid bacteria (LAB), four (LMEM1001, LMEM1002, LMEM1003 and LMEM1004) from fresh idli batter and remaining four isolates

(LMEM1005, LMEM1006, LMEM1007 and LMEM1008) from fermented idli batter sample were isolated. All isolates were Gram positive (Fig. 1), non-spore forming, non-motile rod shaped and were negative to catalase and oxidase tests, and thus recognized as *Lactobacillus*. After interpretation of morphological, cultural, biochemical tests (Table 1), sugar fermentation profile (Table 2) and 16S rRNA sequencing results (Figs. 2, 3 and 4), the isolates were identified as *Lactobacillus pentosus* LMEM1001 (currently *Lactiplantibacillus pentosus*), *Lactobacillus plantarum* LMEM1002 (currently *Lactiplantibacillus plantarum*), *Lactobacillus* sp. LMEM1003 and *Lactobacillus* sp. LMEM1004, *Lactobacillus* sp. LMEM1005, *L. plantarum* LMEM1006, *Lactobacillus* sp. LMEM1007 and *Lactobacillus fermentum* LMEM1008 (currently *Limosilactobacillus fermentum*). The sequences of four LAB strains subjected to molecular identity through 16S rRNA gene sequencing have been submitted to the NCBI (National Centre for Biotechnology Information) GenBank with specific accession numbers: *Lactiplantibacillus pentosus* LMEM1001 (https://www.ncbi.nlm.nih.gov/nuccore/MT783707), *Lactiplantibacillus plantarum* LMEM1002 (https://www.ncbi.nlm.nih.gov/nuccore/MW364384), *Lactiplantibacillus plantarum* LMEM1006 (https://www.ncbi.nlm.nih.gov/nuccore/OR096233) and *Limosilactobacillus fermentum* LMEM1008 (https://www.ncbi.nlm.nih.gov/nuccore/OR096236).

Probiotic property

The probiotic property determining tolerance test results to different stresses (sodium chloride, low-pH, wide range of temperature and bile salts) for the isolated LAB is represented in Table 3.

Antibacterial activity

The idli lactobacilli isolates displayed good antibacterial activity, following agar well diffusion method, against all indicator bacteria (Fig. 5). The *L. pentosus* LMEM1006 isolate showed maximum growth inhibitory activity at highest concentration tested against *Listeria monocytogenes* MTCC 657, *Bacillus cereus* BC2, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* PA3 having ZDIs 23, 20, 20 and 19 mm, respectively, while the *Staphylococcus aureus* had highest sensitivity to *L. fermentum* LMEM1008 isolate (ZDI: 18 mm); the *Lactobacillus* sp. LMEM1004 isolate exhibited poor activity against all indicator bacteria tested with ZDIs 8–12 mm (Fig. 6).

The "R" values and arbitrary unit per mL (AU/mL) of the isolated lactobacilli against indicator bacteria are represented in Fig. 7a, b, respectively. The lowest "*R*" values (1–3 mm) were documented due to the action of *Lactobacillus* sp. LMEM1004, while *L. pentosus* LMEM1006 and *L. fermentum* LMEM1008 had "*R*" values 4.5–8.5 mm and 2.5–6 mm, respectively. Against indicator bacteria, *Lactobacillus* sp. LMEM1004 showed least antagonistic activity (106.66–320 AU/mL); on the other hand, the highest level of growth inhibitory components formed as 640 AU/mL, by *L. plantarum* LMEM1006, and the values ranged from 240 to 480 AU/mL for both *Lactobacillus* sp. LMEM1007 and *L. fermentum* LMEM1008.

Safety profiling

All the isolated idli lactobacilli showed no activity in the production of gelatinase and DNase enzyme except *Lactobacillus sp.* LMEM1004 and had gelatine liquefaction capacity in order to synthesis gelatinase. The antibiotic susceptibility test results of all the isolated bacteria are represented in Fig. 8. All the Lactobacillus isolates had resistance to *Cx*, and *L. plantarum* LMEM1002 had Gn resistance, in addition to the *Cx*. All the isolated LAB showed mixed level of sensitivity to Ak, C and Te, while sensitivity to *Cp* and Va was exhibited by *L. plantarum* LMEM1002 and *Lactobacillus sp.* LMEM1004. High level of resistance pattern showed by *Lactobacillus* sp. LMEM1005 to six antibiotics among ten tested.

Cumulative probiotic potential (CPP)

The individual CPP for the *Lactobacillus* isolates was 62.5% for *Lactobacillus sp.* LMEM1004 and 100% for the rest seven isolates: *L. pentosus* LMEM1001, *L. plantarum* LMEM1002, *Lactobacillus* sp. LMEM1003, *Lactobacillus* sp. LMEM1005, *L. plantarum* LMEM1006, *Lactobacillus* sp. LMEM1007 and *L. fermentum* LMEM1008 (Table 4).

Discussion

As per European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC), group of bacteria, viz. *Staphylococcus aureus*, *Enterococcus* spp., *Enterobacteriaceae* (except *Shigella* and *Salmonella*), *Pseudomonas aeruginosa* and *Acinetobacter* spp., are responsible for nosocomial infections in terms of multidrug resistance (MDR). The increasing number of MDR strains can eventually occupy higher resistance capacity with recognition of extensively drug-resistant (XDR) and pandrug-resistant (PDR), respectively [42]. From the global perspective, acceleration of basic and applied researches is warranted to discover new therapeutics against ESKAPE as well as twelve species of bacteria with critical, high, and medium antibiotic resistance (AR) specified by WHO [43, 44]. To handle the situation of AR, bacteriocins producing probiotic lactic acid bacteria (LAB) strains might be a noble choice for administration against MDR bacteria [45]. As like as dairy-based fermented products, non-dairy-based fermented foods can also be used as the source of

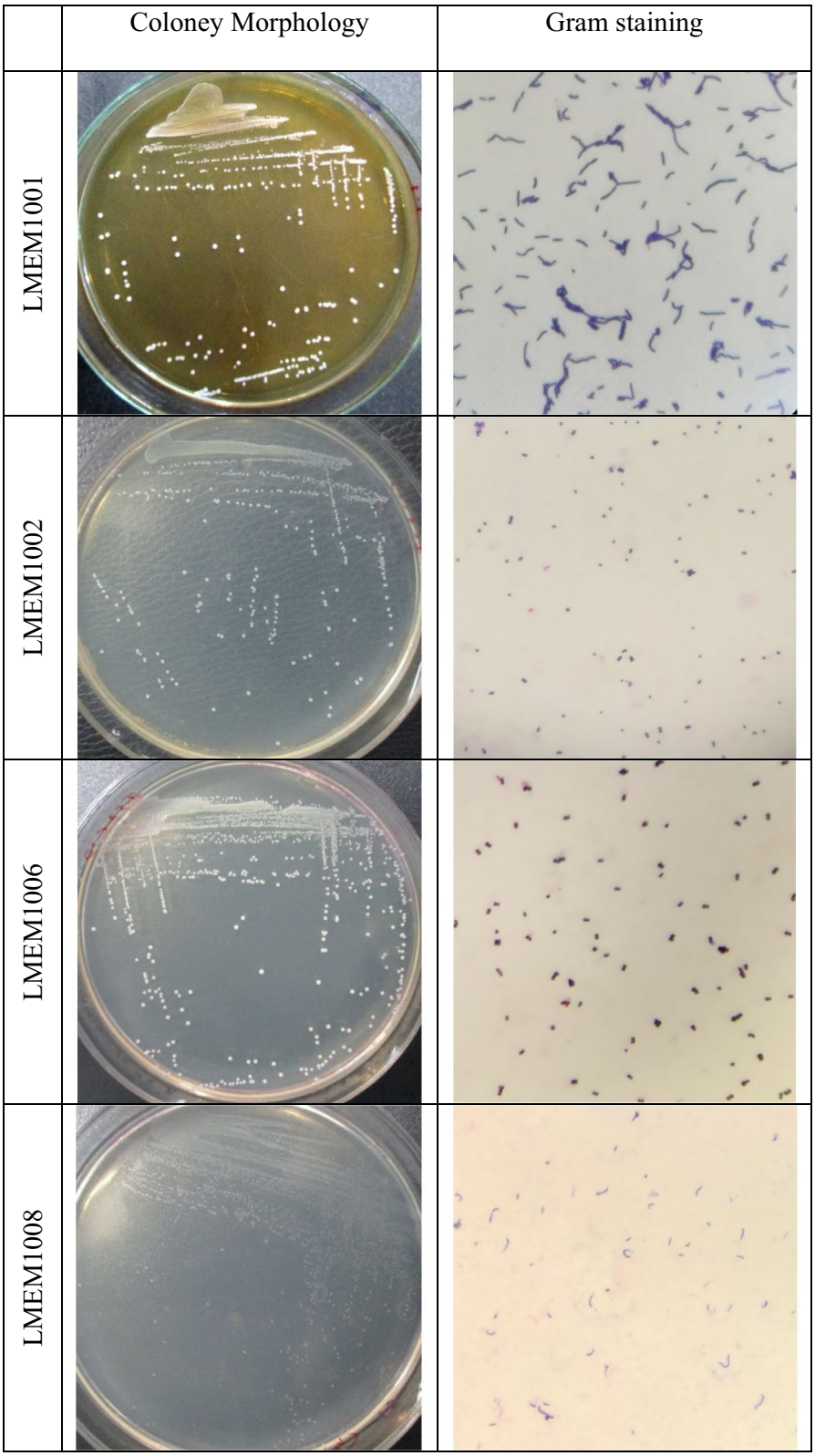

Fig. 1 Photographs showing morphological characteristics of selected isolated bacteria. All isolates were gram-positive, non-spore forming, non-motile rod-shaped

Table 1 Biochemical test results of isolated lactic acid bacteria

Isolates Code	Biochemical tests								
	Catalase	Oxidase	Indole	MR	VP	Urease	Citrate utilization	Nitrate reduction	TSI
LMEM1001	–	–	–	+	–	–	–	–	NC/Y
LMEM1002	–	–	–	–	+	–	–	–	–
LMEM1003	–	–	–	+	–	–	–	–	NC/Y
LMEM1004	–	–	–	+	–	+	–	–	–
LMEM1005	–	–	–	+	–	–	–	–	–
LMEM1006	–	–	–	+	–	–	–	–	NC/Y
LMEM1007	–	–	–	–	–	–	–	–	–
LMEM1008	–	–	–	+	–	–	–	–	NC/Y

NC No change in the slant portion, *Y* Yellow, '+' denotes positive results and '–' means negative results

Table 2 Sugar fermentation profile (24–72-h incubation) of idli lactobacilli

Isolates code	Ad	Ar	De	Du	Fc	Ga	Is	In	La	Ma	Mn	Mo	Mb	Rf	Rh	Sa	Sb	Su	Tr	Xy
LMEM1001	–	–	+	–	–	+	–	–	W	+	+	+	+	–	–	+	–	+	+	–
LMEM1002	–	–	–	–	–	–	–	–	–	–/+	–	–/+	–	–	–	–	–	–/+	–	–
LMEM1003	–	–	+	–	+	+	–	–	W	+	+	+	+	–	–	+	–	+	+	–
LMEM1004	–	–	–	–	+	–/+	–	–	W	–	–	–/+	–	–	–	–	–	–	–	–
LMEM1005	–	+	W	–	+	+	+	–	+	–	–	–/+	–	–/+	–	–	–	–	–	+
LMEM1006	–	–	+	–	+	+	–	–	W	+	–/+	+	++	–/+	–	+	–/+	+	+	–
LMEM1007	–	–	W	–	+	W	–	–	+	+	–	–/+	W	+	–	–	–	–	–	–
LMEM1008	–	–	+	–	+	W	–	–	–/+	+	–	+	+	+	–	–	–	+	–	–

Ad Adonitol, *Ar* Arabinose, *De* Dextrose, *Du* Dulcitol, *Fc* Fructose, *Ga* Galactose, *Is* Inositol, *In* Inulin, *La* Lactose, *Ma* Maltose, *Mn* Mannitol, *Mo* Mannose, *Mb* Melibiose, *Rf* Raffinose, *Rh* Rhamnose, *Sa* Salicin, *Sb* Sorbitol, *Su* Sucrose, *Tr* Trehalose, *Xy* Xylose; *W* Weakly positive, '+' denotes positive results and '–' means negative results, '/' denotes after 48 h positive observation

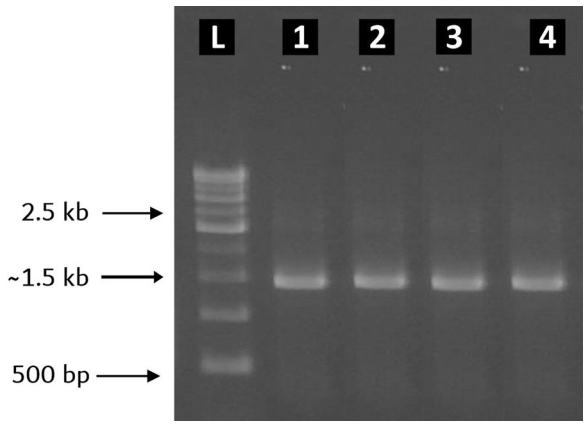

Fig. 2 Agarose gel electrophoresis of 16S rRNA gene PCR amplification showing ~ 1.5-kb amplified fragment. Lane L: ladder (500 bp), Lane 1: LMEM1001, 2: LMEM1002, 3: LMEM1006, 4: LMEM1008

isolation of probiotic microorganism, and lactobacilli is the most preferred one among them [46]. Several investigations approved that the presence of different yeasts and LAB strains in idli batter might be responsible for the beneficial nutritive as well as bioactive values including antimicrobial properties [47, 48]. Sharma et al. [49] studied microbiological dynamics of blends of buttermilk and pearl millet flour using number of techniques, viz. yeast and mould count, aerobic plate count, *Escherichia coli* count, and LAB count. Two probiotic bacteria *L. plantarum* and *Lactococcus lactis* have been isolated from idli batter and identified earlier by physiological and biochemical characterization, and both showed antibacterial activity against gram-positive (*B. cereus* MTCC 1272) and gram-negative (*E. coli* NRRL 3008) bacteria [50]. Total of 354 bacterial isolates identified by Mandhania et al. [51] on the basis of culture-dependent method through spread plate technique and colony characters were obtained from 3 fully fermented idli batter samples. Kadirvelu [52] noticed new antibacterial compound, 2-hydroxy indole propanamide formed by *L. plantarum* isolated from idli batter using phenotypic characterization.

For probiotic characterization of lactobacilli, acid tolerance (low pH), wide range of temperature tolerance,

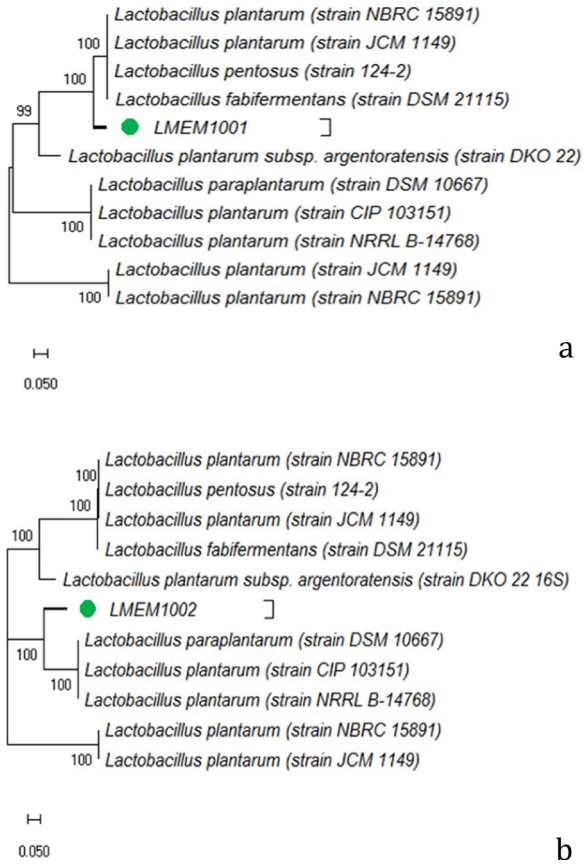

Fig. 3 The 16S rRNA gene sequence-based phylogenetic tree for lactobacilli isolated from fresh idli batter. **a** LMEM1001 showed maximum (96.81%) similarity with *Lactobacillus pentosus* (strain 124-2), **b** LMEM1002 showed maximum (95.20%) similarity with *Lactobacillus plantarum* (strain JCM 1149)

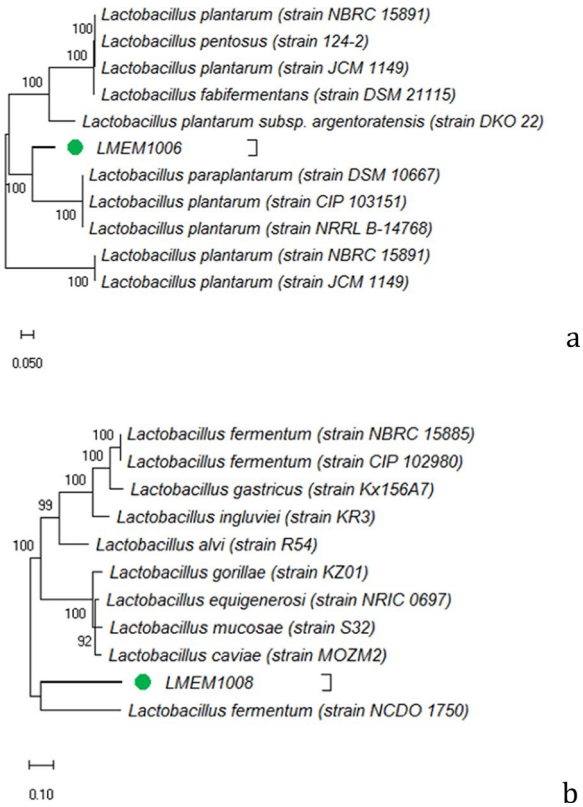

Fig. 4 The 16S rRNA gene sequence-based phylogenetic tree for isolated from fermented idli batter. **a** LMEM1006 showed maximum (96.11%) similarity with *Lactobacillus plantarum* (strain CIP 103151), **b** LMEM1008 maximum (98.40%) similarity with *Lactobacillus fermentum* (strain NBRC 15885)

survivability in presence of NaCl, bile salt are important criteria, and the pH value of 3.0 has been considered standard for such investigation of probiotic strains [15, 53]. In previous investigations on fermented food products, as have been conducted by Haldar et al. [15], Thakkar et al. [54] and Agaliya and Jeevaratnamthe [22] LAB tolerated and survived in MRS broth at pH ranged between 3–4, 3–6.5 and 3.5–9.5, respectively. Balasingham et al.'s [55] study showed significant growth at pH ≥ 3 of LAB isolated from swine intestine, while reduction in viability has been seen at pH 2. Two strains of *L. plantarum* subsp. *argentoratensis* (LQC 2520 and LQC 2320) isolated from spontaneously fermented Greek wheat sourdoughs showed survivability in the presence of NaCl (6.5%), indicating their high sodium chloride tolerance [56]. The lactobacilli isolated from fermented idli batter tested in the presence of 4, 6.5 and 10% of NaCl, whereas survived at 4 and 6.5% concentration of NaCl [22]. In the human intestine, the presence of bile salt is approximately 0.3% that is the reason good probiotic strain should reflect tolerance level greater than this physiological concentration of bile [57]. Iyer et al. [50] found two strains *L. plantarum* IB-1 and *Lactococcus lactis* IB-2 from idli batter, and both the strains showed good viability (58.11% and 60.84%, respectively) at high bile salt concentration (2%). As per the research of Mandhania et al. [51], total of seventy two isolates of LAB from fermented idli batter had tolerance to 2% bile salt, whereas some other researchers reported that total of thirty-four probiotic strains showed survivability and growth at the bile salt concentrations of 0.3–1% [21]. Like our study, temperature tolerance tests of isolated lactobacilli from fermented idli batter were executed and confirmed by Agaliya and Jeevaratnamthe [22] determining the growth at temperature ranged between 15 and 45 °C. The diverse range of tolerance to NaCl, bile salts, low pH (pH: 2–4) and temperature have been confirmed in the seven lactobacilli of the current study: *L. pentosus* LMEM1001, *L. plantarum* LMEM1002, *Lactobacillus* sp. LMEM1003, *Lactobacillus* sp. LMEM1005, *L. plantarum* LMEM1006, *Lactobacillus* sp. LMEM1007 and *L. fermentum* LMEM1008.

Table 3 Physiological stress tolerance test results (24–72-h incubation) for idli lactobacilli

Isolated LAB Strains	NaCl (%)				pH			Bile Salt (%)			Temperature (°C)		
	2	4	6	8	2	3	4	0.125	0.25	0.5	17	45	60
L. pentosus LMEM1001	+	+	+	+	−	w	+	+	+	+	+	+	−
L. plantarum LMEM1002	+	+	+	+	−	w	+	+	+	+	+	+	−
Lactobacillus sp. LMEM1003	+	+	+	+	−	−	+	+	+	+	+	+	−
Lactobacillus sp. LMEM1004	+	+	+	+	−	−	+	+	−	−	−	+	−
Lactobacillus sp. LMEM1005	+	+	+	+	−	−	+	+	+	+	+	+	−
L. plantarum LMEM1006	+	+	+	+	−	w	+	+	+	+	+	+	−
Lactobacillus sp. LMEM1007	+	+	+	+	−	−	+	+	w	−	+	+	−
L. fermentum LMEM1008	+	+	+	+	−	w	+	+	w	−	+	+	−

"+": resistant/tolerant; "−": sensitive/non-tolerant; "w": weakly tolerant

Due to the capacity to produce antimicrobials, probiotic is a potential source to substitute the synthetic antibiotics. The *Lactobacillus* isolates (*L. animalis* LMEM6, *L. plantarum* LMEM7, *L. acidophilus* LMEM8 and *L. rhamnosus* LMEM9) from curd samples had broad antibacterial spectrum with ZDI ranged between 11.33 ± 0.58 and 35.67 ± 2.52 mm (ZDI in mm ± standard deviation), against gram-negative bacterial pathogens, as has been informed by Haldar et al. [15]. According to Katepogu et al. [58], fermented idli batter contains potential LAB *Pediococcus* spp. and has maximum growth inhibition property against *E. coli* (ZDI: 14 mm) and *Bacillus subtilis* (ZDI: 21 mm) at cell-free suspension of 100 µl. Nine strains of *Bacillus* and one *Leuconostoc* strain procured from idli batter showed varied antibacterial activity against food-borne bacteria (ZDI: 6–22 mm) [59]. Two strains *L. plantarum* IB-1 and *Lac. lactis* IB-2 also isolated from idli batter by other researcher in other part of the globe, had good antibacterial activity against *E. coli* NRRL 3008 and *B. cereus* MTCC 1272 with ZDIs > 9 mm, respectively [50]. The probiotic *Pediococcus pentosaceus* strains ($n = 6$), isolated from idli batter, showed growth inhibitory activity against *S. aureus* MTCC 737, *Listeria monocytogenes* MTCC 657, *Bacillus cereus* MTCC 1272, *Aeromonas hydrophila* MTCC 1739, *Vibrio parahaemolyticus* MTCC 451 and *Escherichia coli* MTCC 728 species having ZDIs 11–22 mm [60]. Dubey and Jeevaratnam [61] study revealed two *L. pentosus* isolates (AJ7 and AJ82), from uttapam fermented batter, which was supplemented with *Piper betle* L. leaves displayed in situ growth inhibitory activity against *Listeria monocytogenes* MTCC657 determined using CFU count. The lactobacilli from idli fresh and fermented samples herein had good antibacterial activity with ZDI ranged between 6–16, 6–21 and 8–23 mm at increasing concentration (25, 50 and 75 µl), with "*R*" values 1 to 8.5 at highest concentration. As has been described by Haldar et al. [15], the bacteriocin production, in terms of antagonistic activity, for the test lactobacilli ranged 410.4–649.2 AU/mL. Five LAB isolates exhibited strong bacteriocin activity ranged between 800 to 1600 AU/mL against *Klebsiella pneumoniae* ATCC 12296 and *E. coli* [62]. In the current assay, the growth inhibitory activity of lactobacilli was recorded as 106.67–640 AU/mL, against the test bacterial pathogens at highest concentration.

Probiotic bacteria considered as safe if they show less antibiotic resistance. That is the reason, every strain of lactobacilli should be tested for antibiogram in assessing the safety profile to qualify as safe probiotics [63]. Two *L. pentosus* strains were reported to be sensitive to cephalexin (Cfx), cephradine (Ced), cloxacillin (Clox), co-trimazine, co-trimoxazole, nitrofurantoin (Nfn) and norfloxacin (Nor), while both strains showed resistance to cefuroxime (Cxm), mecillinam (Mec), nalidixic acid (Na) [61]. The *L. plantarum* isolated from idli batter showed resistance to Gn, Cp, Na and Nor [52]. Kandasamy et al. [21] found 34 probiotic microflora from fermented idli batter in which one strain was sensitive to Am and rifampicin (Rfm), two strains were sensitive to sulphadiazinee and five strains displayed sensitivity

(See figure on next page.)

Fig. 5 Through agar well diffusion method, idli lactobacilli isolates showed sensitivity against all test bacteria. **a** activity of *L. pentosus* LMEM1001 against BC2 (upper part) and LM (lower part), **b** against SA (upper part) and BC1 (lower part), **c** activity of *Lactobacillus* sp. LMEM1003 and *Lactobacillus* sp. LMEM1004 against EC3, **d** activity of *Lactobacillus* sp. LMEM1005 and *L. plantarum* LMEM1006 against PA1, **e** activity of *Lactobacillus* sp. LMEM1005 and *L. plantarum* LMEM1006 against PA3, **f** activity of *Lactobacillus* sp. LMEM1007 and *L. fermentum* LMEM1008 against SA. BC1: *Bacillus cereus* 1, BC2: *B. cereus* 2, SA: *Staphylococcus aureus*, LM: *Listeria monocytogenes* MTCC 657, PA1: *Pseudomonas aeruginosa* 1, PA3: *P. aeruginosa* 3, EC3: *Escherichia coli* 3. A: 25 µl, B: 50 µl and C: 75 µl

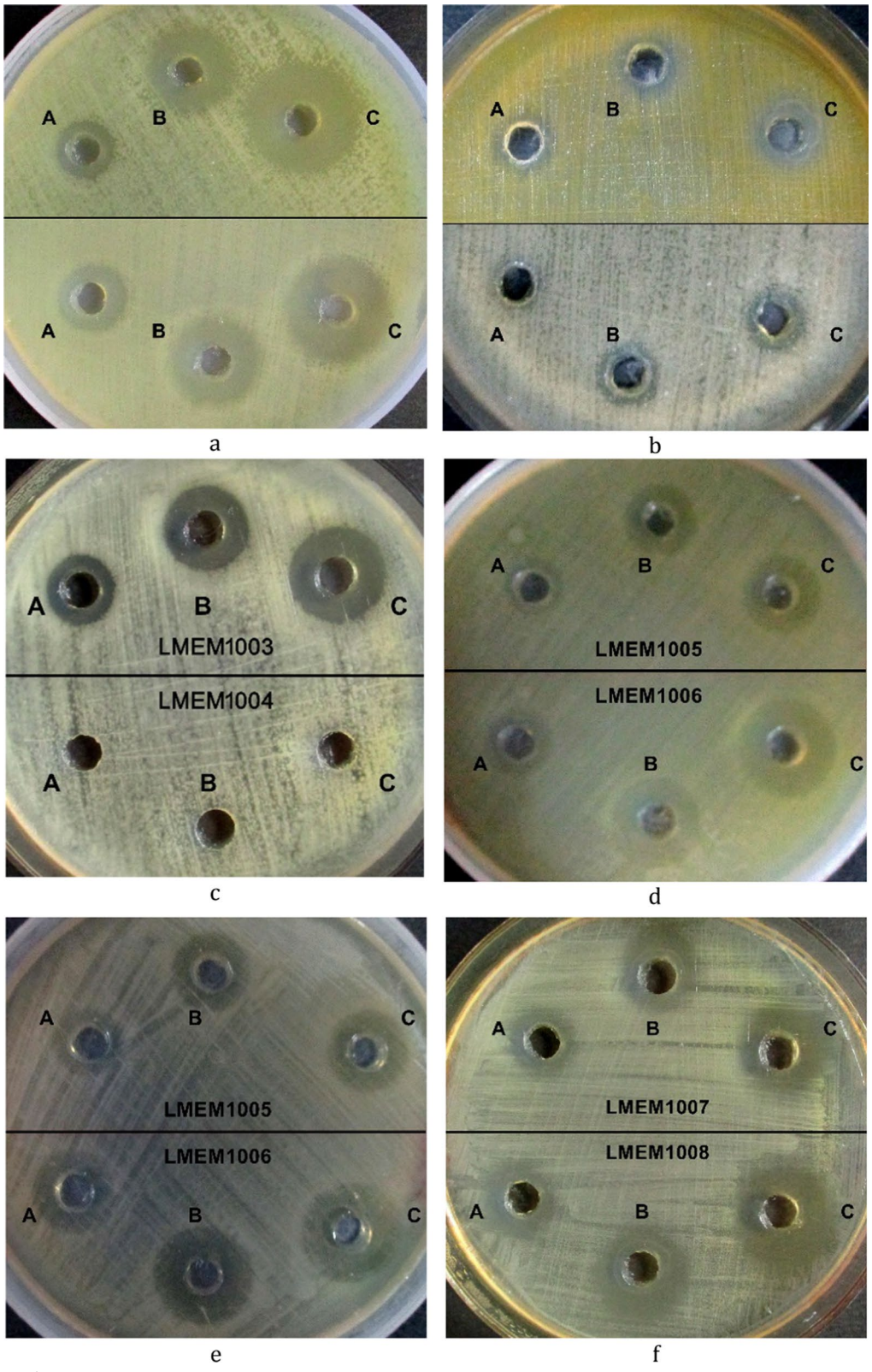

Fig. 5 (See legend on previous page.)

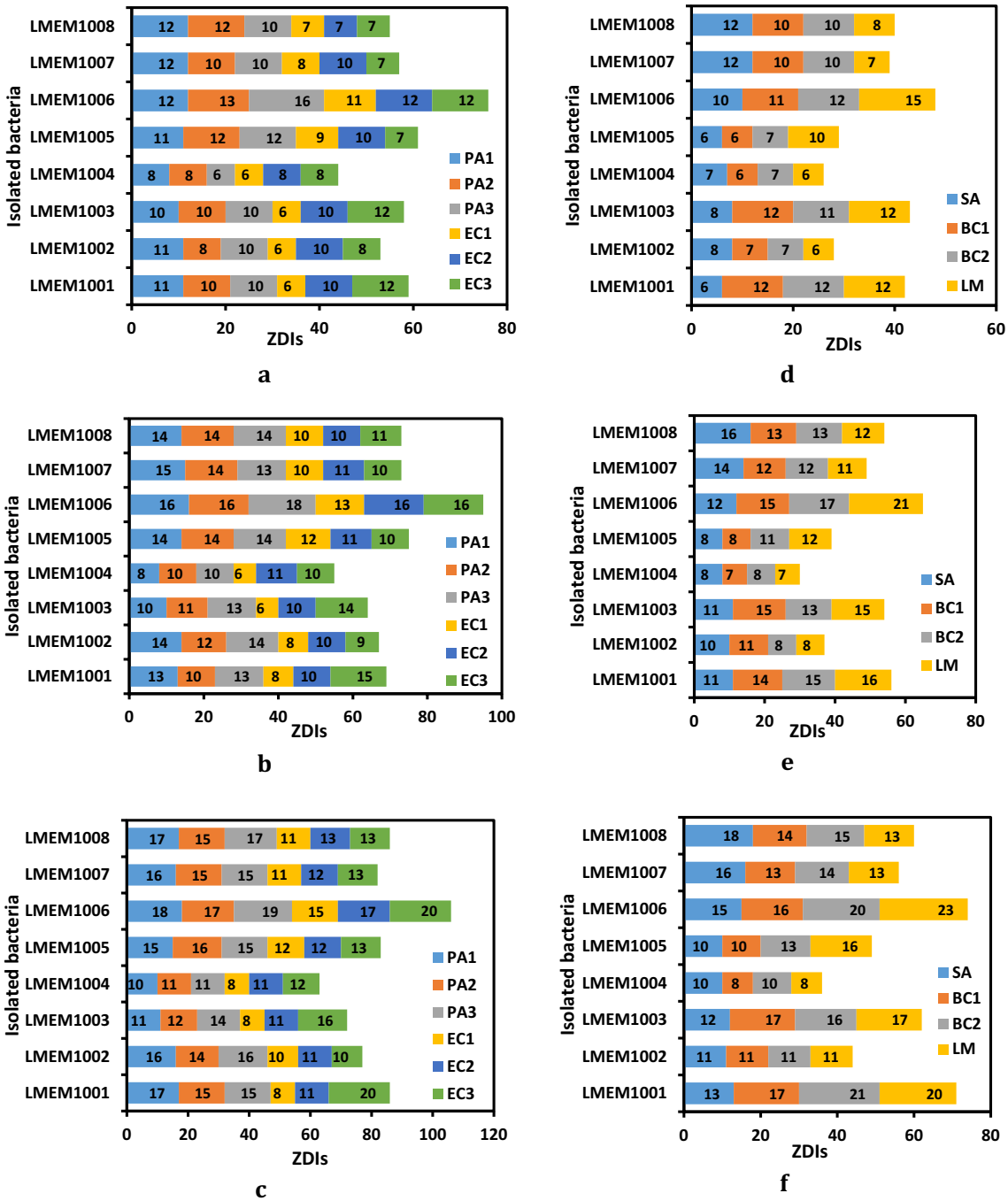

Fig. 6 The *L. pentosus* LMEM1006 isolate exhibited top growth inhibitory activity at highest concentration against indicator bacteria. **a**–**c** Antibacterial activity of isolated strains (*a*: 25, *b*: 50 and *c*: 75 µl/well) against test gram-negative bacteria, **d**–**f** Antibacterial activity of isolated bacteria (*d*: 25, *e*: 50 and *f*: 75 µl/well) against test gram-positive bacteria. PA1: *Pseudomonas aeruginosa* 1, PA 2: *P. aeruginosa* 2, PA3: *P. aeruginosa* 3, EC1: *Escherichia coli* 1, EC2: *E. coli* 2, EC3: *E. coli* 3, BC1: *Bacillus cereus* 1, BC2: *B. cereus* 2, SA: *Staphylococcus aureus*, LM: *Listeria monocytogenes* MTCC 657

to sulphamethizole. In the current study, isolates of idli lactobacilli were sensitive and intermediately sensitive to most of the antibiotics tested with a common resistance to *Cx*.

To validate probiotic, the cumulative probiotic potential (CPP) is a developed criterion for the native lactobacilli [40]. The CPP of *L. animalis* LMEM6 was 80%, and 100% showed by three isolates: *L. plantarum* LMEM7, *L.*

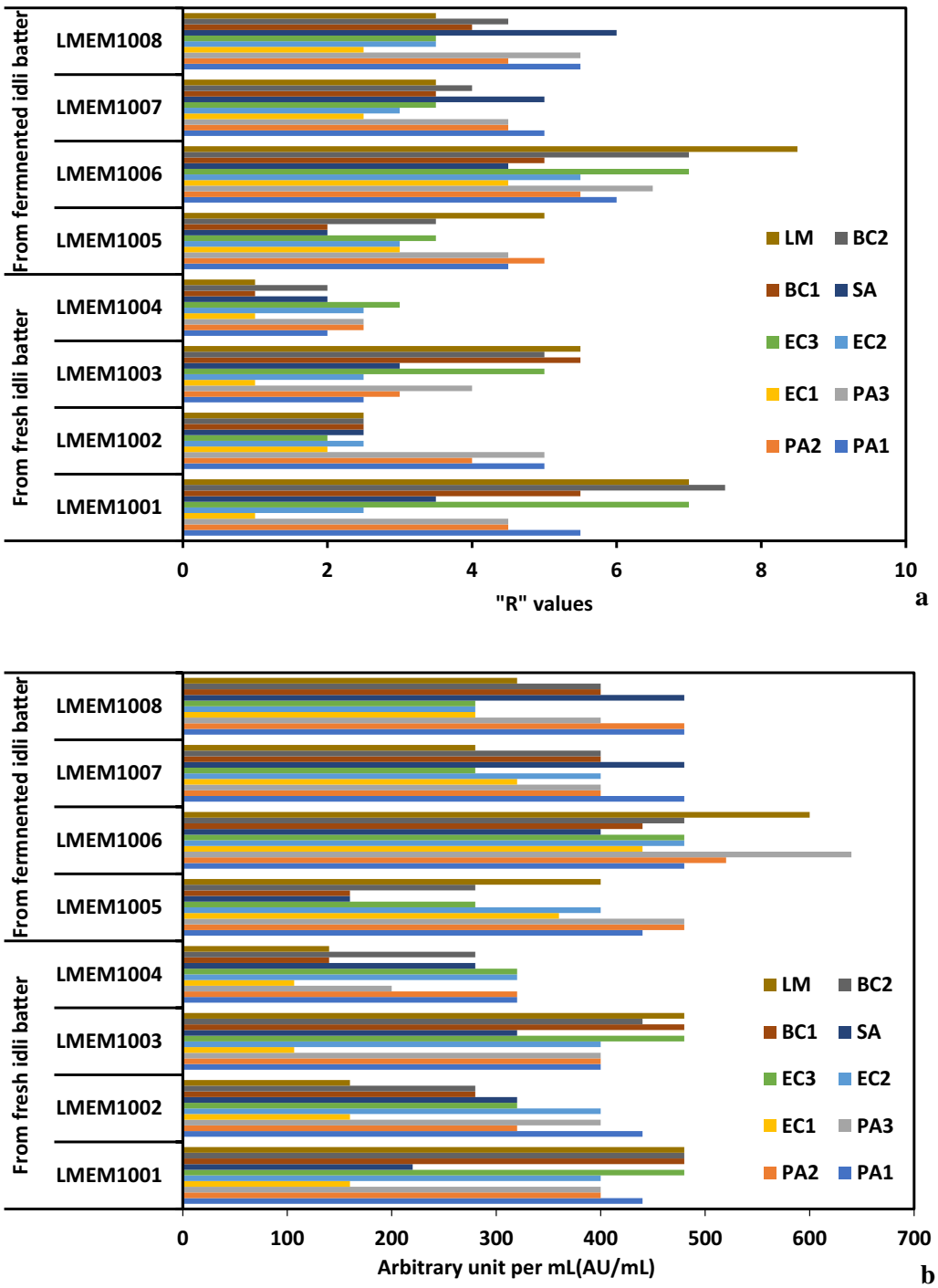

Fig. 7 The lactobacilli from idli fresh and fermented samples displayed antibacterial activity with "*R*" values 1–8.5, while growth inhibitory capacity of lactobacilli was recorded as 106.67-640 AU/mL at highest concentration, against the test bacterial pathogens. a: "*R*" values (mm) showed by lactobacilli against the test bacteria, b: Growth inhibitory capacity of lactobacilli expressed in "AU/mL" for indicator bacteria

acidophilus LMEM8 and *L. rhamnosus* LMEM9 isolated from curd samples [15]. Wadoum et al.'s [41] study revealed *Lactobacillus* isolated from faecal samples of chickens and ducks and had good CPP values (82% for *L. paracasei* MW-38CGZ, *L. plantarum* MW-48CGZ and 100% for *L. paracasei* MW-37CGZ, *L. plantarum* MW-18CGZ) as a potential probiotic fulfilling different probiotic properties. *L. brevis* UN isolated from Dhulliachar (which is a

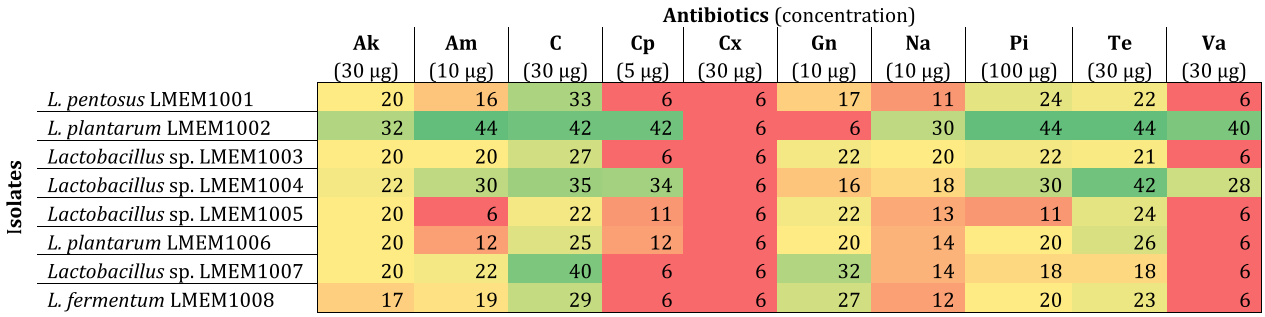

Fig. 8 Heatmap represents antibiotic susceptibility test results of isolated idli lactobacilli. Ak: Amikacin, Am: Ampicillin, C: Chloramphenicol, Cp: Ciprofloxacin, Cx: Cefoxitin, Gn: Gentamicin, Na: Nalidixic acid, Pi: Piperacillin, Te: Tetracyclines, Va: Vancomycin. ZDI: zone diameter of inhibition. Green gradient: antibiotic sensitive (greater ZDI values), yellow gradient: intermediately sensitive to antibiotics (ZDI values intermediate between sensitivity and resistance), red gradient: resistant (lesser ZDI values)

Table 4 Cumulative probiotic potential (CPP) score for the isolated idli lactobacilli

Individual Isolate Score									
	Indicator Score	***L. pentosus* LMEM1001**	***L. plantarum* LMEM1002**	***Lactobacillus* sp. LMEM1003**	***Lactobacillus* sp. LMEM1004**	***Lactobacillus* sp. LMEM1005**	***L. plantarum* LMEM1006**	***Lactobacillus* sp. LMEM1007**	***L. fermentum* LMEM1008**
Probiotic Characters									
Acidic pH tolerance	Resistant = 1 Sensitive = 0	1	1	1	1	1	1	1	1
Bile salt tolerance	Resistant = 1 Sensitive = 0	1	1	1	0	1	1	1	1
Temperature (45 °C) tolerance	Resistant = 1 Sensitive = 0	1	1	1	1	1	1	1	1
Sodium chloride (NaCl) tolerance	Resistant = 1 Sensitive = 0	1	1	1	1	1	1	1	1
Safety profile									
Gelatinase activity	Activity = 0 No activity = 1	1	1	1	0	1	1	1	1
DNase activity	Activity = 0 No activity = 1	1	1	1	1	1	1	1	1
Antibiotic sensitivity	Intrinsic resistance/ Sensitive = 1 Other resistance = 0	1	1	1	1	1	1	1	1
Bioactivity									
Antimicrobial activity	ZDI ≤ 15 mm = 0 ZDI > 15 mm = 1	1	1	1	0	1	1	1	1
Total Score		8	8	8	5	8	8	8	8
CPP		100%	100%	100%	62.5%	100%	100%	100%	100%

powdered mixture of seeds of *Cucurbita pepo* and *Sesamum indicum*) exhibited good CPP value of about 95.83% [64]. In the current study, all the idli lactobacilli strains had CPP value 100% except *Lactobacillus* sp. LMEM1004 and that means seven lactobacilli among eight, were eligible for the criteria [65], in defining the grade of a safe probiotic.

Conclusion

To overcome global bacterial antibiotic resistance, treatment or therapeutic measures depend on the antibiotic alternatives. Probiotic formulations could be a standard bio-weapon against bacterial infection. However, the isolation and screening of lactobacilli from various locally available non-dairy-based natural sources plausibly be a better choice to develop non-antibiotic antibacterials of medical relevance. However, further investigations are recommended in dose determination and particular efficacy of individual LAB strains.

Abbreviations

Ad	Adonitol
Ak	Amikacin
Am	Ampicillin
AMR	Antimicrobial resistance
AR	Antibiotic resistance
Ar	Arabinose
ATCC	American Type Culture Collection
AU/mL	Arbitrary unit per mL
BLAST	Basic Local Alignment Search Tool
C	Chloramphenicol
CDC	Centers for Disease Control and Prevention
Ced	Cephradine
Clox	Cloxacillin
CLSI	Clinical and Laboratory Standards Institute
Cp	Ciprofloxacin
CPP	Cumulative probiotic potential
Cfx	Cephalexin
Cx	Cefoxitin
Cxm	Cefuroxime
De	Dextrose
Du	Dulcitol
ECDC	European Centre for Disease Prevention and Control
ESKAPE	*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species
FAO	Food and Agriculture Organization
Fc	Fructose
Ga	Galactose
Gn	Gentamicin
h	Hour
In	Inulin
Is	Inositol
La	Lactose
LAB	Lactic acid bacteria
Ma	Maltose
Mec	Mecillinam
Mb	Melibiose
µg	Microgram
MDR	Multidrug-resistant
mm	Millimetre
mL	Millilitre
Mn	Mannitol
Mo	Mannose
MR	Methyl-red
MRS	De Man, Rogosa and Sharpe
MRSA	Methicillin-resistant *Staphylococcus aureus*
MTCC	Microbial Type Culture Collection and Gene Bank
Na	Nalidixic acid
NaCl	Sodium chloride
NCBI	National Center for Biotechnology Information
Nfn	Nitrofurantoin
Nor	Norfloxacin
Pi	Piperacillin
PCR	Polymerase chain reaction
PDR	Pandrug-resistant
Rf	Raffinose
Rfm	Rifampicin
Rh	Rhamnose
rRNA	Ribosomal ribonucleic acid
Sa	Salicin
Sb	Sorbitol
Su	Sucrose
Te	Tetracyclines
Tr	Trehalose
Va	Vancomycin
VP	Voges–Proskauer
WHO	World Health Organization
XDR	Extensively drug-resistant
Xy	Xylose
ZDI	Zone diameter of inhibition

Acknowledgements

Not applicable.

Author contributions

BS performed experimental works and wrote the paper; SM designed the study, analysed, discussed and approved the paper.

Funding

Not applicable.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare no conflict of interest.

Received: 3 March 2023 Accepted: 22 June 2023
Published online: 28 June 2023

References

1. Ghatak S, Armstrong CM, Reed S, He Y (2020) Comparative methylome analysis of *Campylobacter jejuni* strain YH002 reveals a putative novel motif and diverse epigenetic regulations of virulence genes. Front Microbiol 11:610395. https://doi.org/10.3389/fmicb.2020.610395
2. Touimi GB, Bennani L, Berrada S, Moussa B, Bennani B (2020) Prevalence and antibiotic resistance profiles of *Staphylococcus* Spp isolated from food, 9 food contact surfaces and food handlers in a Moroccan hospital kitchen. Lett Appl Microbiol 70(4):241–251. https://doi.org/10.1111/lam.13278
3. Medina E, Romero C, Brenes M, Castro AD (2007) Antimicrobial activity of olive oil, vinegar, and various beverages against foodborne pathogens. J Food Prot 70(5):1194–1199
4. Yi L, Qi T, Ma J, Zeng K (2020) Genome and metabolites analysis reveal insights into control of foodborne pathogens in fresh-cut fruits by *Lactobacillus pentosus* MS031 isolated from Chinese Sichuan Paocai. Postharvest Biol Technol 164:111150
5. IPPL (Indian Priority Pathogen List) DBT and WHO India, (2021). Link: https://dbtindia.gov.in/sites/default/files/IPPL_final.pdf (Dated 26 March, 2022)
6. Khan J, Ahmad I, Basharat N, Khan A, Sadiq A, Ali S, Sohail M, Khan AN, Ahmad I, Saddam S, Aziz A, Khan H, Ali I (2021) Prevalence, risk factors and antibiogram analysis of nosocomial infection in tertiary care hospital of Rawalpindi, Pakistan. Res Sq. https://doi.org/10.21203/rs.3.rs-145858/v1

7. Santajit S, Indrawattana N (2016) Mechanisms of antimicrobial resistance in ESKAPE pathogens. BioMed Res Int. https://doi.org/10.1155/2016/2475067
8. Fuller R (1989) Probiotic in man and animals: a review. J Appl Bacteriol 66(5):365–378
9. Vaughan A, Eijsink VG, O'Sullivan TF, O'Hanlon K, Sinderen DV (2001) An analysis of bacteriocins produced by lactic acid bacteria isolated from malted barley. J Appl Microbiol 91(1):131–138
10. Altuntas EG, Cosansu S, Ayhan K (2010) Some growth parameters and antimicrobial activity of a bacteriocin-producing strain *Pediococcus acidilactici* 13. Int J Food Microbiol 141(1–2):28–31
11. Karovicova J, Kohajdova Z (2003) Lactic acid fermented vegetable juices. Hortic Sci 30(4):152–158
12. Silva J, Carvalho AS, Teixeira P, Gibbs PA (2002) Bacteriocin production by spray-dried lactic acid bacteria. Lett Appl Microbiol 34(2):77–81
13. Urbonaviciene D, Viskelis P, Bartkiene E, Juodeikiene G, Vidmantiene D (2015) The use of lactic acid bacteria in the fermentation of fruits and vegetables—technological and functional properties, Chapter 7 in book Biotechnology. https://doi.org/10.5772/59938
14. Cai Y, Yuan W, Wang S, Guo W, Li A, Wu Y, Chen X, Ren Z, Zhou Y (2019) *In vitro* screening of putative probiotics and their dual beneficial effects: To white shrimp (*Litopenaeus vannamei*) post larvae and to the rearing water. Aqua 498:61–71. https://doi.org/10.1016/j.aquaculture.2018.08.024
15. Halder D, Mandal M, Chatterjee SS, Pal NK, Mandal S (2017) Indigenous probiotic *Lactobacillus* isolates presenting antibiotic like activity against human pathogenic bacteria. Biomedicines 5(2):31. https://doi.org/10.3390/biomedicines5020031
16. Yang H, Sun Y, Cai R, Chen Y, Gu B (2020) The impact of dietary fiber and probiotics in infectious diseases. Microb Pathog 140:103931. https://doi.org/10.1016/j.micpath.2019.103931
17. Patel A, Cecilia L, Arti P, Prajapati JB, Olle H (2012) Probiotic properties of exopolysaccharide producing lactic acid bacteria isolated from vegetables and traditional Indian fermented foods. Int J Fermented Foods 1(1):87–101
18. Reale A, Konietzny U, Coppola R, Sorrentino E, Greiner R (2007) The importance of lactic acid bacteria for phytate degradation during cereal dough fermentation. J Agric Food Chem 55(8):2993–2997
19. Akweya BA, Mghalu JM, Yusuf RUM, Bitange T (2020) Probiotic potential of lactic acid bacteria isolated from traditionally fermented legume products of Western Kenya. Afr J Food Sci 14(10):373–377
20. Hwang CE, Haque MA, Hong SY, Kim SC, Cho KM (2019) Origin of lactic acid bacteria in mulkimchi fermentation. J Appl Biol Chem 62(4):441–446
21. Kandasamy S, Sharma S, Halady PKS (2016) Isolation of fermentation micro flora from idli batter and their probiotic characterization. Int. Conference on Food, Energy and Water Microbiology. Periyar University, Salem. https://www.researchgate.net/publication/312218385
22. Agaliya PJ, Jeevaratnam K (2013) Molecular characterization of lactobacilli isolated from fermented idli batter. Braz J Microbiol 44(4):1199–1206
23. Halder D, Mandal S (2015) Curd lactobacilli with probiotic potentiality. Transl Biomed 6(2):1–6
24. Forbes AB, Sahm DF, Weissfeld AS (2007) Overview of bacterial identification methods and strategies. Bailey & Scott's diagnostic microbiology, 12th edn. Elsevier, Philadelphia, pp 311–322
25. Holt JG, Krieg NR (1984) Bergey's manual of systematic bacteriology. Williams and Wilkins, Baltimore
26. Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35(6):1547–1549
27. Nei M, Kumar S (2000) Molecular evolution and phylogenetics. Oxford University Press, New York
28. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biol Evol 4(4):406–425
29. Liong MT, Shah NP (2005) Acid and bile tolerance and cholesterol removal ability of lactobacilli strain. J Dairy Sci 88(1):55–66
30. Chowdhury A, Hossain MN, Mostazir NJ, Fakruddin Billah M, Ahmed M (2012) Screening of *Lactobacillus* spp. from buffalo yoghurt for probiotic and antibacterial activity. J Bacteriol Parasitol 3(8):156
31. Tagg JR, McGiven AR (1971) Assay system for bacteriocins. Appl Microbiol 21:943–944
32. Mandal S, Halder D (2018) Exploring anti-*Klebsiella pneumoniae* activity of probiotic lactobacilli of curd origin. Acta Sci Microbiol 1(4):49–53
33. Halder D, Mandal S (2016) Antibacterial potentiality of commercially available probiotic lactobacilli and curd lactobacilli strains, alone and in combination, against human pathogenic bacteria. Transl Biomed 7(2):61
34. Carasi P, Díaz M, Racedo SM, Antoni GD, Urdaci MC, Serradell MDLA (2014) Safety characterization and antimicrobial properties of kefir-isolated *Lactobacillus kefiri*. Biomed Res Int 2:1–7
35. Pisano MB, Viale S, Conti S, Fadda ME, Deplano M, Melis MP, Deiana M, Cosentino S (2014) Preliminary evaluation of probiotic properties of *Lactobacillus* strains isolated from Sardinian dairy products. Biomed Res Int 2:1–8. https://doi.org/10.1155/2014/286390
36. Dela Cruz TEE, Torres JMO (2012) Gelatin hydrolysis test protocol. American Society for Microbiol. pp 1–10
37. Bauer AJ, Kirby WM, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by standardized single disc method. Am J Clin Pathol 45:493–496
38. Clinical and Laboratory Standards Institute (CLSI) (2020) Performance standards for antimicrobial susceptibility testing, CLSI supplement M100. Wayne, PA. 30th edition
39. Sircar B, Mandal S (2017) Indian Olive, *Elaeocarpus floribundus* Fruit: perspective to the antioxidative capacity and antibacterial activity. EC Microbiol 12(6):273–282
40. Tambekar DH, Bhutada SA (2010) An evaluation of probiotic potential of *Lactobacillus* sp. from milk of domestic animals and commercially available probiotic preparations in prevention of enteric bacterial infections. Recent Res Sci Technol 2(10):82–88
41. Wadoum REG (2019) *In vitro* antimicrobial characterization of *Lactobacillus* Isolates towards their use as probiotic alternatives to antibiotic growth promoters. Int J Microbiol Biotechnol 4(3):72–86
42. Magiorakos AP (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 18(3):268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x
43. Asokan GV, Ramadhan T, Ahmed E, Sanad H (2019) WHO Global priority pathogens list: a bibliometric analysis of Medline-pubmed for knowledge mobilization to infection prevention and control practices in Bahrain. Oman Med J 34(3):184–193
44. De Oliveira DMP, Forde BM, Kidd TJ, Harris PNA, Schembri MA, Beatson SA, Paterson DL, Walker MJ (2020) Antimicrobial resistance in ESKAPE pathogens. Clin Microbiol Rev 33:e00181-e219. https://doi.org/10.1128/CMR.00181-19
45. Halder D, Mandal M, Pal NK, Mandal S (2020) Insights into the broad antibacterial spectrum of bacteriocin isolated from probiotic *Lactobacillus fermentum* LMEM22 Strain, 14 July, Preprint (Version 1) available at Research Square. https://doi.org/10.21203/rs.3.rs-41220/v1
46. Thakur M, Deshpande HW, Bhate MA (2017) Isolation and identification of lactic acid bacteria and their exploration in non-dairy probiotic drink. Int J Curr Microbiol App Sci 6(4):1023–1030
47. Shaikh A, Siddique I, Shaikh M, Shetty S (2021) Isolation and enumeration of probiotic microorganisms from fermented idli batter. Afr J Bio Sc 3(3):59–63. https://doi.org/10.33472/AFJBS.3.3.2021.59-63
48. Teli M, Mahajan S, Mahajan I, Rajput J, Mahajani S (2017) Screening of *Lactobacillus* from Curd and Idli Batter for Production of Bacteriocins. Int J Adv Sci Eng Technol 5(2):30–32
49. Sharma M, Samtiya M, Rana A, Dhewa T, Mishra V (2022) A preliminary study to evaluate the physicochemical and microbial population dynamics of a blend of pearl millet flour and buttermilk during spontaneous fermentation. J Food Diets Res 1(3):1–5. https://doi.org/10.25259/JFDR_13_2021
50. Iyer BK, Singhal RS, Ananthanarayan L (2013) Characterization and in vitro probiotic evaluation of lactic acid bacteria isolated from idli batter. J Food Sci Technol 50:1114–1121. https://doi.org/10.1007/s13197-011-0445-6
51. Mandhania MH, Paul D, Suryavanshi MV, Sharma L, Chowdhury S, Diwanay SS, Diwanay SS, Shouche YS, Patole MS (2019) Diversity and succession of microbiota during fermentation of the traditional Indian food idli. Appl Environ Microbiol 85(13):e00368-e419. https://doi.org/10.1128/AEM.00368-19
52. Kadirvelu J (2012) A novel antibacterial compound, 2-hydroxy indole propanamide produced by *Lactobacillus plantarum* isolated from idli batter with probiotic potential. J Biotechnol Biomater 2(6):97. https://doi.org/10.4172/2155-952X.S1.013

53. Sahadeva RPK, Leong SF, Chua KH, Tan CH, Chan HY, Tong EV, Wong SYW, Chan HK (2011) Survival of commercial probiotic strains to pH and bile. Int Food Res J 18(4):1515–1522
54. Thakkar P, Modi HA, Prajapati JB (2015) Isolation, characterization and safety assessment of lactic acid bacterial isolates from fermented food products. Int J Curr Microbiol App Sci 4(4):713–725
55. Balasingham K, Valli C, Radhakrishnan L, Balasuramanyam D (2017) Probiotic characterization of lactic acid bacteria isolated from swine intestine. Vet World 10(7):825–829
56. Syrokou MK, Paramithiotis S, Drosinos EH, Bosnea L, Mataragas M (2022) Comparative genomic and safety assessment of six *Lactiplantibacillus plantarum* subsp. Argentoratensis strains isolated from spontaneously fermented Greek wheat sourdoughs for potential biotechnological application. Int J Mol Sci 23(5):2487. https://doi.org/10.3390/ijms23052487
57. Sjovall J (1959) On the concentration of bile acids in the human intestine during absorption, bile acids and sterioids 74. Acta Physiol Scand 46(4):339–345
58. Katepogu H, Wee YJ, Almaary KS, Elbadawi YB, Gobinath R, Chinni SV, Lebaka VR (2022) Isolation and characterization of *Pediococcus* sp. HLV1 from fermented idly batter. Fermentation 8(2):61. https://doi.org/10.3390/fermentation8020061
59. Saravanan C, Shetty PH (2014) Screening of Antagonistic Activity and Antibiotic Resistance of Microflora Isolated from Idli Batter. J Pure Appl Microbiol 8(5):4195–4199
60. Vidhyasagar V, Jeevaratnam K (2013) Evaluation of *Pediococcus pentosaceus* strains isolated from idly batter for probiotic properties *in vitro*. J Funct Foods 5(1):235–243
61. Dubey AK, Jeevaratnam K (2015) Probiotic screening of Lactobacilli isolates from Uttapam batter fermented supplementing with *Piper betle* L. leaves. Adv Microbiol 5(13):858–870. https://doi.org/10.4236/aim.2015.513090
62. Shehata MG, Sohaimy SAE, El-Sahn MA, Youssef MM (2016) Screening of isolated potential probiotic lactic acid bacteria for cholesterol lowering property and bile salt hydrolase activity. Ann Agric Sci 61(1):65–75
63. Liu X, Liu W, Zhang Q, Tian F, Wang G, Zhang H, Chen W (2013) Screening of lactobacilli with antagonistic activity against enteroinvasive *Escherichia coli*. Food Control 30(2):563–568
64. Gautam N, Sharma N (2015) Characterization of bacteriocin producer "*Lactobacillus brevis* un" as potential probiotic strain. J Microbiol Biotech Food Sci 5(3):216–220
65. FAO and WHO (2002) Guidelines for the Evaluation of probiotics in food: Report of a joint FAO/WHO working group on drafting guidelines for the evaluation of probiotics in food; Food and agriculture organization; World Health Organization: London, ON, Canada

Publisher's Note