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Research Article

SCREENING OF POTENTIAL PROBIOTIC *LACTOBACILLUS* STRAINS ISOLATED FROM FERMENTED FOODS, FRUITS AND OF HUMAN ORIGIN

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ABSTRACT

Objective: To screen and characterize *Lactobacillus* strains having potential probiotic properties from fermented foods and fruits, and to investigate cell surface characteristics and antimicrobial activity against food-borne and gastrointestinal tract (GIT) pathogens.

Methods and Results: In the present study, twenty five *Lactobacillus* strains were isolated from fruits, fermented foods and human origin on De Man Rogosa and Sharpe (MRS) agar plates and identified on the basis of their phenotypic characteristics and 16S rDNA sequencing. Isolated *Lactobacillus* strains tolerated inhibitory substances like bile (up to 4%), NaCl (up to 8%), phenol (up to 0.6%) and low pH (2.0). Six of the isolates exhibited inhibitory activity against food-borne and GIT pathogens by well diffusion method. The isolates appeared to possess hydrophilic cell surface as determined by microbial adhesion to n-hexadecane. Isolates auto-aggregated and also co-aggregated with pathogens tested. The isolates were not susceptible to antibiotics like vancomycin, tobramycin, kanamycin and norfloxacin while were highly susceptible to chloramphenicol, clindamycin, erythromycin, oleandomycin and penicillin G. None of the isolates showed haemolytic activity.

Conclusion: Six strains (*Lactobacillus paracasei* HML1, *L. paracasei* CR7, *L. plantarum* BRMV1, *L. plantarum* F1, *L. plantarum* OC6 and *L. rhamnosus* Di7) showed potential probiotic properties as well as strong *in vitro* antibacterial activity against various food-borne and gastrointestinal tract (GIT) pathogens.

Keywords: Antimicrobial activity, *Lactobacillus*, Acid tolerance, Bile tolerance, Hydrophobicity, Probiotics

INTRODUCTION

Lactic acid bacteria (LAB) are one of the groups of microorganisms that dominate fermented foods [1]. LAB are a part of human microbiota and widely used as starter cultures in food industry [2]. From the health point of view, ingestion of live cells of certain species and strains of lactobacilli in adequate amounts is believed to confer several beneficial physiological effects on the host [3] such as maintaining a healthy and equilibrated intestinal microbiota and reducing incidence of intestinal infection [4]. Various metabolic and enzymatic activities of LAB lead to production of volatile substances, which contribute to flavor, aroma and texture developments [5]. The *Lactobacillus* genus consists of a genetically and physiologically diverse group of rod-shaped, Gram-positive, non-spore forming, nonpigmented [6], catalase negative and microaerophilic to strictly anaerobic [7]. LAB are considered as "generally recognized as safe" (GRAS) organisms and can be safely used for medical and veterinary applications [8]. Certain LAB strains characterized by their ability to transform lactose improve the digestibility of fermented dairy products [9] as well as their preservation [10]. The ability of LAB to produce probiotics [11] and stimulate the immune system [12] renders this group of microorganisms essentially important for dairy industry.

The criteria for the *in vitro* selection of lactobacilli to be used as health-promoting, probiotic ingredients, in food and pharmaceutical preparations include antibiotic tolerance as well as the production of lactic acid that inhibits the growth of other microorganisms, which allow them to be established in the intestinal tract [13]. Bile tolerance [14] and gastric juice resistance [15] are other important characteristics of probiotic lactic acid bacteria used as adjuncts enabling them to survive, grow and exert their beneficial influence in the gastrointestinal tract (GIT). Although the degree of tolerance required for maximum growth in the GIT is not known, it seems reasonable that the most bile and acid-resistant species should be selected [16].

Adhesion to intestinal epithelial cells is an important prerequisite for colonization of probiotic strains in the GIT, preventing their immediate elimination by peristalsis and providing a competitive advantage in this ecosystem [17]. Adherence of bacterial cells is related to cell surface characteristics [18]. Bacterial aggregation between microorganisms of the same strain (auto-aggregation) or between different species and strains (co-aggregation) is of considerable importance in several ecological niches, especially in the human gut where probiotics are to be active [19]. Auto-aggregation of probiotic strains appear to be necessary for adhesion to intestinal epithelial cells, and co-aggregation abilities may form a barrier that prevents colonization by pathogenic microorganisms [20]. A correlation between adhesion ability and hydrophobicity, as measured by microbial adhesion to hydrocarbons, has been reported for some lactobacilli and physicochemical characteristics of the cell surface such as hydrophobicity may affect auto-aggregation and adhesion of bacteria to different surfaces [21, 22]. The proteinaceous nature of some surface components has been demonstrated, and surface layer (S-layer) proteins detected in some *Lactobacillus* strains may be involved in adherence [23, 24].

Probiotics can be used in the treatment and prevention of enteric infections and chronic inflammatory disorders of the GIT [25]. They are non-pathogenic, acid and bile tolerant, adhere to gut epithelial tissue and produce antimicrobial substances, including organic acids, hydrogen peroxide and bacteriocins [26]. Bacteriocins are proteinaceous antibacterial compounds that are bactericidal to many pathogens associated with food spoilage and food borne illnesses including *Escherichia coli, Salmonella sp., Shigella sp., Bacillus cereus, Clostridium botulinum, Staphylococcus aureus,* etc [27]. They are degraded by the proteolytic action of GIT and seem to be non-toxic and non-antigenic to the animals. Thus, bacteriocins can be used to enhance the safety and shelf life of many foods [28].

The aim of this study was to screen *Lactobacillus* strains from fermented foods, fruits and healthy humans and to determine tolerance of each probiotic strain to bile salts and acidity to demonstrate its survivability in the small intestine and colon to contribute in the balance of the intestinal microbiota. Moreover, their antimicrobial activity against pathogens, antibiotic susceptibility, cell surface characteristics (hydrophobicity, salt aggregation, auto-aggregation, and co-aggregation abilities), and

haemolytic activity was also investigated to screen potential probiotic isolates.

MATERIALS AND METHODS

Sample collection

A total of nine samples were collected from human origin (infant feces and oral cavity), fruits (grapes, orange, sweetlime and pine-apple) and fermented foods (cheese, curd and shrikhand). *Lactobacillus* strains were isolated using De Man Rogosa and Sharpe (MRS) broth [29] and preserved in 10% skim milk at 4°C.

Isolation of lactic acid bacteria

The samples were aseptically weighed (1 g) and enriched in MRS broth for 48 h. From each sample, a 1:10 dilution was subsequently made using sterile phosphate buffer saline (PBS: pH 7.0. 0.1 M, containing 0.85% (w/v) NaCl) followed by making a 10 fold serial dilution. 0.1 ml from the appropriate dilutions were plated on MRS agar and incubated at 37°C for 24-48 h. Individual isolates from countable MRS agar plates were randomly-picked, representatives from all morphologically distinct colonies and were sub-cultured and isolated 3 times on the MRS agar medium. Pure strains, as judged by microscopic observations for homogeneity of cellular morphology, were maintained in 10% skim milk at 4°C. Eighty five *Lactobacillus* strains were isolated from the natural sources and fermented foods, and 25 isolates were further tested for Gram reaction, catalase, oxidase, motility and cell morphology according to the methods described by Kebede et al. [30]. 25 isolates that were Gram-positive, catalase-negative, very short to very long rods, occurring singly, in pairs or in chains, were randomly selected as presumptive LAB.

Identification of the bacterial strains

The cultures were identified according to their morphological, cultural, physiological and biochemical characteristics [31, 32].

Phenotypic characterization

The growth of bacterial strains at 15 and 45°C was visually confirmed by the changes in turbidity of MRS broth after 24 and 48 h of incubation. MRS broth tubes containing inverted Durham's tubes were used for the evaluation of gas production. Evaluation of ammonia production, citrate utilization and acetoin production was performed by peptone nitrate broth, Simmon's citrate agar (Himedia, India), and glucose phosphate broth, respectively.

Carbohydrates fermentation by *Lactobacillus* strains

Fermentation of sugars was assessed by using MRS broth devoid of glucose and beef extract with Andrade's indicator. Carbon sources were added individually to this medium as filter sterilized solutions to a final concentration of 1%. Carbohydrate utilization was determined after 48 h of incubation.

Screening of potential probiotic *Lactobacillus* strains

In vitro assessment of probiotic *Lactobacillus* strains involve resistance to low pH, bile salt, NaCl and phenol. Bile salt, pH, and salt tolerance (sodium chloride) was evaluated as described by Ambalam et al. [33] with some modifications.

MRS broth varying in pH (2-4), bile salt (1-4%) and NaCl (2-8%) was inoculated with 0.1 ml of 18 h old culture of *Lactobacillus s*trains and incubated at 37°C for 24 h. The growth of microorganisms was visually evaluated. Tolerance to low pH, bile salt and NaCl was evaluated by inoculating 50 µl of culture from respective tube to MRS broth. Phenol tolerance was tested by cultivating the organisms in skim milk containing 0.4 and 0.6% phenol. All the tubes including control were incubated (37°C, 24 h) and observed for growth as development of turbidity. In the case of skim milk-phenol medium, clotting of milk was considered as positive growth.

Molecular identification

Molecular identification of six isolates HML1, F1, OC6, Di7, CR7 and BRMV1 having potential probiotic properties was carried out. Identification of these isolates was determined on the basis of 16S rDNA gene sequence. The DNA of isolates was extracted and amplified using PCR with 8f (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492r (5'-GGT TAC CTT GTT ACG ACT T-3') universal primers according to Turner et al [34], using BDT v3.1 cycle sequencing kit on ABI 3730xl genetic analyzer. Consensus sequence of approximately 1300 bp 16S rDNA gene were generated from forward and reverse sequence data using aligner software. Alignment of the 16S-rRNA sequence was conducted by using the BLASTN program from NCBI web site (http://www.ncbi.nlm.nih.gov). Based on maximum identity score, the sequences were selected and aligned using multiple alignment software program Clustal W.

Acid and bile tolerance

Acid and bile tolerance was determined as described previously by Liong MT et al. (2005) with certain modifications [35]. In brief, the cell density of each culture grown in MRS broth was adjusted to A_{620} of 2.0 in pre-reduced MRS broth with pH 2.5 adjusted with 2 M HCl, and incubated at 37°C for 90 min. 100 µl of culture broth at time 0, 30, 60 and 90 min was serially diluted, spreaded on MRS agar and incubated at 37°C for 48 h to determine the viable cell counts and expressed as log cfu/ml. For bile tolerance, the cell density of each strain was adjusted to an A_{620} of 2.0 in a pre-reduced MRS broth with 1 and 2% bile salts (Himedia, India) and incubated at 37°C for 4 h. 100 µl of culture broth at time 0 and 4 h was serially diluted and plated on MRS agar as above.

Hydrophobicity

Microbial adhesion to solvents (MATS) was measured according to the method of Rosenberg et al. with some modifications [36-37]. Bacteria growing in the stationary phase were centrifuged at 5000 *g* for 15 min, washed twice, and resuspended in 0.1 M KNO_3 (pH 6.2) to approximately 10^8 cfu/ml. The absorbance of the cell suspension was measured at 620 nm (A_0). 1 ml of solvent (n-hexadecane, chloroform and ethyl acetate) was added to 3 ml of cell suspension. After 10 min pre-incubation at room temp., the two-phase system was mixed by vortexing for 2 min. The aqueous phase was removed after 20 min of incubation at room temperature, and its absorbance at 620 nm (A_1) was measured. The percentage of bacterial adhesion to solvent was calculated as $(1-A_1/A_0) \times 100$. Cell surface characteristics of *Lactobacillus* strains were determined with reference to standard strain *Lactobacillus* rhamnosus GG (ATCC 53103).

Salt Aggregation Test

The cell surface hydrophobicity of lactobacilli was also determined by salt aggregation test (SAT) as previously described [38]. 10 µl aliquot of fresh cell suspension in PBS was mixed on a glass slide with 10 µl of ammonium sulphate (pH 6.8) of various molarities (0.02-4 M). The formation of cell aggregates was observed after 1 min by visual reading. The lowest concentration of ammonium sulphate giving visible aggregation was scored as the SAT hydrophobicity value.

Auto-aggregation

Strains were grown in MRS broth for 24 h at 37°C. The cells were harvested, washed and resuspended in sterile PBS and adjusted to A_{620} of 1. After 60 min, the cultures were centrifuged at 300×*g* for 2 min at 20°C and the A_{620} was recorded. Auto-aggregation was determined using the following equation.

$$\% \text{ Auto - aggregation} = [(A_{t0}-A_{t60})/A_{t0}] \times 100$$

A_{t0} refers to the initial A_{620}, and A_{t60} refers to the A_{620} determined after 60 min [39].

Co-aggregation

Lactobacillus strains grown in 10 ml MRS broth at 37°C, and pathogenic strains grown in 10 ml nutrient broth at 37°C were used. Cells were harvested after 24 h, washed, resuspended in sterile PBS

and A_{620} of 1 was adjusted. 1 ml of each cell suspension was transferred and the A_{620} recorded over 60 min using spectrophotometer. Cells were harvested at 300×*g* for 2 min at 20°C and the A_{620} of the supernatant was determined. Co-aggregation was calculated using the following equation.

% Co-aggregation = $[(A_{tot}-A_s)/A_{tot}] \times 100$

A_{tot} refers to the initial A_{620} taken immediately after the relevant strains were paired. A_s refers to the A_{620} of the supernatant after 60 min.

Antimicrobial Activity

Antimicrobial effects of presumptive strains of *Lactobacillus* strains on food-borne and GIT pathogens were determined by agar diffusion method [40]. *Enterobacter aerogenes* MTCC 111, *Salmonella typhi* MTCC 98, *Serratia marcescens* MTCC 97, *Pseudomonas aeruginosa* MTCC 2587, *Bacillus cereus* MTCC 430, *Bacillus subtilis* MTCC 441, *Escherichia coli* MTCC 1697 and *Staphylococcus aureus* MTCC 1144 were obtained from MTCC (Microbial Type Culture Collection Centre, Chandigarh, India). *Bacillus megaterium, Shigella sp., Klebsiella pneumonia and Micrococcus leutus* were obtained from Government Hospital, Rajkot, India. All pathogens were maintained on Nutrient agar (Himedia, India) slants at 4°C. From this slant loopful culture was inoculated in Nutrient broth and for activation, incubated at 37°C for 18 h.

Lactobacillus strains were inoculated in MRS broth and incubated at 37°C for 24 h. The cultures were centrifuged (10,000 rpm, 20 min, 4°C) and supernatant was filtered through a 0.45 µ Millipore filter. Sterile filtrate was used as cell free culture (CFC) filtrate. Furthermore, to rule out the possibility of organic acids accounting for the inhibitory action, the CFC of the LAB isolates were neutralized with 1 M NaOH and the remaining activity determined. Briefly, the test pathogen culture (A_{620}-0.2) was added in molten nutrient agar and poured in sterile Petriplate and allowed to solidify. Wells measuring 7 mm were made and filled with 100 µl of CFC filtrate and neutralized CFC filtrate. The plates were pre-incubated at 4°C for 2 h to allow diffusion of the sample before incubating the plates at 37°C for 24 h and the diameter of the inhibition zone was measured. Acetate buffer (pH 4.5, 10 mM) was used as control.

Haemolytic activity

The isolates were streaked on MRS agar supplemented with 5% blood and incubated at 37°C for 72 h and observed for haemolytic activity [41].

Antibiotic susceptibility test

The antibiotic susceptibility of isolated *Lactobacillus* strains was determined using antibiotic discs (Himedia Laboratories Pvt. Ltd. Mumbai, India) on MRS agar plates. The antibiotic discs were placed on agar surface and incubated at 37°C for 24 h [42]. The diameter (mm) of inhibition zones was measured. The antibiotics used in the study were ampicillin (10 µg), cephalothin (30 µg), chloramphenicol (30 µg), clindamycin (2 µg), erythromycin (15 µg), gentamicin (10 µg), oxacillin (1 µg), vancomycin (30 µg), cefaloridin (30 µg), kanamycin (30 µg), lincomycin (2 µg), methicillin (5 µg), norfloxacin (10 µg), oleandomycin (15 µg), Penicillin G (10 µg) and tobramycin (10 µg).

RESULTS

Isolation of *Lactobacillus* strains

Twenty five *Lactobacillus* strains were isolated (Table 1) that formed round, creamy white colonies on MRS agar plate. Isolates were divided into three groups on the basis of their ability to grow at 15 and 45°C. (1) Group 1: '*Thermobacteria*' (2) Group 2: '*Streptobacteria*' and (3) Group 3: '*Betabacteria*'. Morphological, cultural, physiological and biochemical characteristics of the isolates were determined for their identification (Table 2). The isolates showed varying sugar fermentation pattern (Table 3-5). *Lactobacillus* strains isolated from food origin CR7, Di3, HML1, BRMV1, Di7 and SpiG3 fermented inulin.

Table 1: It shows source of sample of different *Lactobacillus* strains

Source of Sample	*Lactobacillus* strains
Cheese	Di3, HML1, Di7, BM3 & BM8
Curd	CR2, CR7, CG1 & CL3
Shri-khand	BRMV1, SMG6, SPiG1, SPiG3, SPL3 & SML3
Human oral cavity	OC1, OC6
Infant feces	F1
Grapes	GG1 & GG2
Orange	Ora& OG3
Pine-apple	PL1
Sweet-lime	SW & SWL1

Table 2: It shows phenotypic characterization of *Lactobacillus* strains

	Group 1 Thermo bacteria	**Group 2 Strepto bacteria**	**Group 3 Beta bacteria**
***Lactobacillus* strains**	**BM3, BM8, SML3, SPiG3, SPiG1**	**Di3, HML1, CR7, CR2, BRMV1, F1, SMG6, OC6, Di7, OC1**	**CL3, SW, Ora, PL1, SPL3, GG2, SWL1, GG1, CG1, OG3**
Growth at	45°C	15°C	15°C and 45°C
Gram Reaction	Positive	Positive	Positive
Catalase	Negative	Negative	Negative
Motility	Non-motile	Non-motile	Non-motile
Ammonia	Negative	Negative	Negative
Citrate	Negative	Negative	Negative
Acetoin	Negative	Negative	Negative
Gas from glucose	Negative	OC1	Ora, CL3, CG1 & SW

Table 3: It shows sugar fermentation pattern of *Lactobacillus* strains belonging to Group 1

Sugars	BM3	BM8	SML3	SPiG3	SPiG1
Arabinose	-	-	-	-	-
Mannitol	-	-	-	+	+
Xylose	-	-	-	-	-
Rhamnose	-	-	-	-	-
Ribose	+	+	-	+	+
Sorbitol	-	-	-	+	-
Fructose	+	+	+	+	+
Galactose	+	+	+	+	+
Glucose	+	+	+	+	+
Lactose	+	+	+	+	+
Sucrose	+	+	+	+	+
Cellobiose	-	-	-	+	+
Esculin	+	+	+	+	+
Maltose	+	+	+	+	+
Mannose	+	+	+	+	+
Melibiose	+	+	+	-	+
Raffinose	+	+	+	-	-
Salicin	-	-	-	+	+
Trehalose	-	-	+	+	+
Starch	-	-	-	-	-
Inulin	-	-	-	+	-

Sugar fermenter (+), Sugar non-fermenter (-)

Screening of potential probiotic *Lactobacillus* strains

Growth and survival of *Lactobacillus* strains in the presence of bile salt, low pH, NaCl and phenol is shown in Tables (6-8).

Influence of pH

The isolates were able to grow at pH 4. The only isolate CR7 showed growth at pH 3 and the rest except CR2 and GG1 only survived.

Table 4: It shows sugar fermentation pattern of *Lactobacillus* strains belonging to Group 2

Sugars	Di3	HML1	CR7	CR2	BRMV1	SMG6	F1	OC6	Di7	OC1
Arabinose	-	-	-	-	-	-	-	-	+	-
Mannitol	+	+	+	+	+	+	+	+	+	-
Xylose	-	-	-	+	-	-	+	-	-	-
Rhamnose	-	-	-	-	-	-	-	-	+	-
Ribose	+	+	+	+	+	+	+	+	+	+
Sorbitol	+	+	+	-	+	+	+	+	+	-
Fructose	+	+	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+
Cellobiose	+	+	+	-	+	+	+	+	+	-
Esculin	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+	+	+	+
Melibiose	-	-	-	-	-	-	+	-	-	+
Raffinose	-	-	-	-	-	-	+	-	-	+
Salicin	+	+	+	+	+	+	+	+	+	-
Trehalose	+	+	+	-	+	+	+	+	+	-
Starch	-	-	-	-	-	-	-	-	-	-
Inulin	+	+	+	-	+	-	-	-	+	-

Sugar fermenter (+), Sugar non-fermenter (-)

Table 5: It shows sugar fermentation pattern of *Lactobacillus* strains belonging to Group 3

Sugars	CL3	SW	Ora	PL1	SPL3	GG2	SWL1	GG1	CG1	OG3
Arabinose	-	-	-	-	-	-	+	-	-	-
Mannitol	+	-	+	+	+	+	+	+	+	+
Xylose	+	-	-	-	-	-	-	-	+	-
Rhamnose	-	-	-	-	-	-	-	-	-	-
Ribose	+	+	+	+	+	+	+	+	+	+
Sorbitol	+	-	-	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+
Cellobiose	+	-	+	+	+	+	+	+	+	+
Esculin	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+	+	+	+
Melibiose	+	-	+	+	-	-	+	+	+	+
Raffinose	-	+	-	-	-	-	-	-	-	+
Salicin	+	-	+	+	+	+	+	+	+	+
Trehalose	+	-	+	+	+	+	+	+	+	+
Starch	-	-	-	-	-	-	-	-	-	-
Inulin	-	-	-	-	-	-	-	-	-	-

Sugar fermenter (+), Sugar non-fermenter (-)

Isolates CR7, HML1, F1, BRMV1, OC6 and Di7 did not grow but survived and retained viability at pH 2.

Influence of bile salt

The Thermobacteria BM3 and SPiG1 were able to grow in the presence of 4% bile while the isolates only survived except SML3. The Streptobacteria Di3, HML1, Di7 and OC1 and the isolates belonging to Betabacteria, were able to grow in MRS containing 4% bile salts except SW which survived in the presence of 4% bile salts.

Influence of NaCl

Isolates CR2, Di7, BRMV1, CL3, PL1, GG2, SWL1, GG1 and OG3 were able to grow at 8% NaCl while the other isolates survived in MRS containing 8% NaCl. All the isolates were able to grow in the presence of 4% NaCl.

Influence of Phenol

Isolates CR2, CR7, OC6, Ora, CL3, CG1 and SPL3 were able to grow at 0.6% phenol and rest of the isolates were able to grow at 0.4% and survived at 0.6% phenol.

Table 6: It shows growth and survival of *Lactobacillus* strains Group 1 in the presence of bile salt, NaCl, pH and phenol after 24 h.

S.No.	*Lactobacillus* strains	Bile Salt (%)			NaCl (%)				pH			Phenol (%) in Skim milk	
		1	2	4	2	4	6	8	2	3	4	0.4	0.6
1	BM3	+	+	+	+	+	S	S	-	S	+	+	S
2	BM8	+	S	S	+	+	S	S	-	S	+	+	S
3	SML3	+	S	-	+	+	S	S	-	S	+	+	S
4	SPiG3	+	+	S	+	+	+	S	-	S	+	+	S
5	SPiG1	+	+	+	+	+	+	S	-	S	+	+	S

Growth (+); Survival (S); No survival (-)

Table 7: It shows growth and survival of *Lactobacillus* strains Group 2 in the presence of bile salt, NaCl, pH and phenol after 24 h.

S. No.	*Lactobacillus* strains	Bile Salt (%)			NaCl (%)				pH			Phenol (%) in Skim milk	
		1	2	4	2	4	6	8	2	3	4	0.4	0.6
1	Di3	+	S	-	+	+	S	-	-	S	+	+	S
2	HML1	+	+	S	+	+	+	S	S	S	+	+	S
3	F1	+	+	+	+	+	+	S	S	S	+	+	S
4	CR7	+	+	+	+	+	+	S	S	+	+	+	+
5	CR2	+	+	+	+	+	+	+	-	-	+	+	+
6	BRMV1	+	+	+	+	+	+	+	S	S	+	+	S
7	SMG6	+	+	+	+	+	+	S	-	S	+	+	S
8	OC6	+	+	+	+	+	+	S	S	S	+	+	+
9	Di7	+	+	S	+	+	+	+	S	S	+	+	S
10	OC1	+	+	S	+	+	S	S	-	S	+	+	S

Growth (+); Survival (S); No survival (-)

Table 8: It shows growth and survival of *Lactobacillus* strains Group 3 in the presence of bile salt, NaCl, pH and phenol after 24 h.

S. No.	*Lactobacillus* Strains	Bile Salt (%)			NaCl (%)				pH			Phenol (%) in Skim milk	
		1	2	4	2	4	6	8	2	3	4	0.4	0.6
1	CL3	+	+	+	+	+	+	+	-	S	+	+	+
2	SW	+	+	S	+	+	S	S	-	S	+	+	S
3	Ora	+	+	+	+	+	+	S	-	S	+	+	+
4	PL1	+	+	+	+	+	+	+	-	S	+	+	S
5	SPL3	+	+	+	+	+	+	S	-	S	+	+	+
6	GG2	+	+	+	+	+	+	+	-	S	+	+	S
7	SWL1	+	+	+	+	+	+	+	-	S	+	+	S
8	GG1	+	+	+	+	+	+	+	-	-	+	+	S
9	CG1	+	+	+	+	+	+	S	-	S	+	+	+
10	OG3	+	+	+	+	+	+	+	-	S	+	+	S

Growth (+); Survival (S); No survival (-)

Molecular identification

Molecular identification of the isolates was done on the basis of 16S rDNA sequence analysis. Isolates HML1 and CR7 were identified as *Lactobacillus paracasei*, BRMV1, F1 and OC6 were identified as *Lactobacillus plantarum* and Di7 was identified as *Lactobacillus rhamnosus.* The GenBank accession numbers for the sequences are KC884268 (*Lactobacillus paracasei* HML1) and KC884269 (*Lactobacillus plantarum* BRMV1).

Acid tolerance

Isolates Di7, BRMV1 and HML1 showed >90% and >80% viability after 30, 60 and 90 min of exposure to pH 2.5 respectively (Figure 1). Isolate OC6 showed 88% viability after 30 min while the viability was upto 79% after 60 and 90 min of exposure to pH 2.5. As compared to other isolates, isolate CR7 showed viability upto 50% after 90 min. Viability of CR7 decreased rapidly in the first 30 min and then it decreased very little.

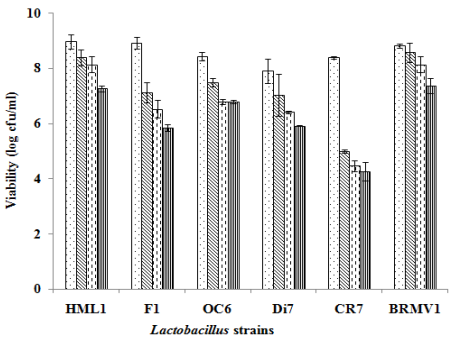

Fig. 1: It shows survival (log cfu/ml) of *Lactobacillus* strains after 0(□), 30(▨), 60(▩) & 90(▥) minutes of exposure to pH 2.5 as determined by viable plate count method on MRS medium. Error bar indicates the standard deviation (SD) of three independent experiments.

Bile tolerance

Survival of six *Lactobacillus* strains after 4 h exposure to 1 and 2% bile salt is shown in Figure 2. Isolates HML1, F1 and BRMV1 showed 100% viability in both 1 and 2% bile salt compared to the other three strains. Viability of OC6 decreased to approximately 90% in both 1 and 2% bile salt. Viability of Di7 and CR7 decreased by one order of magnitude in the presence of 1 and 2% bile salt. Di7 and CR7 showed 90 and 74% viability in presence of 1% bile salt, while 69 and 58% viability in presence of 2% bile salt respectively.

Hydrophobicity

Three different solvents were tested in this study: an apolar solvent, hexadecane, a monopolar and acidic solvent, chloroform, and a monopolar and basic solvent, ethyl acetate. Microbial adhesion to hexadecane reflects cell surface hydrophobicity or hydrophilicity because electrostatic interactions are absent. The values of hydrophobicity obtained with the two other solvents, chloroform and ethyl acetate, were regarded as a measure of electron donor/basic and electron acceptor/acidic characteristics of bacteria, respectively. Furthermore, it should be noted that all these three solvents have similar van der waals properties.

First, direct measurements of the cell surface hydrophobicity and hydrophilicity were carried out by the partitioning of cells between aqueous and hexadecane partitions. The very low percentages of bacteria which adhered to this apolar solvent, ranging from 8.5 to 25.5%, demonstrated a hydrophilic surface, regardless of the *Lactobacillus* strains tested. As compared to other isolates, *L. plantarum* BRMV1, *L. plantarum* F1 and *L. plantarum* OC6 showed 25.5, 16.2 and 13.0% hydrophobicity.

The results showed an overall strong affinity of lactobacilli to chloroform, an acidic solvent and electron acceptor (Table 9). These higher values of adhesion were compared with those obtained for hexadecane because both solvents possess the same van der waals properties. The important difference observed was due to the implication of Lewis acid-base interactions resulting from the electron donor and basic character of bacterial strains. The data obtained for ethyl acetate, a strongly basic solvent and electron

donor, produced results contrary to those encountered with chloroform: the bacterial adhesion to this third solvent was low, ranging from 0.9 to 15.1%. It confirmed the nonacidic cell surface character of the bacterial strains studied.

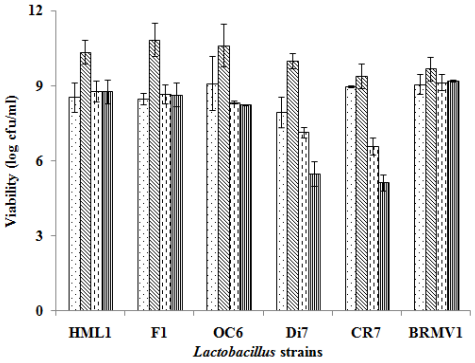

Fig. 2: It shows survival (log cfu/ml) of *Lactobacillus* strains after 4 h of exposure to bile salt (1 and 2%) as determined by viable plate count method on MRS medium. Error bar indicates the standard deviation (SD) of three independent experiments. 0 h, control (□), 4 h, control (▨), 4 h, 1% bile salt (▤) and 4 h, 2% bile salt (▥)

Table 9: It shows cell surface hydrophobicity of *Lactobacillus* strains by microbial adhesion to solvents assay

Lactobacillus strains	% adhesion (± SD)[a] to:		
	n-hexadecane	chloroform	ethyl acetate
HML1	9.1 ± 1.6	62.0 ± 2.2	0.9 ± 0.1
F1	16.2 ± 0.9	52.7 ± 2.7	4.5 ± 0.6
OC6	13.0 ± 1.8	46.2 ± 0.8	8.6 ± 1.1
Di7	8.5 ± 0.1	59.2 ± 5.1	9.5 ± 1.2
CR7	8.9 ± 0.1	63.5 ± 5.6	7.2 ± 3.1
BRMV1	25.5 ± 4.0	61.9 ± 3.4	7.8 ± 5.4
GG	10.0 ± 0.3	84.1 ± 4.9	15.1 ± 4.3

[a] shows ± standard deviations of three independent experiments

Salt Aggregation Test

Bacterial strain OC6 showed high hydrophobicity by SAT assay with a minimum ammonium sulfate concentration of 0.02 M and isolate CR7 showed lowest hydrophobicity with 4 M ammonium sulfate (Table 10).

Table 10: It shows % Auto-aggregation after 1 h and salt aggregation test (SAT) value of *Lactobacillus* strains

Lactobacillus strains	(%)Auto-aggregation (± SD)[a]	SAT value[b]
HML1	86.77 ± 1.97	1.6
F1	86.4 ± 1.35	3.2
OC6	88.37 ± 0.4	0.02
Di7	85.63 ± 0.64	0.8
CR7	82.0 ± 1.75	4
BRMV1	88.53 ± 0.21	3.2
GG	72.1 ± 0.4	0.8

[a] shows ± standard deviations of three independent experiments; [b] shows SAT value was scored as the lowest concentration of ammonium sulfate giving visible aggregation after 1 min

Auto-aggregation and Co-aggregation

Bacterial aggregation between microorganisms of the same strain (auto-aggregation) or between genetically different strains (co-aggregation) is of considerable importance in several ecological niches, especially in the human gut. All *Lactobacillus* strains showed high auto-aggregation abilities (Table 10). In the present study, auto-aggregation of *Lactobacillus* strains ranged from 72-88%. Among all the isolates, *L. rhamnosus* GG showed lowest auto-aggregation (72%) and *L. plantarum* OC6 and *L. plantarum* BRMV1 showed highest auto-aggregation (88%) abilities. *Lactobacillus* strains showed higher coaggregation with Gram positive microbes as compared to Gram negative microbes. The *Lactobacillus* strains coaggregated with *Serratia marcescens* and *Enterococcus faecalis* more and with *Staphylococcus aureus* less (Table 11).

Table 11: It shows % Co-aggregation of *Lactobacillus* strains with Gram positive and Gram negative pathogens after 1 h of co-incubation

% Co-aggregation (±SD)[a]							
	HML1	**F1**	**OC6**	**Di7**	**CR7**	**BRMV1**	**GG**
Gram Positive organisms							
Staphylococcus aureus	46.2 ± 5.5	42.2 ± 2.6	55.6 ± 2.5	31.7 ± 4.2	50.6 ± 3.4	40.4 ± 4.3	58.1 ± 3.6
*Micrococcus leutus**	60.6 ± 2.0	71.0 ± 2.2	60.3 ± 1.1	52.9 ± 3.2	71.1 ± 2.6	62.5 ± 2.0	75.8 ± 2.2
*Bacillus megaterium**	57.8 ± 3.4	60.0 ± 3.9	53.3 ± 4.1	57.1 ± 3.4	53.1 ± 4.2	69.6 ± 2.4	69.7 ± 1.2
Bacillus cereus	56.1 ± 2.5	70.0 ± 0.2	42.5 ± 1.3	67.2 ± 2.0	50.6 ± 0.8	35.3 ± 2.7	44.6 ± 1.2
Bacillus subtilis	60.9 ± 5.5	70.2 ± 3.2	36.5 ± 2.8	67.7 ± 1.5	59.5 ± 2.0	40.7 ± 0.4	27.0 ± 2.8
Gram Negaitive organisms							
Pseudomonas aeruginosa	42.6 ± 3.9	65.0 ± 1.9	48.2 ± 5.1	54.7 ± 5.0	57.6 ± 2.6	51.0 ± 0.6	68.2 ± 0.6
*Proteus vulgaris**	43.4 ± 1.0	69.9 ± 1.5	53.6 ± 4.0	45.1 ± 4.9	49.6 ± 3.1	55.3 ± 1.8	37.3 ± 3.8
*Shigella sp.**	69.9 ± 5.1	68.2 ± 3.8	58.4 ± 2.6	80.2 ± 0.4	55.0 ± 5.5	57.5 ± 2.6	54.6 ± 3.8
Escherichia coli	71.9 ± 2.1	74.5 ± 1.9	78.3 ± 3.3	77.6 ± 4.7	57.1 ± 0.2	60.5 ± 2.0	77.0 ± 0.5
Serratia marcescens	72.8 ± 3.7	73.1 ± 0.2	68.3 ± 4.5	78.3 ± 0.7	72.6 ± 1.9	73.5 ± 0.6	75.7 ± 0
*Enterococcus faecalis**	63.4 ± 4.1	76.7 ± 3.0	68.5 ± 2.2	77.0 ± 1.9	73.6 ± 3.0	76.2 ± 0.7	77.6 ± 4.9

[a] shows ± standard deviations of three independent experiments; *shows clinical strains were obtained from Government Hospital, Rajkot, India. Other strains were obtained from MTCC (Microbial Type Culture Collection Centre, Chandigarh, India)

Antimicrobial activity

Supernatants of the cultures of *Lactobacillus* strains were investigated for antibacterial activity against pathogenic bacteria. All the isolates exhibited higher antimicrobial activity against Gram positive organisms as compared to Gram negative organisms except CR7 (Table 12). Among Gram positive organisms, *Lactobacillus* strains showed the higher inhibitory effect against *Micrococcus luteus* (≥20 mm) while the least activity was found against *Bacillus subtilis* (≤18). In case of Gram negative organisms, all the isolates exhibited high antimicrobial activity against *Shigella sp.* (≥18) while the least activity was demonstrated against *Salmonella typhi* (≤15) and *Enterobacter aerogenes* (≤16).

Neutralized CFC filtrate of six of the isolates HML1, F1, OC6, Di7, CR7 and BRMV1 showed inhibitory effect against few pathogens. All six isolates showed inhibitory effect against *E. coli*. Neutralized CFC filtrate of *Lactobacillus paracasei* HML1 exhibited inhibitory activity against *B. subtilis, B. cereus, B. megatarium*, *M. luteus* and *Ent. aerogenes*. While inhibitory effect of neutralized CFC filtrate of *L. plantarum* BRMV1 was also found against *Ent. aerogenes*. As compared to other isolates, these six isolates showed high antimicrobial activity against pathogens.

Table 12: It shows antimicrobial spectrum of CFC filtrate of *Lactobacillus* strains (zone of inhibition in mm including 7 mm well)

	Gram positive microbes					Gram negative microbes						
Isolates	***B. cereus***	***B. megaterium***	***B. subtilis***	***M. leutus***	***Staph. aureus***	***K. pneumoniae***	***P. aeruginosa***	***E. coli***	***Ser. marcescens***	***S. typhi***	***Shigella sp.***	***Ent. aerogens***
BM3	17	14	11	21	15	17	12	15	17	12	18	13
BM8	12	12	12	20	18	11	12	12	14	13	19	0
SML3	14	15	16	20	18	12	12	13	16	13	18	13
SPiG3	19	17	12	21	12	13	14	14	13	11	18	13
SPiG1	18	18	17	22	21	15	19	19	19	12	20	12
Di3	12	15	13	21	17	17	14	14	17	12	20	14
HML1*[a]	16	19	14	22	17	17	15	17	16	15	18	14
F1*	19.5	18	16	24	17	18.5	16	17	18	12	20	16
OC6*	18	18	16	23.5	18	18	16	13	19	13	20	16
Di7*	16	17	15	20	20	16	12	16	17	15	21	0
OC1	14	16	16	20	19	0	12	0	13	13	19	14
CR2	18	15	11	21	17	14	14	13	14	12	19	13
CR7*	14	13	12	20	13	15	13	15	16	14	24	12
BRMV1*[b]	20	19	18	23	20	18	13.5	19	18	15	22	15
SMG6	17	15	13	21	19	11	12	11	15	11	20	12
CL3	16	16	15	22	18	21	13	14	18	14	23	11
SW	12	15	12	21	14	12	11	12	13	13	21	0
Ora	11	13	12	20	16	15	12	14	17	11	18	0
PL1	17	16	18	23	19	12	19	15	17	15	20	12
SPL3	13	14	12	22	18	14	14	15	19	13	18	0
GG2	14	16	13	21	15	13	12	13	12	14	21	0
SWL1	16	17	13	24	18	14	19	15	17	15	20	14
GG1	18	18	18	26	18	16	20	18	17	15	20	12
CG1	19	13	12	23	17	16	19	19	18	15	23	14
OG3	16	18	16	22	16	14	19	18	19	15	25	14

*** Neutralized CFCs of isolates HML1, F1, OC6, Di7, CR7 and BRMV1 also showed inhibitory activity against *E. coli*; [a] Neutralized CFC of *Lactobacillus paracasei* HML1 showed inhibitory activity against *Bacillus* spp., *Ent. aerogens* and *M.leutus;* [b] Neutralized CFC of *L. plantarum* BRMV1 showed inhibitory activity against *Ent. aerogens.***

Table 13: It shows antibiotic susceptibility of *Lactobacillus* strains (zone of inhibiton in mm)

Antibiotics (µg)	**HML1**	**CR7**	**BRMV1**	**F1**	**OC6**	**Di7**
Inhibitors of cell wall synthesis						
Penicillins						
Penicillin G (10)	24	21	17	20	24	25
Ampicillin (10)	15	17	NS	15	21	22
Methicillin (5)	11	10	NS	NS	NS	13
Oxacillin (1)	18	18	10	18	19	24
Cephalosporins						
Cephalothin (30)	17	16	19	NS	15	20
Cephaloridine (30)	14	15	18	14	19	NS
Glycopeptides						
Vancomycin (30)	NS	NS	NS	NS	NS	NS
Inhibitors of protein synthesis						
Aminoglycosides						
Gentamycin (10)	10	11	11	18	13	10
Kanamycin (30)	NS	10	NS	NS	10	NS
Tobramycin (10)	NS	13	11	NS	12	NS
Tetracyclines						
Chloramphenicol (30)	20	20	19	20	20	21
Lincomycins						
Clindamycin (2)	26	18	14	26	18	25
Lincomycin (2)	10	13	NS	10	10	12
Macrolides						
Oleandomycin (15)	24	23	24	22	22	27
Erythromycin (15)	24	24	22	24	22	23

Inhibitors of nucleic acid synthesis						
Norfloxacin (10)	10	NS	NS	NS	NS	12

NS-Not susceptible

Haemolytic activity

None of the isolates showed haemolytic activity on blood agar.

Antibiotic susceptibility

Susceptibility of *Lactobacillus* strains to sixteen antibiotics was determined. Vancomycin did not inhibit the growth of most of the isolates tested. The isolates were less susceptible to tobramycin, kanamycin and norfloxacin while highly susceptible to the antibiotics chloramphenicol, clindamycin, erythromycin, oleandomycin and penicillin G indicated by the size of inhibition zone. As compared to other isolates, BRMV1 and F1 were observed to be non-susceptible to six of the antibiotics (Table 13).

DISCUSSION

Lactobacilli populating in the human body such as oral cavity, GIT, fruits and fermented foods are an important source of probiotics. Therefore, focus of the present study was to screen *Lactobacillus* strains belonging to different habitats such as human origin strains, fruits and fermented foods. It is indispensible to define novel probiotic candidates by *in vitro* characterization from large number of strains. Many studies have reported human origin strains, however it would be noteworthy to assay the functional features of lactobacilli, which originate from the fruits and Indian fermented foods such as raw cheese, shrikhand and curd.

Twenty-five isolates that were Gram-positive rods, non-motile, non-spore forming and catalase negative were considered lactobacilli. All the strains were further identified by phenotypic characterization such as sugar fermentation profile and growth pattern at 15 and 45°C. Another striking feature of the isolates CR7, Di3, HML1, BRMV1, Di7 and SpiG3 is their ability to ferment prebiotic such as Inulin. Such property would be beneficial when considering the probiotic strains to be used in the preparation of synbiotics and confer competitive advantage over other microbes by selective utilization of nutrient source. Molecular identification of potent probiotic strains was done by 16S rDNA sequence analysis. Potential probiotics strains obtained from different habitats such as oral cavity, infant feces, shrikhand, raw cheese and curd belonging to the species *L. plantarum* OC6, *L. plantarum* F1, *L. plantarum* BRMV1, *L. paracasei* CR7, *L. paracasei* HML1 and *L. rhamnosus* Di7 respectively.

The probiotic strain should possess healthy origin and must be non-pathogenic; lactobacilli have a long history of being safe for humans and have been conferred GRAS status. An important step towards the selection of probiotic candidate is to investigate the strain behavior under condition, mimicing the GIT. Second, the strain has to survive in harsh condition during gastrointestinal transit; gastric fluid is the crucial barrier to overcome prior to reaching the site of action [43], which enable their viable passage through the GIT allow them to establish and multiply in the existing nutritional and ecological conditions. Isolates were evaluated under *in vitro* conditions for their probiotic properties *viz.* acid, bile, NaCl and phenol tolerance, antagonistic activity, auto-aggregation, co-aggregation and cell surface characteristics. OC6, F1, BRMV1, CR7, HML1 and Di7 isolates were able to tolerate pH 2 even after exposure of 24 h where as other strains tolerated up to pH 3. Such survival would be promising as probiotic bacteria are not directly exposed to such low pH [44]. Acid tolerance of the isolates is not only important to withstand the gastrointestinal environment but also for their use as dietary adjuncts in acidic food preparations [45].

Ingested microbes must endure numerous environmental extremes to survive in the human GIT, such as after overcoming the harsh condition of gastric fluid, they exposed to bile salt. Bile acids are secreted from gall bladder into the duodenum, helpful in the digestion of fat. The bile salt concentration fluctuates from 1.5 to 2% (w/v) in the first hour of digestion, and decreases thereafter to *ca*. 0.3% [46]. Our isolates OC6, F1, BRMV1, and CR7 able to grow in the presence of 4% bile salt, while HML1 and Di7 tolerated 4% bile salt.

NaCl tolerance of the strains is essential for their survival during processing of fermented vegetable which is generally carried out in the presence of *ca*. 6-8% NaCl. It shows similarities with the findings of Elezete and Carlos [47], in case of lactobacilli isolated from GIT of swine that tolerated to 4-8% NaCl. Such potential would be important in food processing, food preservation and confer competitive advantage over other undesirable organisms. In addition to this the strains also tolerated 0.6% phenol, which is a toxic metabolite produced upon deamination of some aromatic amino acids during putrefaction by intestinal bacteria [48].

The functional advantages of the isolated strains are their potential probiotic features such as acid, bile salt, NaCl and phenol tolerance regardless of their habitat differences. Such positive merits of the isolated strains enable their viable passage through GIT.

Adherence of bacterial cells is usually related to cell surface characteristics. Cell surface hydrophobicity is a nonspecific interaction between microbial cells and host. The initial interaction may be weak, often reversible and precedes subsequent adhesion processes mediated by more specific mechanisms involving cell surface proteins and lipoteichoic acids [49-50]. The determination of microbial adhesion to hexadecane as a way to estimate the ability of a strain to adhere to epithelial cells is a valid qualitative phenomenological approach [51]. The results indicated that the cell surface of microorganisms studied were relatively hydrophilic. In our present studies *L. plantarum* showed higher hydrophobicity than *L. rhamnosus* GG, similar results were recorded by Todorov and dicks; 2008 [52]. Microbial adhesion to two other solvents was also investigated. All lactobacilli tested here displayed maximal affinity for an acidic solvent such as chloroform and low affinity for a basic solvent such as ethyl acetate. These results demonstrated that lactobacilli are strong electron donors and weak electron acceptors, as confirmed by their hydrophilic cell surface properties. In other words, lactobacilli have a strong basic and a weak acidic character.

Aggregation is an important feature for biofilm formation. However, co-aggregation between LAB and other cells, especially *Listeria monocytogenes*, may be considered a positive characteristic, as it is one of the steps required for the elimination of non desirable strains from the GIT. Auto-aggregation and co-aggregation are strain-specific and most probably involve species-specific surface proteins. In our studies, *L. plantarum* BRMV1 and *L. plantarum* OC6 showed higher autoaggregating abilities (88%), and other isolates showed >70%. *L. plantarum* has a number of genes encoding for surface proteins that could function in recognition of, or binding to components in the environment. Several of these genes are homologous to proteins with predicted functions, such as mucus binding, aggregation promoting and intracellular adhesion [53]. Furthermore, it has been suggested that inhibitor producing lactic acid bacteria, which co-aggregate with pathogens, may constitute an important host defence mechanism against infection in the urogenital tract [54]. Also, a similar protective mechanism could operate in the GIT [55]. Co-aggregation with potentially gut pathogens could therefore contribute to the probiotic properties ascribed to LAB.

Lactobacilli exhibited broad antimicrobial spectrum against Gram-positive and Gram-negative organisms, including food-spoilage organisms and human pathogens, which is observed to be multifactorial and attributed to extracellular metabolites as evidenced from the cell free culture filtrate of the isolates. The antimicrobial activity is due to presence of various metabolites such as organic acids (lactic and acetic acid), H_2O_2, and or other antibacterial molecules such as bacteriocins, and low molecular peptides. Retention of antimicrobial activity even after neutralization of CFC provides the evidence of the presence of antimicrobial peptides other than organic acid, which act synergistically. Antimicrobial activity of *Lactobacillus* strains against pathogens has also been reported earlier [56]. Probiotic supplements (*Bifidobacteria spp* and *Lactobacilli)* are known to improve resistance to gut infections by inhibiting the growth of

harmful bacteria, to reduce cholesterol levels, improve the immune response and produce vitamins [57]. Moreover, antioxidant and lipid lowering effect of *L. acidophilus* has been reported [58] which will be a better therapy option for atherosclerosis and dyslipidemia. None of the isolated *Lactobacillus* strains showed haemolytic activity, indicating absence of haemolysis.

Safety of the strains intended to be used as probiotic must evaluated for antibiotic susceptibility profile and verification of antibiotic resistance genes. Our isolates exhibited similar antibiotic susceptibility pattern with few exceptions. None of them were susceptible to vancomycin. All are non-susceptible to kanamycin, methicillin, norfloxacin and tobramycin. Resistance of the probiotic strains to some antibiotics could be used for both preventive and therapeutic purposes in controlling intestinal infections. Moreover, their resistance to antibiotics was clarifying their potential in minimizing the negative effects of antibiotic therapy on the host bacterial ecosystem [59]. This investigation revealed that, some antibiotics such as chloramphenicol, clindamycin, erythromycin, oleandomycin and penicillin G intake can drastically drop the *Lactobacillus* spp. from intestinal microflora, on the other hand four antibiotics *viz*. tobramycin, kanamycin, norfloxacin and vancomycin will not influence the growth of lactobacilli population. The antibiotic resistance data supports that all the isolates are different with respect to antibiotic sensitivity pattern.

CONCLUSIONS

In conclusion, isolated *Lactobacillus* strains are from natural sources like human origin, fruits and fermented foods possess potential probiotic properties as evidenced by its ability to resist low pH, growth in the presence of bile salt. These characteristics may be advantageous for a probiotic culture to be successful in colonizing and compete with pathogens in gastrointestinal environment. These indigenous probiotic isolates possess antagonistic activities against Gram positive as well as Gram negative food-borne and GIT pathogens, and is beneficial both in food industries and in medical sector. Our results indicate that the ability to auto-aggregate, together with cell surface hydrophobicity and co-aggregation abilities with pathogen strains, can be used for preliminary screening in order to identify potentially probiotic bacteria suitable for human or animal use.

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