The Pharma Innovation Journal 2022; SP-11(7): 200-206

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(7): 200-206

www.thepharmajournal.com
Received: 11-05-2022
Accepted: 14-06-2022

Malashree
Assistant Professor, Department of Dairy Microbiology, Dairy Science College, Karnataka Veterinary Animal and Fisheries Sciences University, Bangalore, Karnataka, India

Akshaykumar
Scientist, Department of Animal Science, Indian council of Agriculture Research (ICAR)-Krishi Vigyan Kendra (KVK), Janawada Road, Bidar, Karnataka, India

Corresponding Author
Malashree
Assistant Professor, Department of Dairy Microbiology, Dairy Science College, Karnataka Veterinary Animal and Fisheries Sciences University, Bangalore, Karnataka, India

In vitro evaluation of probiotic properties of lactic acid bacteria isolated from human and dairy origin

Malashree and Akshaykumar

Abstract
Probiotics are live microorganisms which when consumed in large number together with a food promote the health of the consumer. The aim of this study was to evaluate *in vitro* probiotic properties of lactic acid bacteria (LAB) isolated from Human feces and dairy products such as raw milk and dahi samples. A total of 40 isolates were selected from the samples. These isolates of Human origin (H1 –H20) and dairy origin (D1-D20) were initially screened as Gram-positive, rods, catalase-negative isolates. Human isolates H4, H8, H9, H10, H14 and D6, D7, D9, D12, D13 confirmed the *Lactobacillus* genus identification. Further these 10 isolates were subjected for *in vitro* probiotic properties. Isolate H9 and D12 were able to survive at pH 3.0 for 120 min as indicated by log counts up to 5.01 to 5.2, whereas D9 showed low viability at pH 2.0 at 120 min. For bile tolerance study isolate H9, H10, D12 and D13 were able to tolerate 1% bile for 3 hrs with a log count varying from 6 to 6.3 whereas D9 was found to be bile sensitive with log count of 2.8. Almost all the isolates were moderately or highly sensitive towards all the antibiotics used in this study, except ofloxacin. All the isolates were resistant to vancomycin except H9 and D9. Maximum cell surface hydrophobicity was observed for D9 (52%) while it was minimum for H9 (4%). In case of antibacterial activity against pathogenic bacteria, majority of the isolates showed inhibition against *S. aureus* and *E. coli* (73%) followed by B. cereus and S. dysenteriae (70%) and S. typhi (33%). Only 3 out of 10 isolates namely H4, H9 and D7 exhibited a moderate level of BSH activity (++) as indicated by the dense precipitation of the sodium taurodeoxycholate. Among 10 isolates 4 (H4, H8, H9, H14) were identified as *Lactobacillus plantarum* with the amplification of 248 bp product using primer L pla 3/ L pla 2.The sequence analysis showed that D6, D7, D9, D12, and D13 belonged to *Lactobacillus rhamnosus*, whereas H10 as *L. plantarum*. Studies revealed that the *Lactobacillus plantarum* and *Lactobacillus rhamnosus* strains were found to be potentially useful to produce probiotic products.

Keywords: Human origin, dairy products, lactic acid bacteria, probiotics and lactic cultures

Introduction
Lactic acid bacteria (LAB) are a diverse group of microorganisms consisting of Gram-positive, aerotolerant, acid-tolerant, usually nonsporulating and nonrespiring rod or cocci microorganisms. These play an important role in the process of fermentation of food by inhibiting spoilage/pathogenic bacteria and by producing excellent flavor, aroma, and texture of fermented foods (Ricci *et al*. 2019) [18].

LAB could be isolated from many kinds of sources such as milk products, fermented foods, animal intestines or Human intestines, freshwater fishes, soil samples, sugar cane plants, and poultry farms (El-Rab *et al*. 2011) [7]. The most common types of probiotic LAB include different *Lactobacillus* spp. (*Lb. acidophilus*, *Lb. johnsonii*, *Lb. casei*, *Lb. rhamnosus*, *Lb. gasseri*, and *Lb. reuteri*) and genus *Bifidobacteria* (*Bf. bifidum*, *Bf. animalis subsp. lactis*, *Bf. longum* subsp. *longum*, and *Bf. longum* subsp. *infantis*) (Chassard *et al*. 2011) [5].

There is a general global interest in the use of probiotics in food, in feeds and as supplements to enhance human and animal health. Probiotics are live microorganisms which when administered in adequate amounts confer health benefits to the host (FAO/WHO 2006) [9]. Some of the health benefits include the following: prevention of antibiotic related diarrhea, treatment of irritable bowel syndrome, production of B vitamins, prolongation of life, production of antioxidants and other geroprotectors, serum cholesterol reduction, prevention of cancers, treatment of *Helicobacter pylori*, relief from lactose intolerance, and improved immune response, among others (Nangia *et al*. 2014) [15]. Therefore, the isolation and characterization of LAB from different traditional fermented foods and products have gained research interest in recent years (Alonso *et al*. 2018) [1].

Guidelines for screening candidate microorganisms for probiotic activity have been developed by the Joint FAO/WHO Working Group (FAO/WHO, 2002) [12] and Ganguly *et al*. (2011) [10]. Also Byakika *et al*. (2019) [4] recently reviewed these guidelines. A potent probiotic isolate must possess certain characteristics like survival and colonizing ability under different environmental conditions (Palachum *et al*. 2018) [17]. The isolates should be able to withstand low pH of gastric juice with resistance to bile salts and also adhere to epithelial cells. They should also offer certain health benefits like antimicrobial activity, anticancer activity, toxin-reducing effects, and boosting immune response. Hence, bacteria adhering to suitable surfaces and survival in the gastrointestinal tract should be confirmed by *in vitro* evaluation prior to using them as probiotics (Berardi *et al*. 2013) [2].

Probiotic strains isolated from traditionally fermented foods and drinks could have application as a starter culture for large-scale production of the traditional product and have a desirable functional property for their application as probiotics against food-borne pathogens.

Probiotics can be found in many environments such as dairy products, fermented, food and humans. However, the use of probiotics of human origin for use in humans is frequently proposed (Sanders, 2008).

The aim of this study is to identify indigenous bacterial strains from healthy human individuals and dairy products that can be used as potential probiotics for the treatment and prevention of various human ailments and also could translate into technological applications that improve the safety and functionality of fermented foods as well as overall consumer health. In order to be recognized as potential probiotics, bacterial strains that are isolated from various sources must meet specific criteria. For this purpose, a series of standard tests are usually carried out to identify and characterize potential probiotic strains.

Materials and Methods

Isolation and Phenotypic Characterization of LAB

For about 60 samples, 30 each of dairy products (Raw milk and Dahi) and Human feces, were randomly obtained from the institute's experimental dairy plant located at NDRI, in sterile corked plastic tubes followed by immediate storage in a 4 °C icebox. Further transported to the laboratory, and examined for the presence of LAB upon arrival. To identify LAB, 10 mL of each sample was enriched in 40 mL of de Man, Rogosa, and Sharpe (MRS) broth and stirred overnight in a shaking incubator at 37 °C under aerobic conditions. All tubes with visible turbidity were further cultured on MRS agar plates followed by incubation for 24 to 72 h at 37°C under aerobic conditions. Then, individual colonies from each plate were selected and purified through 3 successive transfers on MRS agar. Finally, 40 pure isolates (H1-H20 and D1-D20) were characterized as LAB by Gram staining, cell morphology, catalase test, carbohydrate fermentation profiles according to standard procedures (Sharpe, 1979), wherein gram-positive and catalase -ve isolates were selected before being stored at −20 °C in MRS broth plus 28% glycerol (El Soda *et al*. 2003) [8].

Genus identification

Genomic DNA was extracted as per the method of Somashekaraiah *et al*. (2019). Genus specific PCR was carried out with forward primer LbLMA-1 (CTCAAAACTAAACAAAGTTTC) and reverse primer R-161 (CTTGTACACACCGCCCGTCA). Amplification reactions were performed in a thermal cycler: initial denaturation at 95 ^{0}C/5 min followed by 35 cycles of 30 sec each at 95 ^{0}C for denaturation, 55 ^{0}C for annealing, 72 ^{0}C for extension, and a final extension of 7 min at 72 ^{0}C. The amplified PCR products were resolved by Agarose gel electrophoresis on 1.5% agarose gel containing ethidium bromide (0.5µg/mL) in 1X TAE buffer. A 100-bp DNA marker was used as the size standard. The gel was visualized and photographed on gel documentation system.

In vitro characterization of probiotic properties

Tolerance to acid and Bile

In order to determine the acid and bile tolerance of the 10 Lactobacilli isolates, MRS broth was used to simulate acidic conditions of gut after adjusting to different pH values namely 2.0, 3.0, and 4.0 and with 1.0 N HCl. Another set of broth was adjusted to neutral pH (7.0) to serve as a control. For bile resistance MRS broth was supplemented with 0%, 1.0% and 2.0% (w/v) oxbile. MRS broth without bile salt served as control. All the broth tubes were inoculated @ 1.0% (at 10^9cfu/ml) with overnight grown cultures of lactobacilli and incubated at 37 ^{0}C. One ml of the culture was taken from each tube immediately (0 hr) and 10-fold serial dilutions were prepared in 9 mL of 0.1% peptone water. Pour plating was done using MRS agar. Similarly, one ml of culture was taken from each tube after an interval of 1, 2 and 3 h followed by plating. The plates were incubated at 37 ^{0}C for 24 to 48 h and the colony forming units (cfu) were counted (Haghshenas *et al*. 2016).

Antibiotic susceptibility of lactobacilli isolates

For determining the antibiotic susceptibility of lactobacilli isolates against different antibiotics, disc diffusion method was used. For this, an aliquot of 15 mL of tryptone glucose extract agar (TGE) was poured in petri plate and was allowed to solidify, followed by over laying with 4 mL of soft agar (TGE with 0.75% agar at 45 ^{0}C) seeded with 100 µl of actively growing isolates. The plates were allowed to stand at room temperature for 15 min before dispensing the antibiotic discs into agar by forcep aseptically. After dispensing the discs, the plates were incubated at 37 ^{0}C for 24 hours. Diameter (mm) of zone of inhibition was measured using antibiotic zone scale and results were expressed as resistance (R), intermediate susceptible (I) and susceptible (S) as given in Hi Media Catalogue.

Antimicrobial activity of lactobacilli isolates against pathogenic organisms

The antimicrobial activity of lactobacilli isolates were tested using agar well diffusion assay as described by Zangh *et al*. (2016). An aliquot of 15 mL of TGE agar was poured in petri plate and allowed to solidify followed by over laying with 4 mL of soft agar (TGE with 0.75% agar at 45 ^{0}C) seeded with 100 µl Brain heart infusion broth (BHI) activated 100µL of the indicator organism, stirred gently, poured into the agar plates and allowed to solidify. The plates were marked into five different zones to represent the four lactobacilli isolates and one for control in the centre of the plate. Wells of 6mm diameter were bored on the solidified agar medium and were filled with 50µL of supernatant of 24 hours old culture of lactobacilli grown in MRS broth. The central well was used as a control and filled with sterile water. The plates were

incubated at 37 ^{0}C for 48 hours and the diameter (mm) of inhibition zone measured.

Cell Surface Hydrophobicity

The ability of microorganism to adhere to selected hydrocarbons was tested using hydrophobicity assay. For this, isolates were grown MRS broth and harvested after 24h by centrifugation at 12000 rpm for 5min at 5 ^{0}C, washed twice in 50 mM KH_2PO_4 buffer (pH 6.5) and suspended the cell pellet in 10mL of the same buffer. The optical density of cell suspension was adjusted to approximately 0.6-0.7 at 610 nm in the buffer. In a sterilized glass tube, 3 mL of cell suspension and 1mL of test hydrocarbon (n-hexadecane) were added. The mixture was vortexed for 90 seconds and incubated at 37 ^{0}C for 10min followed by again vortexing and incubating at 37 ^{0}C and allowed to stand for separation of two phases. The optical density of aqueous phase was measured at 610 nm against blank phosphate urea magnesium (PUM) buffer. The percent hydrophobicity was calculated from the decrease in optical density of original bacterial suspension due to partitioning, and calculated as per the following equation:

$$\text{Percent hydrophobicity (H \%)} = \frac{(\text{Initial OD}_{610}) - (\text{Final OD}_{610}) \text{ X } 100}{(\text{Initial OD}_{610})}$$

The higher the value for percent hydrophobicity indicates the higher adherence or colonization potential of probiotic in the gut.

Bile salt hydrolase activities

A direct plate assay was applied for detecting of the bile salt hydrolase activity of the isolates. BSH activity was examined by streaking over night grown culture of lactobacilli on MRS agar with 0.037g calcium chloride containing 0.5% biles like sodium taurocholate (TC) and sodium taurodeoxycholate (TDCA). The petriplates were then anerobically incubated at 37 ^{0}C for three days. *Enterococcus faecium* was taken as positive and *Pediococcus acidilactici* LB-42as negative control for the test. BSH activity was indicated when the hydrolyzed products of the salts, *viz*. cholic acid or deoxycholic acid, precipitated in the agar medium in and around the culture spots.

Species level identification of the isolates

On the basis of sugar fermentation profiles, tentatively identified species were confirmed by employing species specific PCR. The primer used for species specific identification was Lu5 (F) CTAGCGGGTGCGACTTTGTT and Rha 11 (R) GCGATGCGAATTTCTATTATT (Song *et al*. 2004) with the annealing temperature of 62 ^{0}C and product size of 113 bp. PCR amplified products obtained with different templates were electrophoresed on agarose gel following standard procedures. Further the unidentified isolates were subjected for 16S rRNA sequence analysis.

Statistical Analysis

Statistical analyses were carried out using one-way analysis of variance (ANOVA) and SPSS software version 25. Each test was performed in triplicate.

Results and Discussion

Phenotypic and Genus identification of Isolates

About 40 isolates were Gram-positive, rods and catalase-negative and were considered for testing as presumptive LAB isolates. Genus specific PCR of isolates showed the desired 250 bp PCR product, confirming only H4, H8, H9, H10, H14 and D6, D7, D9, D12, D13 as lactobacilli.

Acid Tolerance

At pH 2.0 almost all the isolates lost their viability up to 60 min except H9, H14, D9 and D13 where they have showed the viability of 1.0 to 2.0 log counts even after 120 min exposure. All the five human isolates showed 0.89 to 3.6 log count reduction while all five dairy isolates showed 3.2 to 5.6 log reductions. At pH 3.0 most of the isolates showed survival up to 120 min except H4. Whereas H8, H9, H10, D12 and D13 showed good survivability with the log count varying from 2.0 to 5.2. Human isolates showed 2.98 to 5.65 log reduction and dairy 2.7 to 6.73 log reduction. At pH 4.0 all the isolates were exhibiting good tolerance with the log count varying from 5.5 to 8.9 log count except H4 and D6.Human isolates showed 0.2 to 3.5 log reduction and dairy 0.16 to 2.88 log reduction. At pH 7.0, human isolates showed 0.28 to 0.73 log count increase and dairy 0.02 to 0.37 increased log counts (Fig 1 and 2). Our results on acid tolerance of Lactobacilli isolates are, however consistent with the findings of Mantzourani *et al*. (2019) [13] who in general recorded a decrease in the number of survivability of *Lb. acidophilus* strain during 3 hrs of incubation at all pH conditions used in their study.

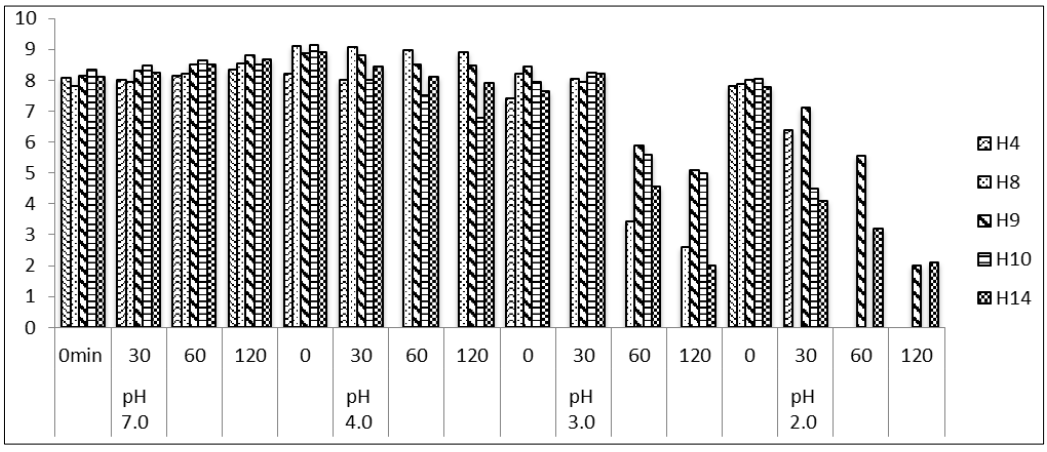


Fig 1: Acid tolerance of Lactobacilli isolates of human origin

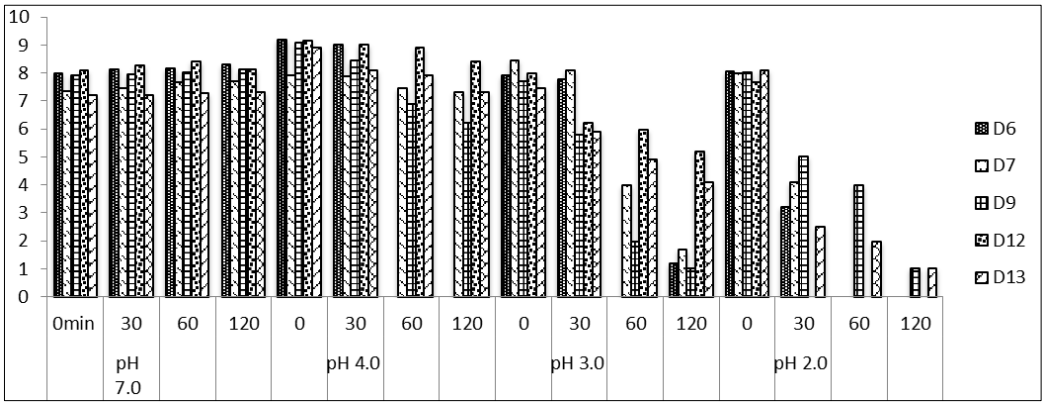


Fig 2: Acid tolerance of Lactobacilli isolates of Dairy origin

Bile Tolerance

The data pertaining to the same has been presented in Fig 3 and 4. Majority of the isolates at 2% bile concentration lost their viability at 2hr and 3hr exposure except H4, H10 and H14 with a log count of 2.5 to 4.10 at 2hr exposure. At 1% bile all the isolates were able to tolerate at 3 hrs exposures with the log count of average 6.0. At 1% bile concentration human isolates showed 1.5 to 2.5 log reductions and dairy isolates 1.6 to 3.8 log reduction. At 2% bile concentration human isolates showed 1.4 to 4.5 log reduction and dairy isolates lost the viability. Our results in this regard are consistent with the observations of other investigators who also reported variations in the bile tolerance among their probiotic strains after exposure different exposure times (Mantzourani *et al*. 2019) [13].

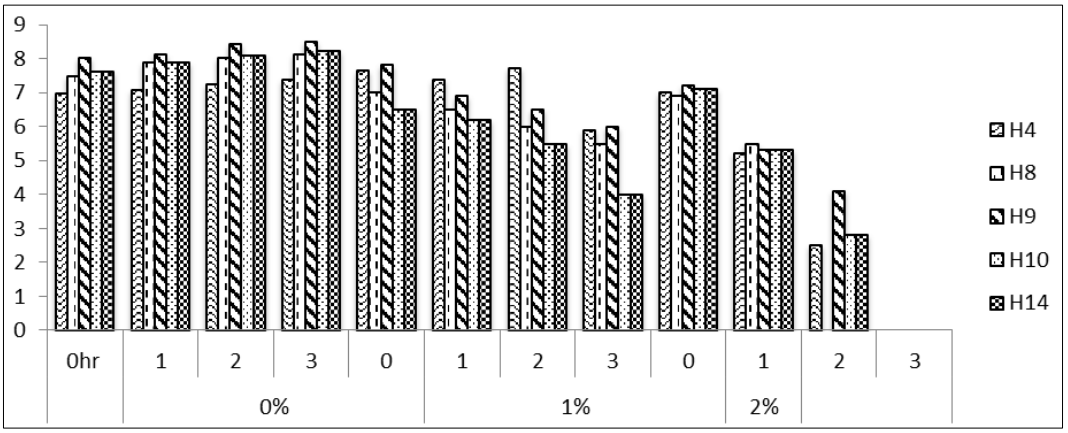


Fig 3: Bile tolerance of Lactobacilli isolates of Human origin

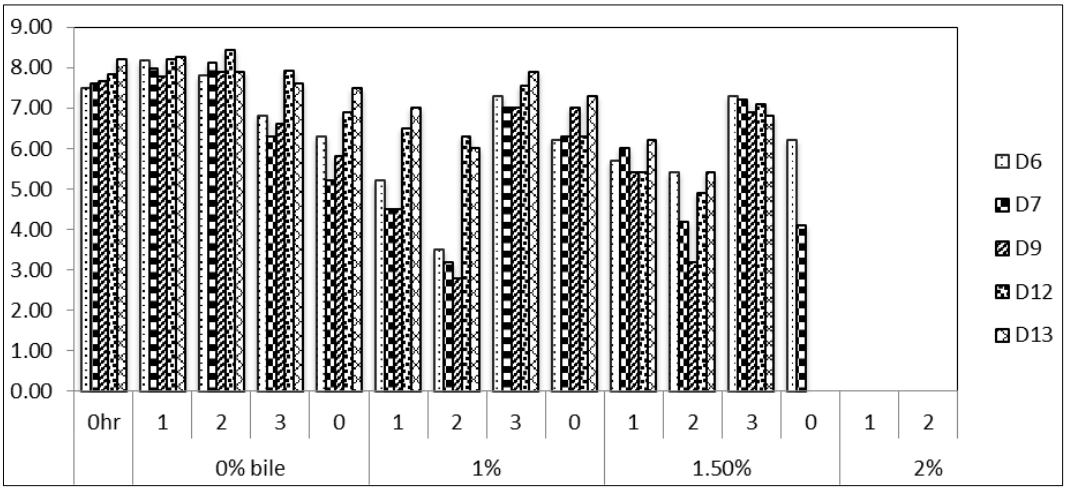


Fig 4: Bile tolerance of Lactobacilli isolates of dairy origin

Antibiotic Resistance of Lactobacilli Isolates

The study revealed that almost all the lactobacilli isolates were highly susceptible to antibiotic used namely Clindamycin, Co-trimoxazole, Gentamycin, Tetracycline, Chloramphenicol, Amikacin where as isolates are moderately sensitive to erythromycin and ampicillin. All isolates exhibited resistance against ofloxacin, Penicillin and Vancomycin. Isolate H9 and D9 were sensitive to Vancomycin (Table 1 and Fig 5).

Our results in this regard are in agreement with Mishra (2001)

[14] who also recorded vancomycin resistance against all the lactobacillus examined in this study. Similarly Neelakanteshwara (2005) [16] also reported vancomycin resistance against Lactobacillus. Based on our results and similar observations made by other investigators, it can be stated that vancomycin resistance is a widespread phenomenon among lactobacilli as was reported earlier also (Dowarah *et al*. 2018) [6].

Table 1: Inhibition zone of Lactobacilli Isolates (dia in mm)

Isolates	V	G	P	E	Chl	T	Ce	CM	O	CX	AM	A
H 4	6	13	6	19	13	22	6	17	11	17	35	12
H 8	6	15	6	20	25	24	8	22	11	16	28	19
H 9	6	14	6	20	24	25	12	26	10	18	30	17
H 10	6	15	8	23	24	24	17	28	10	16	32	16
H 14	6	17	12	24	21	23	20	23	17	25	22	15
D 6	6	13	21	21	24	25	16	25	10	11	22	16
D 7	9	13	19	23	28	28	19	26	12	6	24	14
D 9	10	16	16	21	25	23	20	17	15	17	17	17
D 12	6	15	14	22	22	23	16	23	12	18	18	16
D 13	6	19	17	21	20	25	20	23	12	10	20	17
	R=14 I=15-16 S=17	R=12 I= 13-15 S= 16	R=16 I=14-15 S=17	R =13 I= 13-15 S= 23	R=12 I=13-17 S=18	R=14 I=15-18 S=19	R= 14 I=15-17 S=18	R=14 I=15-20 S=21	R=12 I=13-15 S= 16	R=10 I=11-15 16	R=13 I=22 S=17	R=14 I= 15-16 S=17

V: Vancomycin, G: Gentamycin, P: Penicillin, E: Erythromycin, Chl: Chloramphenicol,
T: Tetracyclin, Ce: Cephal-othin, CM; Clinda-mycin, O; Oflaxa-cin, CX: Cotrimo-xazole,
AM: Ampicil-lin, A:Amika-cin.

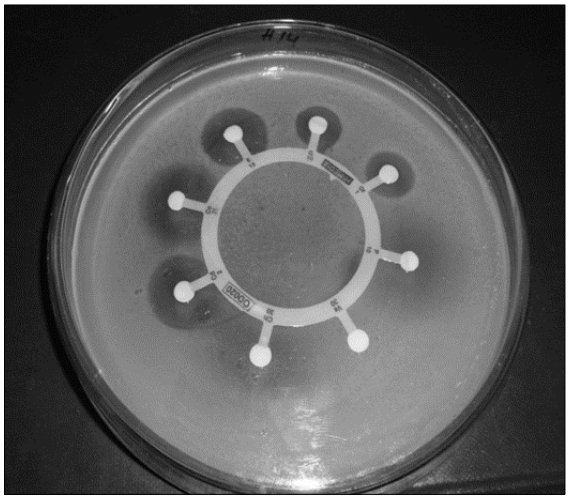


Fig 5: Antibiotic Susptibility of Lactobacill Isolate

Antimicrobial activity of Lactobacillus isolates

From the data presented in Table 2 and Fig 6, it can be concluded that all most all the isolates exhibited a moderate to slightly higher antibacterial activity against *E. coli* and *S. aureus* except H10, H14, D13 and D12. The maximum activity with the zone of inhibition *viz*.14mm and 16 mm was recorded in case of H9 against *E. coli* and *S. aureus* respectively. Apart from this, it was also able to inhibit *B. cereus and S. dysenteriae* where maximum of 14mm zone of inhibition was recorded. However most of the isolates could not demonstrate zone of inhibition against *S. typhi.* Our results in this regard are comparable to those of several other investigators who also recorded strong antibacterial activity of probiotic lactobacilli against Gram positive and Gram negative bacteria (Reuben *et al*. 2020) [19].

Table 2: Antimicrobial activity of Lactobacilli isolates against indicator organisms

Isolates	*E. coli*	*S. aureus*	*B. cereus*	*S. typhi*	*S. dysenteriae*
H 4	12 mm	11 mm	11 mm	No zone	10 mm
H 8	9 mm	10 mm	10 mm	No zone	8 mm
H 9	14 mm	16 mm	13 mm	No zone	12 mm
H 10	10 mm	No zone	11 mm	No zone	11 mm
H 14	12 mm	No zone	14 mm	No zone	10 mm
D 6	14 mm	11 mm	13 mm	12 mm	9 mm
D 7	No zone	12 mm	11 mm	9 mm	8 mm
D 9	6 mm	14 mm	13 mm	No zone	10 mm
D 12	10 mm	No zone	10 mm	No zone	10 mm
D 13	No zone	10 mm	12 mm	No zone	11 mm

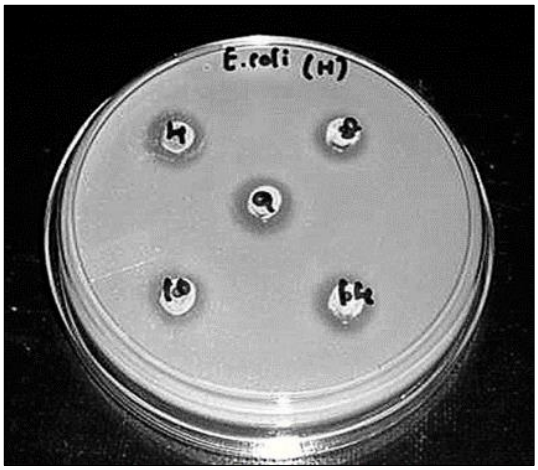


Fig 6: Antibacterial spectrum of Lactobacilli against *E. coli*

Cell surface hydrophobicity of lactobacillus isolates

In this investigation, the hydrophobicity of our lactobacillus isolates was determined with one of the common hydrocarbons namely n-hexadecane. The results concerning the hydrophobicity of the test Lactobacillus cultures are in Table 3.

Lactobacilli isolate showed cell surface hydrophobicity varying from 4% to 51%. Isolate D9 was found to have 51% followed by H4 36%), D6 (33%), H8 (19%) and lowest hydrophobicity of 4% (H9).The results in this regard are inconsistent with those of Somashekaraiah *et al*. (2019) who observed very high hydrophobicity of *Pediococcus pentosaeus* and *Propioni bacterium acidopropinic* and *L. casei.*

Table 3: Cell surface hydrophobicity of Lactobacilli isolates

Isolates	Initial O.D	Final O.D	% Hydrophobicity
H4	0.72	0.602	16.3
H8	0.78	0.6315	19.0
H9	0.685	0.6575	4.0
H10	0.797	0.6215	22.0
H14	0.696	0.4385	36.9
D6	0.674	0.4495	33.3
D7	0.78	0.5675	27.2
D9	0.77	0.3725	51.6
D12	0.694	0.6065	12.6
D13	0.725	0.6525	10

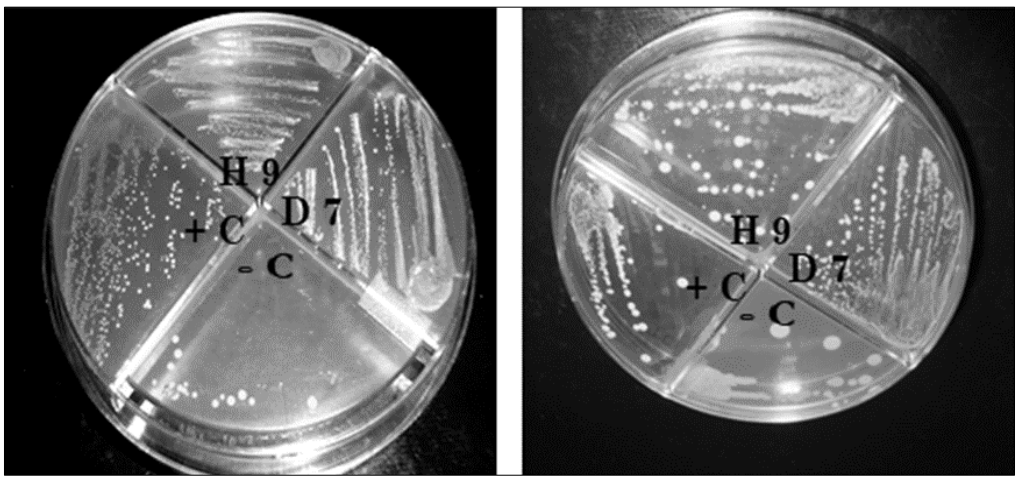
Values are means ± standard deviations of three independent determinations.

Bile salt Hydrolase Activity of Lactobacilli isolates

Lactobacilli have been shown to be predominant producers of BSH activity in the gut. Gastric lactobacilli contribute approximately 86% of the total BSH activity in the ileum and 74% in the caecum of mice. As it is quite evident from the data presented therein, only four out of 10 isolates namely H4,H9 and D7 exhibited a moderate level of BSH activity (++) as indicated by dense precipitation of the sodium taurodeoxycholate (Table 4 & Fig 7). Our results are in this regard are consistent with the observation of Stellah *et al*. (2020).

Table 4: Bile salt hydrolase activity of isolates

Isolates	TDC (Taurodeoxycholate)	TC (Taurocholate)
H4	++	+
H8	+	+
H9	+ +	+
H10	+	+
H14	+	+
D6	+	+
D7	++	+
D9	+	+
D12	+	+
D13	+	+



Simple MRS Plate MRS plate with 0.5% Sodium Taurodeoxycholate (TDC)

Fig 7: BSH activity of Lactobacilli isolate for deconjugation of TDC
Positive Control: *E. faecium*, Negative Control – *Pediococcus acidilactici* LB-42

Molecular characterization of the isolates

Biochemically, Out of 10 isolated none of the isolates fermented mannose except D6 and D7. Human isolates directed to *L. plantarum* except H10 and dairy isolates as *L. casei.* Species specific PCR also confirmed the isolates as *L. rhamnosus* with an amplified product of 113 bp. After ascertaining the identity of isolates as *L. plantarum* and *L. casei*, study directed to confirm them at species level by subjecting them to using L pla 3/ L pla 2 and SS1/ CA. Among 10 isolates, 4 isolates (H4, H8, H9, H14) could amplify 248 bp product specific for *L. plantarum* and none of the isolate could amplify 1200 bp product specific for *L. casei*. In our study, blast analysis of sequence clearly revealed 99% homology with *L. rhamnosus* for D6, D7, D9, D12 and D13. whereas H10 was recorded for *L. plantarum* with 99% homology.

Conclusion

Given the results of this study, These isolated probiotic bacteria of dary and human origin can be raised for the production of various kinds of food and pharmaceutical products. They can also be used for the production of new functional foods. Therefore, increasing use of dairy products containing probiotics, identification and production of foods containing highest and most effective lactobacilli are recommended in daily diet.

Acknowledgment

I would like to acknowledge all the teaching staff and PhD scholars of the Dairy Microbiology Division, NDRI (ICAR) Karnal, Haryana for their whole hearted support in executing this research work.

References

1. Alonso S, Carmen CM, Berdasco M, Banda IG. Isolation and partial characterization of lactic acid bacteria from the gut microbiota of marine fishes for potential application as probiotics in aquaculture. Probiotics Antimicrob Proteins. 2018;11:569-579.
2. Berardi C, Solovey W, Cummings ML. Investigating the efficacy of network visualizations for intelligence tasks. Proceedings of the IEEE International Conference on Intelligence and Security Informatics, 2013, 278-283.
3. Boubezari MT, Idoui T, Hammami R, Fernandez B, Gomaa A, Fliss I. Bacteriocinogenic properties of *Escherichia coli* P2C isolated from pig gastrointestinal tract: purification and characterization of microcin. V Arch Microbiol. 2018;200:771-782.
4. Byakika S, Mukisa IM, Byaruhanga YB, Muyanja C. A review of criteria and methods for evaluating the probiotic potential of microorganisms. Food Rev Int. 2019;35:427-466.
5. Chassard C, Grattepanche F, Lacroix C. Probiotics and health claims: challenges for tailoring their efficacy. Probiotics and Health Claims. 2011;8:49-74.
6. Dowarah R, Verma AK, Agarwal N, Singh P, Singh BR. Selection and characterization of probiotic lactic acid bacteria and its impact on growth, nutrient digestibility, health and antioxidant status in weaned piglets. PLoS ONE. 2018;13(3):0192978.
7. El-Rab GD, Barakat G, Ibrahim N, Tawfik W, El-Kholy. Identification and probiotic characteristics of Lactobacillus strains isolated from traditional Domiati cheese. Int J Microbiol Resear. 2011;3(1):59.
8. El Soda M, Ahmed N, Omran N, Osman G, Morsi A. 2003. Isolation, identification and selection of lactic acid bacterial cultures for cheese making. Emir. J Food Agric. 2003;15:51-71.
9. FAO/WHO. Evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria, 2006.
10. Ganguly NK, Bhattacharya SK, Sesikeran B. ICMR-DBT guidelines for evaluation of probiotics in food. Ind J Medi Resear. 2011;134:22-25.
11. Haghshenas M, Haghshenas Y, Nami1 AY, Khosroushah i N, Abdullah A, Barzegari R. Probiotic assessment of *Lactobacillus plantarum* 15HN and *Enterococcus mundtii*50H isolated from traditional dairies Microbiota, 2017.
12. Joint FAO/WHO. Working Group, Report on Drafting Guidelines for the Evaluation of Probiotics in Food. FAO and WHO, London, UK, 2002.
13. Mantzourani I, Chondrou P, Bontsidis C. Assessment of the probiotic potential of lactic acid bacteria isolated from kefir grains: evaluation of adhesion and antiproliferative properties in *in vitro* experimental systems. Ann Microbiol. 2019;69:751-763.
14. Mishra V. Studies on probiotics attributes in selected strains of *Lactobacillus casei.* Ph.D. Dissertation, NDRI Karnal, India, 2001.
15. Nangia T, Setia V, Kochhar G, Kaur K, Bansal R, Sharma R. Probiotics: Review of literature, J Period MediClini Practice. 2014;1:144-151.
16. Neelakanteshwara G. PCR identification of bile salt hydrolase positive Lactobacilli. M.Sc. Thesis submitted to NDRI Karnal, India, 2005.
17. Palachum W, Chisti Y, Choorit W. *In-vitro* assessment of probiotic potential of *Lactobacillus plantarum* WU-P19 isolated from a traditional fermented herb. Ann Microbiol. 2018;68:79-91.
18. Ricci A, Cirlini A, Maoloni. Use of dairy and plant-derived lactobacilli as starters for cherry juice fermentation. Nutrients. 2019;11(2):213.
19. Reuben RC, Roy PC, Sarkar SL, Rubayet ASM. Characterization and evaluation of lactic acid bacteria from indigenous raw milk for potential probiotic properties. J. Dairy Sci. 2020;103:1223-1237.
20. Sanders ME, Akkermans LM, Haller D, Hammerman C, Heimbach J, Hörmannsperger G. Safety assessment of probiotics for human use. Gut Microbes. 2010;1:1-22.
21. Sharpe ME. Identification of the lactic acid bacteria. Identification Methods for Microbiologists. F. A. Skinner and D. W. Lovelock, ed. Academic Press, London, UK, 1979, 233-259.
22. Song J, Lee SC, Kang JW, Baek HJ, Suh. Phylogenetic analysis of Streptomyces spp. isolated from potato scab lesions in Korea on the basis of 16S rRNA gene and 16S-23S rDNA internally transcribed spacer sequences. Int. J Syst. Evol. Microbiol. 2004;54:203-209.
23. Zolotukhin P, Prazdnova E, Chistyakov V. Methods to assess the antioxidative properties of probiotics. Probiotics Antimicrob Proteins. 2017;1:11.