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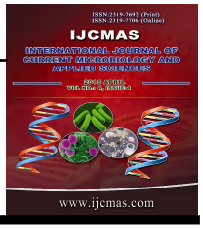

Original Research Article

Isolation, characterization and safety assessment of lactic acid bacterial isolates from fermented food products

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Keywords

Lactic acid bacteria (LAB), Fermented foods, Physiology, Biochemistry, Safety, Probiotics

A B S T R A C T

The purpose of the research was to isolate and characterize lactic acid bacteria from various dairy and non dairy products. A total of 22 samples collected from homemade products or from local market were plated on MRS agar. Total 181 isolates having apparently different colony morphology were collected and screened based on their morphological properties. The selected 29 isolates were further evaluated for their biochemical and physiological characterization. Isolates PD2, PD11, PJ29, PFC21, PSC6, PC6 and PC27 survived incubation periods of 4 h at pH 1.0, and 2.0. Among them, 4 isolates PD2, PSC6, PC6 and PC27 could be able to grow in presence of 0.8% sodium taurocholate, 12% sodium chloride and 0.6% phenol. The highest titratable acidity observed by the curd isolate PC27 (2.52%). Ten isolates showing high potential as probiotic candidate or food cultures were further selected. These isolates showed negative test for hemolytic activity, gelatinase activity and biogenic amine (BA) production and hence considered safe.

Introduction

India is traditionally rich in microbial diversity since ancient times. As the production and consumption of traditional fermented food products become increasingly relevant in the face of rapidly increasing population and food insecurity, more research and development to ensure the safety and nutritional quality of these fermented products is warranted. In addition to preservation, fermented foods can also have the added benefits of enhancing flavor, increased digestibility, improving nutritional and pharmacological values (Jeyaram et al., 2009). Lactic acid bacteria have contributed in increased volume of fermented foods worldwide especially in foods containing probiotics or health promoting bacteria. Probiotics are live microbial, dietary supplements or food ingredients that have a beneficial effect on the host by influencing the composition and metabolic activity of the flora of the gastrointestinal tract.

The Lactic acid bacteria (LAB), generally considered as “food grade” organisms, show special promise for selection and implementation as protective cultures. There

are many potential applications of protective cultures in various food systems (Holzapfel et al., 1995). These organisms have been isolated from grains, dairy products and fermenting vegetables. In the present investigation, we have isolated LAB from vegetables and indigenous fermented food products of Gujarat. In a similar study, LAB were isolated from vegetables and traditional fermented foods including dhokla batter, idli batter, dahi, jalebi batter, lassi, yogurt and cabbage (Patel et al., 2012 and Neha et al., 2014). In another similar study, LAB were isolated from Dosa (Appam) batter and vegetable pickle (Vijai Pal et al., 2005). The Indian population depends heavily on fermented cereal and legume grains which gives higher digestible protein content, and provides a better balanced ratio of amino acids. Cereals such as rice (*Oryza sativum*), ragi (*Eleusine coracana*), wheat (*Triticum* spp.), barley (*Hordeum vulgare*), and pulses such as black gram, red gram, green gram are predominantly used in the preparation of significant number of fermented foods since ancient times. Cereals and legumes are effective substrates for the production of probiotic-incorporated functional food, as they can be used as a source of non digestible carbohydrates which stimulate the growth of *Lactobacilli* and *Bifidobacteria*. They are very rich source of water soluble fibers like ß-glucan, galactooligosaccharides and fructooligosaccharides, which act as prebiotics which are digested by selective groups of LAB (Swennen et al., 2006). Cereals and legumes are fermented by several groups of bacteria in the large intestine and produce SCFA which provides an acidic environment, which stimulates the proliferation of probiotic lactic cultures. Lactic acid bacteria convert milk sugar lactose into lactic acid and also produce anti microbial substances that suppress spoilage bacteria. Dahi or curd is the most popular traditional Indian fermented products prepared by fermentation of milk by lactic acid bacteria. Intake of Dahi has been found to cure diarrhea (Agarwal and Bhasin 2002); and reported to have anti-cholesterolemic (Sinha and Sinha 2000), anticarcinogenic (Arvind et al., 2010) and anti-diabetic effects (Yadav et al., 2007) and few other benefits (Prajapati et al., 2014). The lactic acid fermentation of vegetables, applied as a biopreservation method to enhance their storage life (Kingston et al., 2010).

Considering the rich microbial diversity in such fermented food, and the nutritional and therapeutic potential of the associated microflora, an attempt is made to isolate novel strains and characterize them for their application in functional foods.

Materials and Methods

Isolation of LAB

Rice and pulse based batter of various traditional fermented foods like Dhokla, Dosa, Handva, Jalebi and Dahi, Soy curd, and Cabbage were collected from households and local markets into sterile glass bottles. All the 22 samples were stored at 4°C in refrigerator and processed within two hours. Samples 10 % (w/v) were homogenized in phosphate buffer (0.1M, pH 7.2), serially diluted and pour plated on De Man, Rogosa and Sharpe agar (MRS agar, Hi media, Bangalore) and were incubated at 37°C for 24 to 48 h anaerobically (5% CO_2). Selected colonies with different morphological characteristics were purified by streak plate method and then preserved at -20°C on MRS agar slants, in MRS broth containing 10 % glycerol (v/v) and in freeze-dried form. The isolates were maintained in litmus milk at refrigeration temperature after their overnight growth at 37°C. All the cultures were routinely sub-

cultured at regular intervals and were activated in MRS broth before being used in the experiment.

Physiological characterization

Growth at Different Temperature

Overnight grown active cultures were inoculated at 1% in MRS broth tubes and incubated up to 7 days at 15, 30 and 45 ºC. Extent of growth was visually recorded based on intensity of turbidity.

Growth at Different pH & bile concentrations

To check growth at various pH or bile concentrations, MRS broth with different pH 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 9.0 and with various concentrations of bile salt (0.3, 0.5 and 0.8% Sodium taurocholate) was prepared, inoculated at 1% and then incubated at 37 ºC for 48 h. During incubation, extent of growth was recorded objectively based on visible turbidity marked as double positive sign (++) for maximum growth, single positive sign (+) for normal growth and negative sign (-) for no growth.

Growth at Different Phenol and Salt concentrations

Overnight grown active cultures were inoculated at 1% in MRS broth tubes adjusted to various concentrations of NaCl viz. 4, 8 and 12% (w/v) or phenol level viz. 0.4, 0.5 and 0.6 % (w/v) along with their respective controls.

The cultures were incubated at 37°C. After 24 h of incubation, extent of growth was recorded objectively based on visible turbidity marked as double positive sign (++) for maximum growth, single positive sign (+) for normal growth and negative sign (-) for no growth.

Biochemical characterizations

Sugar fermentation

MRS broth containing different Sugars (HiMedia, Mumbai, India) and phenol red as pH indicator was inoculated with active cultures at 1%, incubated anaerobically at 37°C for 24 h. The cultures were identified as positive or negative based on acid and gas production after the incubation period.

The carbohydrate fermentation profiles of the strains were determined using API 50 and 20 CH system (Biometrix, France). Interpretation of these fermentation profiles were facilitated by systematically comparing all the results obtained for the isolates studied with information from the computer aided database Apiweb^{TM} software.

Milk fermentation

Milk fermentation abilities of LAB isolates were assayed by inoculating litmus milk (10% sterilized skim milk with 0.1% litmus) with 1% active cultures. Observations were made regarding acidification, reduction, coagulation, whey separation and gas production at the end of 24 h incubation at 37°C.

Acid production

Sterilized skim milk (10% reconstituted skim milk powder) was inoculated with 1% active culture and incubated at 37°C for 24 h. Titratable acidity was measured and expressed as % Lactic acid. pH of the sample was also recorded using digital pH meter (Model no. pHTestr 30)

EPS production

The isolates were streaked onto MRS agar plates supplemented with 5 % sucrose and incubated at 30ºC for 48 h. Production of mucoid colonies were considered as the potential EPS production ability of the culture (Patel et al., 2012).

Phytase activity

Phytase activity was tested on a special medium containing glucose (1.5%), calcium phytate (0.5%), NH_4NO_3 (0.5%), KCl (0.05%), $MgSO_4.7H_20$ (0.05%), $MnSO_4.7H_20$ (0.02%), $FeSO_4.7H_20$ (0.001%) and Agar (1.5). Phytase positive colonies were detected by the presence of zone of precipitates around the colonies (Haros et al., 2005).

Protease activity

Plates were prepared with standard plate count agar supplemented with 10% reconstituted sterilized skim milk. The isolates grown in MRS broth were spot inoculated (10 µl) on the agar plates and incubated for 48 h at 37ºC. Colonies with clear zone of milk hydrolysis were considered as proteolytic isolates (Taheri et al., 2009).

Amylase activity

Amylase activity was determined as per the method suggested by (Taheri et al. 2009). Starch agar plates were spot inoculated with 10 µl of active cultures and incubated at 30 ºC for 48 h. Clear zone of amylase activity was detected after flooding the plates with Lugol's solution.

Lipase activity

For the detection of lipase activity, the MRS broth containing olive oil (1%) and Arabic gum (1%) was used to subculture the strains and the activity was detected by using a medium that consisted of tryptone (0.1%), yeast extract (0.5%), NaCl (0.05%), olive oil (0.1, 0.5, or 1%), Arabic gum (1%), and agar (1.5%). Lipase positive colonies were detected by the presence of zone of precipitates around the colonies (Taheri et al., 2009).

Arginine hydrolysis

Production of ammonia from arginine was tested by inoculation of the isolates in arginine broth. After 24 h of incubation, 100 µl of the sample was spotted on white tile and equal volume of Nessler's reagent was added.

Immediate appearance of dark orange color indicates the presence of ammonia due to hydrolysis of arginine (Ghosh Asit R. et al. 2011).

Safety Studies

The selected 10 isolates were further assessed for their safety appraisal for their applications in functional foods.

Haemolytic activity

Haemolysis activity was investigated as described by Gerhardt et al. (1981) with some modification. Three µl of a 6 h old culture broth was spot inoculated into sterile blood agar. The blood agar was prepared by adding 7% sheep-blood (freshly collected & preserved in EDTA), into sterile sheep blood agar base (Hi media) at 45°C.

Plates were incubated anaerobically at 37°C for 48 h after which they were observed for clear zones surrounding colonies (positive reaction for beta haemolysis). A strain of *S. aureus* was used as positive control.

Gelatinase activity

Gelatinase activity of the isolates was investigated as described by Harrigan and McCance (1990) with slight modification. Two µl of a 6 h old culture was spot-inoculated into nutrient gelatin agar (Himedia, Mumbai). The plates were incubated anaerobically for 48 h at 37°C after which they were flooded with saturated ammonium sulfate solution and observed for clear zones surrounding colonies (positive reaction for gelatin hydrolysis). A strain of *Staphylococcus aureus* was used as positive control.

Amino acid decarboxylating activity

The decarboxylase test for the production of Biogenic amines was done by incubation of the isolates in the improved broth medium (Bover-Cid and Holzapfel, 1999) supplemented with 2g/L final concentration of histidine, tyrosine, tryptophan and phenylalanine (HiMedia, Mumbai, India), as precursors. After activation, all strains were streaked in duplicates on the decarboxylase medium plates containing 1% of each amino acid, with and without amino acid (Control), and were incubated for 3 days at 37°C and observed for clear zones surrounding colonies (positive reaction for amino acid decarboxylation).

Result and Discussion

A total of 22 samples comprising of dairy and non dairy fermented foods collected from various sources were plated on MRS agar and after 48 h of incubation, typical colonies showing different morphological characteristics were picked up (Table 1). Total 181 isolated colonies were inoculated in MRS broth and after 24 h of incubation; the cell morphology was studied by Gram staining. The isolates were also tested for Catalase reaction. From among 181 isolates, only 29 were found to be Gram positive rods or cocci and Catalase negative. These isolates were also found to be non motile and non spore formers. Hence, these 29 isolates were further evaluated for different physiological and biochemical characterization

Physiological characterization

All strains grew well at 30◦C, 37◦C and 45◦C and their growth was comparable. None of them except PD11 could grow at 15◦C (Table 2).

Probiotic bacteria mostly delivered in a food system must be acid and bile tolerant to survive in the human gastrointestinal tract. The time from entrance to release from the stomach has been estimated to be approximately 90 min with further digestive processes requiring longer residence time (Berrada et al. 1991). Before reaching the intestinal tract, probiotic bacteria must first survive transit passage through the stomach where the pH can be as low as 1.5 to 2 (Dunne et al, 2001).

All the isolates showed different performance at varying pH. The threshold point to state acid resistance in this research was set at pH 2 and pH 3 for 4 h incubation, as it simulates bacterial residency in the stomach. This is in accordance with findings from Liong and Shah, (2004) which stated that resistance at pH 3 was set as standards for acid tolerance of probiotic culture. In present study, isolates PD2, PD11, PJ29, PFC21, PSC6, PC6 and PC27 survived an incubation periods of 4 h at pH 1.0. Isolates PD2, PD11, PD30, PJ29, PH5, PFC21, PSC6, PC6 and PC27 were able to resist at pH 2 for 4 h and almost all the isolates survived at pH 3 after 4 h of incubation period except PJ38, PH8, PFC18, PSC2 and

PSC5 (Table 2). Similar results were reported by Dunne et al. (2001) and EI-Naggar (2004).

Tolerance to bile salt is a precondition for colonization and metabolic activity of bacteria in the small intestine of the host (Havenaar et al. 1992). This will help *Lactobacilli* to arrive at the small intestine and colon and contribute in balancing the intestinal microflora (Tambekar and Bhatuda, 2010). Bile salts are synthesized in the liver from cholesterol and are secreted from the gall bladder into the duodenum in the conjugated form in volumes ranging from 500 to 700ml per day. The relevant physiological concentrations of human bile range from 0.1 to 0.3% (Dunne *et al.*, 2001) and 0.5% (Mathara *et al.*, 2008) and staying time is proposed to be 4 h (Mishra & Prasad, 2005).

Thus, in present study we checked the growth of lactic acid bacterial strains to various concentrations of bile salts ranging from 0.3 - 0.8% (w/v) for at least 4 h. among 29, 16 isolates were able to tolerate 0.3% sodium taurocholate. Nine isolates namely PD2, PD11, PJ29, PH5, PFC21, PSC6, PC4, PC6 and PC27 have shown resistance to 0.5% sodium taurocholate and only 4 isolates PD2, PSC6, PC6 and PC27 could be able to show turbidity in presence of 0.8% sodium taurocholate as compared to control (Table 2). Similar observation by Abriouel et al. (2012) showed all lactic acid bacteria isolated from fermented olive were able to grow and survive at 0.3% w/v bile salt.

Lactic acid bacteria tolerate high salt concentrations as it allows the bacteria to begin metabolism, which produces acid that further inhibits the growth of undesirable micro organisms. In the present study, all the lactic acid bacterial isolates were able to tolerate 4 % NaCl concentration as shown in Table 2. Among all, 12 isolates namely PD2, PD11, PD30, PJ5, PJ25, PJ29, PH5, PFC21, PSC6, PC4, PC6 and PC27 were able to grow at 8 % NaCl and among 12, seven isolates PD2, PH5, PFC21, PSC6, PC4, PC6 and PC27 tolerated 12 % NaCl. In a similar finding, Schillinger and Lucke (1987) were able to grow lactobacilli isolated from meat and meat products in the presence of 7.5% NaCl.

Tolerance to phenol is a characteristic feature as phenols can be formed in the intestines by bacteria that deaminate few aromatic amino acids delivered by the diet or produced by endogenous proteins (Gilliland & Walker, 1990). In our study, all the lactic acid bacterial isolates were able to tolerate 0.4 % phenol as shown in Table 2. Among 29, dosa batter isolates PD2, PD11 and PD30; fermented cabbage isolate PFC21 and curd isolate PSC6, PC4, PC6 and PC27 showed growth in the presence of 0.5 % phenol. Total 6 isolates, 3 from dosa batter PD2, PD11, PD3 and rest three PC6, PC27 and PSC6 from curd could grow in presence of 0.6% phenol. Our results showed similarities with the findings by Patel et al. (2012).

Biochemical characterizations of the isolates

Lactic acid is the major metabolic end product of carbohydrate fermentation by lactic acid bacteria, responsible for the sour taste and improved the microbiological stability and safety of the food. Almost all the selected isolates were able to utilize hexose sugars like Glucose(G), Lactose(L), Maltose(M), Sucrose(S) and Fructose(F) at different rate. Few isolates PD30, PJ3, PJ4, PJ5, PJ25, PJ29, PJ38, PDH3, PDH8 and PH8 were able to ferment pentose sugar Xylose(X). None of the isolates produced

gas indicating that all were homofermentative (Table 3).

All LAB isolates were subjected to species level biochemical identification based on their carbohydrate fermentation profile using API test stripes. A total forty eight sugars were used to check the characteristics of the isolates and the fermented sugars (+ve results) were detected by the pH indicator present in the chosen medium. Interpretation of the fermentation profiles were facilitated by systematically comparing the results obtained from the isolates studied with information from the computer-aided database API LAB plus software with identification database version 5.1 (BioMerieux, France). The percentage of identification (% id), which is an estimate of how closely the profile corresponds to the taxon relative to all the other taxa in the database (Table 3).

All the cultures were able to ferment milk by forming curd and changed color of the litmus milk from purple to white (Reduction) with a pink color ring at the top of the tube (Oxidation) with slight whey separation (Table 3).

Acid production in the shortest time is the key factor using the cultures for fermented milk manufacturing. The highest titratable acidity observed by the curd isolate PC27 (2.52%) followed by PFC21 (2.02%) and others at 37º C at the end of 24 h incubation whereas, PD30, a dosa batter isolates showed lowest titrable acidity (0.506%) as shown in Table 3.

Table.1 Collection of isolates from various sources

Sample no.	Type of sample	Source	No. of Sample	No. of Isolates collected
1	Dosa Batter	Homemade	1	28
		Local Market	3	15
2	Jalebi Batter	Homemade	2	21
		Local Market	3	9
		Laboratory made	1	18
3	Dhokla Batter	Local Market	1	14
4	Handva Batter	Homemade	3	06
		Local Market	1	07
5	Fermented Cabbage	Homemade	1	11
		Laboratory Made	1	15
6	Soy curd	Laboratory Made	1	08
7	Curd	Homemade	1	03
		Local Market	3	26
Total			**22**	**181**

Table.2 Physiological characterizations

Isolate type	Growth at various Temp.(◦C)				pH Tolerance								Bile tolerance (%)			NaCl Tolerance (%)			Phenol Tolerance (g/100 ml)		
	15	30	37	45	1	2	3	4	5	6.5	8	9	0.3	0.5	0.8	4	8	12	0.4	0.5	0.6
PD2	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PD11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	±	+	+	-	+	+	+
PD18	±	+	+	+	-	-	+	+	+	+	-	-	-	-	-	+	-	-	+	-	-
PD30	-	+	+	+	+	+	+	+	+	+	-	+	+	±	-	+	+	-	+	+	+
PD35	-	+	+	+	-	-	+	+	+	+	±	-	-	-	-	+	-	-	+	-	-
PJ3	-	+	+	+	-	±	+	+	+	+	-	-	+	-	-	+	-	-	+	-	-
PJ4	-	+	+	+	±	±	+	+	+	+	-	-	±	-	-	+	-	-	+	-	-
PJ5	-	+	+	+	±	±	+	+	+	+	-	+	±	-	-	+	+	-	+	±	±
PJ25	-	+	+	+	-	-	+	+	+	+	±	-	+	-	-	+	+	-	+	-	-
PJ29	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	±	-
PJ38	-	+	+	+	-	-	±	+	+	+	±	-	-	-	-	+	-	-	+	-	-
PJ41	±	+	+	+	±	±	+	+	+	+	-	-	±	-	-	+	-	-	+	±	-
PDH2	-	+	+	+	-	-	+	+	+	+	-	-	-	-	-	+	-	-	+	-	-
PDH3	±	+	+	+	-	-	-	+	+	+	-	-	-	-	-	+	-	-	+	-	-
PDH4	±	+	+	+	-	-	-	+	+	+	±	-	±	-	-	+	-	-	+	-	-
PDH8	-	+	+	+	-	±	+	+	+	+	+	-	-	-	-	+	±	-	+	-	-
PDH9	-	+	+	+	-	±	+	+	+	+	+	-	±	-	-	+	±	-	+	-	-
PH4	-	+	+	+	-	-	+	+	+	+	-	-	+	±	-	+	±	-	+	±	-
PH5	±	+	+	+	±	+	+	+	+	+	+	±	+	+	±	+	+	+	+	+	-
PH8	-	+	+	+	-	-	±	+	+	+	+	-	+	-	-	+	-	-	+	-	-
PFC2	±	+	+	+	-	±	+	+	+	+	-	-	+	±	-	+	-	-	+	±	-
PFC18	±	+	+	+	-	±	±	+	+	+	-	-	-	-	-	+	±	-	+	±	-
PFC21	±	+	+	+	+	+	+	+	+	+	+	+	+	+	±	+	+	+	+	+	±
PSC2	-	+	+	+	-	-	-	+	+	+	±	-	+	±	-	+	-	-	+	-	-
PSC5	-	+	+	+	-	-	-	+	+	+	±	-	±	-	-	+	-	-	+	±	-
PSC6	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PC4	±	+	+	+	±	±	+	+	+	+	+	-	+	+	±	+	+	+	+	+	±
PC6	±	+	+	+	+	+	+	+	+	+	+	±	+	+	+	+	+	+	+	+	+
PC27	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+, growth; ±, weak growth; -, no growth

Table.3 Biochemical characterizations

Isolate Type	Sugar Fermentation						% Titrable Acidity	EPS Prod.	Enzyme Activity			Prod. of Ammonia from Arginine	Probable species identified	Carbohydrate fermentation (API Kit)	
	G	L	M	S	F	X			Protease	Amylase	Phytase			Species identity	% Similarity
PD2	+	+	+	+	+	-	0.711	-	+	+	-	-	*L. fermentum*	*L. rhamnosus*	99.8
PD11	+	+	-	+	+	-	1.017	-	+	+	-	-	*L. paracasei*	*L. casei*	99.9
PD18	+	+	-	-	+	-	2.457	-	-	-	-	-	*L. plantarum*	*L. plantarum*	-
PD30	+	-	+	+	+	+	0.506	-	-	-	-	+	*L. amylophilus*	*L. plantarum*	88.6
PD35	+	+	+	+	+	-	1.449	-	-	-	-	-	*L. plantarum*	*L. rhamnosus*	66.7
PJ3	+	+	+	+	+	+	1.35	-	-	-	-	+	*E. faecium*	*G. sanguinis*	85.7
PJ4	+	+	+	+	+	+	1.314	-	-	-	-	+	*E. faecium*	*E. faecium*	91.0
PJ5	+	+	+	+	+	+	0.712	-	-	-	-	+	*E. faecium*	*E. faecium*	99.8
PJ25	+	+	+	+	+	+	1.278	-	-	+	-	+	*E. faecium*	*E. faecium*	97.6
PJ29	+	+	+	+	+	+	1.377	-	-	+	-	+	*E. faecium*	*E. faecium*	95.9
PJ38	+	+	+	+	+	+	0.531	-	-	-	-	+	*L. plantarum*	*L. plantarum*	66.4
PJ41	+	+	-	-	+	-	0.918	-	+	+	-	-	*L. plantarum*	*L. rhamnosus*	-
PDH2	+	+	+	+	+	-	1.314	-	-	-	-	-	*A. urinae*	*A. urinae*	57.8
PDH3	+	+	+	+	+	+	1.089	-	-	-	-	+	*E. faecalis*	*E. faecium*	93.5
PDH4	+	+	+	+	+	-	1.413	-	-	-	-	-	*E. faecium*	*E. faecium*	-
PDH8	+	+	+	+	+	+	0.927	-	-	-	-	+	*E. faecium*	*E. faecium*	99.6
PDH9	+	+	+	+	+	-	1.206	-	-	-	-	-	*Lactococcus lactis*	*A. viridans*	-
PH4	+	+	+	+	+	-	1.161	-	-	+	-	-	*L. plantarum*	*L. plantarum*	57.8
PH5	+	+	+	+	+	-	1.521	-	+	+	-	-	*L. fermentum*	*L. fermentum*	97.4
PH8	+	+	+	+	+	+	0.612	-	-	-	-	+	*L. plantarum*	*L. plantarum*	-
PFC2	+	+	+	+	+	-	1.188	-	-	-	-	-	*E. faecium*	*E. faecium*	99.4
PFC18	+	+	+	+	+	-	1.332	-	-	-	-	-	*E. avium*	*A. viridans*	-
PFC21	+	+	-	-	+	-	1.02	-	+	+	-	-	*L. fermentum*	*L. fermentum*	99.9
PSC2	+	+	+	+	+	-	1.296	-	-	-	-	-	*Leuconostoc*	*Leuconostoc*	-
PSC5	+	+	+	+	+	-	1.332	-	-	-	-	-	*E. faecalis*	*E. faecium*	85.7
PSC6	+	+	+	+	+	-	1.251	-	+	+	-	-	*L. casei*	*L. rhamnosus*	99.9
PC4	+	+	-	-	+	-	2.277	-	-	+	-	-	*L. fermentum*	*L. rhamnosus*	99.9
PC6	+	+	+	+	+	-	2.007	-	+	+	-	-	*L. casei*	*L. casei*	99.8
PC27	+	+	+	+	+	-	1.521	-	+	+	-	-	*L. paracasei*	*L. fermentum*	99.8

G, Galactose, L, Lactose, M, Maltose, S, Sucrose, F, Fructose, X, Xylose, +, growth; ±, weak growth; -, no growth

None of the isolates could be able to produce EPS when streaked on plates containing 5% Sucrose (Table 3).

None of the isolate had displayed halo zones surrounding the colonies showing negative indication of extracellular phytate enzyme. Similarly, no extracellular lipase activity was detected in the strains of this study, which is in agreement with the report of Oterholm et al. (1968), who have found that LAB do not have extracellular lipase production. Eight isolates namely PD2, PD11, PJ41, PH5, PFC21, PSC6, PC6 and PC27 were considered protease positive as they showed clear zone of milk hydrolysis on milk agar plates after 48 h of incubation. Total 12 isolates among 29, two from dosa batter PD2, PD11; three jalebi batter isolates PJ25, PJ29 and PJ41; two handva batter isolates PH4, PH5; fermented cabbage isolate PFC21 and four curd isolates PSC6, PC4, PC6 and PC27 shown clear zone of amylase activity (Table 3).

Among the lactic acid bacterial strains, all jalebi batter isolates except PJ41; PD30 a dosa batter isolate; PDH3 and PDH8 dhokla batter isolates and PH8 handva batter isolate did show ammonia production from arginine as shown in Table 3.

Based on their physiological and biochemical profile, 10 isolates were further selected to study their safety by performing haemolytic activity, gelatinase activity and decarboxylase activity.

Safety Studies

Safety is one of the most recommended criteria in the FAO/ WHO (2002) guidelines on evaluation for probiotics. The mucoid lining constitutes the target across which many important physiological substances are exchanged. Haemolysis activity would break down the epithelial layer while gelatinase activity would derange the mucoid lining. These impairments interfere with the normal functioning of these very important linings and would cause pathways for infections. Absence of haemolytic and gelatinase activity is a selection criteria for probiotic strains, indicating that these bacteria are none virulent (De Vuyst et al., 2003). In our study, all the 10 assayed strains showed no positive haemolysis and gelatinase activity as there was no change in the color of the medium surrounding the colony on blood agar plates and there was no clear zone surrounding the colony on Nutrient Gelatin Agar plates compared to positive control strain of *S.aureus*, respectively. In a similar study, *L. plantarum* isolated from raw cow milk in the western highlands of Cameroon were haemolytic-negative and gelatinase-negative (Sieladie et al., 2011). Similar results were also published by Mami et al., (2008) and Kauli et al., (2009). Amino acid decarboxylation is also considered important criteria for safety and commonly associated with food fermentation which may results in formation of biogenic amines (BA). BA can trigger human health problems including palpitation, hypertension, vomiting and headache (Lonvaud-Funel, 2001). Therefore, a strain with potentiality showing amino acid decarboxylating activity should not be considered as safe for human welfare. In our study, all the 10 strain showed negative response regarding biogenic amine production. Similar negative response has been showed by Tejinder et al. (2014).

Lactic acid bacterial cultures are designed to meet food safety, shelf life, technological effectiveness and economic feasibility criteria. Specially selected strains may also provide probiotic health benefits, and, if properly modified, then may be endorsed with nutraceutical traits. Many isolates displayed varying promising capabilities but

not all the desirable properties were present in a single isolate. Very interestingly, the experimental results indicated that some of the selected isolates can be further used to check their probiotic potential in detail as they were resistance to Gastro-intestinal passage and may be work effectively in human gut by modulating resident microflora.

References

Abriouel, H., Benomar, N., Cobo, A., Caballero, N., Fernández Fuentes, M. A., Pérez-Pulido, R. 2012. Characterization of lactic acid bacteria from naturally-fermented Manzanilla Aloreña green table olives. Food Microbiol 32:308–316.

Agarwal K.N, Bhasin S.K. 2002. Feasibility studies to control acute diarrhoea in children by feeding fermented milk preparations Actimel and Indian Dahi. European Journal of Clinical Nutrition 56:56–59.

Arvind K., Nikhlesh K.S, Pushpalata R.S. 2010. Inhibition of 1,2-dimethylhydrazine induced colon genotoxicity in rats by the administration of probiotic curd. Molecular Biology Report 37:1373–1376.

Berrada N., Lemeland G., Laroch P. Thouveno T.P., Piaia M. 1991. Bifidobacterium fromfermented milks: Survival during gastric transit. Journal of Dairy Science 74:409-413.

Bover-Cid, S. & Holzapfel, W. 1999. Improved screening procedure for biogenic amine production by lactic acid bacteria. International Journal of Food Microbiology 53:33-41.

De Vuyst L., Foulquie M.R., Revets H. 2003. The role and application of enterococci in food and health. International Journal of Food Microbiology 84:299-318.

Dunne C., O'Mahony L., Murphy L., Thornton G., Morrissey D., O'Halloran S., Feeney M., Flynn S., Fitzgerald G., Daly C., Kiely B.C., O'Sullivan G., Shanahan F., & Collins J.K. 2001. In vitro selection criteria for probiotic bacteria of human origin: correlation with in vivo findings. American Journal of Clinical Nutrition 73:386-392.

El-Naggar, M. Y. M. 2004. Identification and Probiotic Characteristics of Lactic Acid Bacteria Isolated from Indonesian Local Beef. Biotechnology 3:173-180.

Gerhardt P., Murray R.G.E., Costilow R.N., Nester E.W., Wood W.A., Krieg N.R., Phillips G.B. 1981. Study of the Antimicrobial and Probiotic Effect of *Lactobacillus Plantarum* Isolated from Raw Goat's Milk from the Region of Western Algeria. Manual of methods for general bacteriology. American society for Microbiology, Washington, DC 20006.

Ghosh Asit R., Shruthy V.V., Pavithra M. Gowri S. (2011). Probiotic potentials among Lactic acid bacteria isolated from curd. International journal of research in ayurveda and pharmacy. 2 (2) 602-609.

Gilliland, S.E. and Walker, D.K. 1990. Factors to consider when selecting a culture of *Lactobacillus acidophilus* as a dietary adjunct to produce a hypocholesterolemic effect in humans.Journal of Dairy Science 73:905-911.

Haros M., Maria B., Yolanda S. (2005). Phytase activity as a novel metabolic feature in Bifidobacterium.FEMS Microbiology Letters, 247, 231–239.

Harrigan W.F., McCance M.E. 1990. Laboratory Methods in Food and Dairy Microbiology. Academic Press, London.

Havenaar, R. and Huis in't Veld, J. H. J. 1992. Probiotics: A general view. In: The Lactic Acid Bacteria in Health & Disease, B. J. B. Wood (Ed.), Elsevier Applied Science, London 151–170.

Holzapfel, W. H., R. Geizen, U. Schillinger. 1995. Biological preservation of foods with reference to protective cultures, bacteriocins and food grade enzymes. International Journal of Food Microbiology 24:343-362.

Jeyaram K., Singh A., Romi W., Devi A.R., Singh W.M., Dayanithi H. 2009. Traditional fermented foods of Manipur. Indian Journal of Traditional Knowledge 8:115–121.

Kalui C.M., Mathara J.M., Kutima P.M., Kiiyukia C., Wongo L. E. 2009. Functional characteristics of Lactobacillus plantarum and Lactobacillus rhamnosus from ikii, a Kenyan traditional fermented maize porridge. African Journal of Biotechnology 8:4363-4373.

Kingston J.J., Radhika M., Roshini P.T., Raksha M.A., Murali H.S., Batra H.V. 2010. Molecular characterization of lactic acid bacteria recovered from natural fermentation of beet root and carrot Kanji. Indian Journal of Microbiology 50:292–298.

Liong M.T. & Shah N.P. 2004. Bile salt deconjugation ability, bile salt hydrolase activity and cholesterol co-precipitation ability of lactobacilli strains. International Dairy Journal 15:391-398.

Lonvaud-Funel, A. 2001. Biogenic amines in wines: role of lactic acid bacteria. FEMS. Microbiology Letter 199:9-13.

Mami A., Henni J.E., Kihal M. 2008. Antimicrobial Activity of Lactobacillus Species Isolated from Algerian Raw Goat's Milk against Staphylococcus aureus. World Journal of Dairy and Food Sciences 3:39-49

Mathara J. M., Schillinger U., Guigas C., Franz C., Kutima P.M., Mbugua S. K., Shin H.-K., Holzapfel W. H. 2008. Functional characteristics of Lactobacillus spp. from traditional Maasai fermented milk products in Kenya. International Journal of Food Microbiology 126:57–64.

Mishra, V. & Prasad, M.N. 2005. "Application of in vitro methods for selection of Lactobacillus casei strains as potential probiotics," International journal of Food Microbiology 103:109-115.

Neha K., Sharma P.K. & Deepak C.S. 2014. Screening and evaluation of Lactobacillus spp. For the development of potential probiotics. African Journal of Microbiological Research 8:1573-1579.

Patel A., Cecilia L., Arti P., Prajapati J.B., Olle H. 2012. Probiotic properties of exopolysaccharide producing lactic acid bacteria isolated from vegetables and traditional Indian fermented foods. International Journal of Fermented Foods 1:87-101.

Prajapati JB, Trivedi Jigar, Nair Baboo, Rickard Oste, Aparnathi KD. 2014. Fermentation kinetics and sensory attributes of milk fermented by probiotic bacteria. International journal of fermented foods 3:75-84.

Schillinger U., Lucke F.K. 1987. Antibacterial activity of Lactobacillus sake isolated from meat. Applied Environmental Microbiology 55:1901–1906.

Sinha P.R, Sinha R.N. 2000. Importance of good quality dahi in food. Indian Dairyman 52:45–47.

Swennen K., Courtin C.M., Delcour J.A. 2006. Heat and pH stability of prebiotic arabinoxylooligosaccharides, xylooligosaccharides and fructooligosaccharides. Critical

Revolution in Food Science Nutrition 46:459–471.

Taheri H.R., Moravej H., Tabandeh F., Zaghari M. and Shivazad M. (2009). Screening of lactic acid bacteria toward their selection as a source of chicken probiotic. Immunlogy, Health, and Disease. 1586-1593.

Tambekar D.H. & Bhutada S.A. 2010. An evaluation of probiotic potential of lactobacillus sp. From Milk of domestic animals and commercial available probiotic Preparations in prevention of enteric bacterial infections. Journal of Microbiology 8:1-6.

Tejinder P.S., Ravinder K.M., Gurpreet K. & Renuka. 2014. Safety assessment and evaluation of probiotic potential of Lactobacillus reuteri strains under in vitro conditions. International journal of current Microbiology and Applied Sciences. 3:335-348.

Vijai P., Marilingappa J. & Kadirvelu J. (2005). Isolation and characterization of bacteriocins producing lactic acid bacteriafrom a South Indian special Dosa (Appam) batter. Journal of Culture Collection. 4, 53-60.

Yadav H., Jain S., Sinha P.R. (2007). Antidiabetic effect of probiotic dahi containing *Lactobacillus acidophilu* sand *Lactobacillus casei* in high fructose fed rats. Nutrition 23, 62–68.