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Probiotic potential of lactic acid bacteria isolated from food samples: an in vitro study

Ram Kumar Pundir[1]*, Satish Rana[1] Neha Kashyap[2] and Amandeep Kaur[2]
[1]Assistant Professor, Department of Biotechnology Engineering, Ambala College of Engineering and Applied Research (ACE), Devsthali (Near Mithapur), PO Sambhalkha-133101, Ambala, Haryana, India
[2]M.Tech. Students, Department of Biotechnology, Ambala College of Engineering and Applied Research (ACE), Devsthali (Near Mithapur), Sambhalkha, Ambala, 133101, Haryana, India

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ABSTRACT

Probiotics are the health promoting viable microorganisms that exhibit a beneficial effect on the health of human being by improving the intestinal microbial balance. Probiotic bacteria may produce various compounds, which are inhibitory to the growth of pathogen, which include organic acids (lactic and acetic acids), bacteriocins, and reuterin. In the present study a total of 17 food samples were collected for isolation of lactic acid bacteria. A total of 26 isolates of lactic acid bacteria were purified and screened for their antimicrobial activity against seven human pathogenic MTCC strains counting three test fungal strains such as *Aspergillus fumigatus*, *Aspergillus* sp. and *Candida albicans* and four test bacterial strains (two Gram-negative namely *Escherichia coli*, *Salmonella enterica ser. typhi* and two Gram-positive *Staphylococcus epidermidis* and *Bacillus amyloliquifaciens*). Out of 26, eight isolates were considered for further analysis of probiotic potential whose antimicrobial activity was found to be good against maximum number of tested strains. The selected lactic acid bacteria exhibited excellent probiotic characteristics and thus can be used as a potential source of probiotic. Study affirms their use in the development of new pharmaceutical preparations and functional foods belonging to vegetables and fruits (as origin) as probiotics for the betterment of public health.

INTRODUCTION

The term probiotic was defined as **"**a live microbial feed supplement which beneficially affects the host animal by improving its microbial balance'' (Aslam and Qazi, 2010). Probiotic bacteria may produce various compounds, which are inhibitory to the pathogen's growth, which include organic acids (lactic and acetic acids), bacteriocins, and reuterin. The organic acids not only lower the pH, thereby affecting the growth of the pathogen, but they can also be toxic to the microbes (Tambekar and Bhutada, 2010). There is increasing evidence that probiotics are beneficial in gastrointestinal disturbances, such as diarrhoea, dysentery, typhoid etc (Tambekar and Bhutada, 2010). It is important to underline when considering the effectiveness and biological activity of probiotics, prebiotics or their combination (synbiotics) that they are food products and not drugs.

* *Corresponding Author*
Dr. Ram Kumar Pundir, Sr. Asst. Professor and Head Department of Biotechnology Ambala College of Engineering and Applied Research Devsthali, PO Sambhalkha, Ambala, Haryana, India. Mob.: 09813267025

Furthermore, in many cases, their effects are mainly prophylactic in nature, rather than therapeutic, *i.e.* preventive rather than curative (Suskovic *et al.*, 2001). Lactic acid bacteria were referred to as probiotics in scientific literature by Lilley and Stillwell. Lactic acid bacteria (LAB) are a group of Gram positive, non-spore forming, cocci or rods which produce lactic acid as major end product from fermentation of carbohydrates. Majority of microorganisms used as probiotics belong to the LAB and bifidobacteria. Within the group of LAB, *Lactobacillus* species are most commonly utilized group of microorganisms for their potential beneficiary properties as probiotics. The antagonistic activity of such bacteria is known to inhibit a large number of enteric and urinary pathogenic bacteria (Hutt *et al*., 2006). Lactic acid bacteria including *Lactobacillus, leuconostoc, Lactococcus, pediococcus* and *Bifidobacterium* are found throughout the gastrointestinal tract. *Lactobacillus* and *Bifidobacterium spp.* are prominent members of the intestinal flora and are the commonly studied probiotics bacteria. They cause reduced lactose intolerance alleviation of some diarrhoeas, lowered blood cholesterol, increased

immune response and prevention of cancer. The selection criteria for probiotic LAB include: safety, viability/activity in delivery vehicles, resistance to acid and bile, adherence to gut epithelial tissue ability to colonise the gastro intestinal tract, production of antimicrobial substances, ability to stimulate a host immune response and the ability to influence metabolic activities such as vitamin production, cholesterol assimulation and lactose activity (Savodago *et al*., 2006). Therefore an attempt was made to isolate LAB from food samples and to evaluate their potential as probiotics.

MATERIALS AND METHODS

Sample collection

A total of 17 samples of fresh foods (Joshi *et al.*, 2006) were collected from the field and market of Ambala, Haryana. Samples consisted of three groups: group I, 8 types of fresh vegetables namely, tomato, carrot, radish, garlic, ginger, cucumber, lemon and sweet potato; group II, 5 types of fresh fruits such as, ras bhari, grapes, orange, apple, and kiwi; group III, packed ready-to-eat foods which were cheese, pickle, chocolate, and curd. Samples of group I and group II were washed with sterile distilled water, chopped with sterile cutter in small pieces and weighed 10g (Trias *et al.,*2008), on other hand the group III samples were taken weighed 10g and 10ml (for curd).

Isolation and purification of lactic acid bacteria from food samples

For isolation of LAB, serial dilution agar technique was used. Ten gram of each sample (except curd taken 10 ml) was dissolved into 90 ml of MRS broth. After dissolving into MRS broth they were shaken homogeneously and were incubated at 37°C for 24 hrs in an aerobic condition. In the serial dilution agar plate technique, 10ml of a stock solution was suspended and agitated in 90 ml water blanks to form a microbial suspension. Serial dilution of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} were made by pipetting 10ml into 90ml water blanks. 10 ml of each dilution was inoculated to MRS agar plates (prepared by pouring 15ml of sterile and cooled molten media in plates) and incubated at 37^0C for 24 hrs for bacterial growth. The plates were observed for appearance of colonies and number of colonies produced on each plate of different dilution was recovered (Hoque *et al.*, 2010). Bacteria were purified by streak plate method on MRS agar and incubated at 37^0C for 24hrs and transferred to MRS agar slants and then maintained in refrigerator at 4^0C till further analysis.

Test microorganisms

A total of seven human pathogenic strains counting three test fungal strains such as *Aspergillus fumigatus*, *Aspergillus* sp. and *Candida albicans* and four test bacterial strains (two Gram-negative namely *Escherichia coli, Salmonella enterica ser. typhi* and two Gram-positive *Staphylococcus epidermidis* and *Bacillus amyloliquifaciens*)were procured from IMTECH, MTCC, Chandigarh, India.

Standardisation of test microorganisms

The tested microorganisms were standardised by using 0.5 Mc Farland standard. Mc Farland Standard was used as reference to adjust the turbidity of microbial suspensions so that their number will be within a given range. 0.5 Mc Farland gives approximate cell density of 1.5×10^8 CFU/ml, having absorbance of 0.132 at wavelength of 600 nm. The microbial suspensions were prepared in their respective sterile nutrient broth and are compared either visually or by measuring the absorbance with that of the standard (Andrews, 2001).

Production and evaluation of antimicrobial metabolite from lactic acid bacteria

MRS broth was used for antimicrobial metabolite production from lactic acid bacteria, 500mL Erlenmeyer's flasks each containing 200 ml MRS broth autoclaved at 121^0C for 15 minutes and inoculated with colony of a LAB isolate grown on MRS agar. The inoculated flasks were incubated at 37^0C for 2-3 days under stationary condition. Then centrifuged at 10000 rpm for 10 min. Antimicrobial activity of culture supernatant (100µl/well) and broth (100µl/well) was tested by agar well diffusion method.

Screening of isolated bacterial cultures for antimicrobial activity

For screening of isolated bacterial cultures, these cultures were inoculated to MRS broth incubated at 37^0C for 24-48 hrs on shaker to carry out the fermentation process. After incubation, 2 ml of each fermented culture broth and supernatant was taken to test the antimicrobial activity by agar well diffusion method against the seven test microorganisms. Each isolated culture was screened against every test microorganism. An overnight culture of pathogens grown in their respective medium at 37 °C was diluted to a turbidity equivalent to that of a 0.5 McFarland standard (Khunajakr *et al.*, 2008).

Protocol for agar well diffusion method for antibacterial and antifungal activity

Petri plates were prepared by pouring 20ml of respective sterile molten media for test microorganisms and allowed it to solidify

↓

Spreading of agar plates with 100 µl of each standardised tested microorganisms

↓

Plates were allowed to dry and two wells (each 7mm in diameter) made into agar plates with sterile borer

↓

Wells loaded with 100 µl of isolated bacterial culture filtrate supernatant) and 100 µl sterile broth

↓

Plates were incubated at 37^0C for 24 hrs for test bacterial and 25^0C for 3-5 days for fungal isolates

↓

Measurement of diameter of zone of inhibition

Evaluation of probiotic potentials of isolated bacterial cultures

Bacterial cultures with good antimicrobial activity were selected for further determination of probiotic potential as follows:

pH tolerance

The isolated bacterial cultures were inoculated into sterile MRS broth tubes of varying pH, i.e. pH 2, 3, 4, 5, 6, and 7 and incubated at 37^0C for 2-3 days. Then 0.1ml inoculums from each tube was poured to MRS agar medium by pour plate method and incubated at 37^0C for 48hrs. The growth of LAB on MRS agar was used to designate isolates as pH tolerant (Tambekar and Bhutada, 2010).

Bile salt tolerance

The medium with varying concentrations of bile salt (0.5, 1.0, 1.5 and 2.0%) was inoculated with each selected bacterial culture and incubated at 37^0C for 48hrs. Then 0.1ml inoculums was transferred to MRS agar by pour plate method and incubated at 37^0C for 48hrs. The growth of LAB cultures on agar plates was used to designate isolates as bile salt tolerant (Tambekar and Bhutada, 2010).

Temperature sensitivity

The selected bacterial cultures were grown at varying temperatures, i.e. 25, 30, 37 and 40^0C for 48-72 hrs. Then 0.1ml inoculum was transferred to MRS plates by pour plate method and incubated at 37^0C for 48hrs. The growth of LAB on MRS agar plates was used to designate isolates as temperature tolerant (Tambekar and Bhutada, 2010).

Lactose utilization

The acid production by selected bacterial cultures was detected by observing the change in colour of the medium. Sterilized fermentation medium (10g peptone, NaCl 15g, phenol red 0.018g, lactose 5g, for 1L distilled water and final pH 7.0) was inculated with different cultures and incubated at 35^0C for 24-48 hrs. Change in colour from red to yellow indicates the production of acid (Ahmed and Kanwal, 2004).

NaCl tolerance

Salt tolerance of selected bacterial cultures was assessed after 3days of incubation at concentration of 1-6.5% NaCl in MRS broth (Adebayo-tayo and Onilude, 2008; Hoque *et al.*, 2010).

Antagonistic activities

Agar well diffusion method was used to detect the antimicrobial activity as described above.

Antibiotic susceptibility

The antibiotic susceptibility of isolated LAB was assessed using antibiotic discs diffusion method on MRS agar plates. Broth cultures of LAB was prepared using MRS and adjusted to 0.5 McFarland standards. A 100µl suspension of freshly grown bacterial cultures was spread on MRS agar plates. The antibiotic discs were placed on the surface of agar and the plates were incubated at 37^0C for 48 hrs. Susceptibility pattern was assessed using Penicillin G (10 units), Cephalothin (30µg), Oxacillin (1µg), Clindamycin (2µg), Erythromycin (15µg), and Amoxyclav (30µg).

RESULTS AND DISCUSSION

Isolation and antimicrobial activity of lactic acid bacteria

A total of 26 bacterial cultures were isolated from food samples. Out of 26 isolates tested, some isolates were found to exhibit antimicrobial activity against indicator strains as shown in Table 1. The isolates B_3, B_5 and B_{14} showed inhibitory activity against all seven tested pathogenic strains whereas isolates B_4, B_6, B_7, B_{11} and B_{12} exhibited activity against 6 tested strains out of 7 and rest of the isolates showed activity either against 1, 2, 3, 4 or 5 tested strains as shown in Fig 1. On the basis of maximum zone of inhibition and inhibition against almost all tested microorganisms, eight isolates (B_3, B_4, B_5, B_6, B_7, B_{11}, B_{12} and B_{14}) out of 26 isolates were selected for further evaluation of probiotic properties.

Out of the 8 isolates, B_3 isolate showed maximum zone of inhibition against *Aspergillus fumigatus* of 31mm followed by *Escherichia coli* (30mm), *Candida albicans* (27mm), *Aspergillus* sp. (23mm), *Bacillus amyloliquifaciens* (22mm), *Staphylococcus epidermidis* (19mm) and minimum against *Salmonella enteric ser. typhi* (12mm); B_5 isolate exhibited maximum zone of inhibition against *E.coli* of 30mm, followed by *B.amyloliquifaciens* (29mm), *A.fumigatus* (28mm), *Aspergillus* sp. (24mm), *C.albicans* (24mm), *S.epidermidis* (22mm) and minimum against *S.typhi* (12mm) and B_{14} isolate showed maximum zone of inhibition against *A.fumigatus* of 26mm, followed by *Aspergillus* sp. (24mm), *C.albicans* (18mm), *S.epidermidis* (18mm), *B.amyloliquifaciens* (17mm) and minimum against *E.coli* and *S.typhi* with zone of inhibition 15mm.

The isolates which showed activity against 6 tested strains were B_4, B_6, B_7, B_{11} and B_{12} isolates. Isolate B_4 showed maximum zone of inhibition against *E.coli* and *A.fumigatus* with zone of inhibition of 28mm, followed by *B.amyloliquifaciens* (26mm), *Aspergillus* sp. (25mm), *C.albicans* (23mm) and minimum against *S.epidermidis* with zone of inhibition of 20mm in diameter but did not show inhibition against *S.typhi*; B_6 showed maximum inhibition against *B. amyloliquifaciens* with zone of inhibition of 30mm, followed by *A.fumigatus* (27mm), *Aspergillus* sp. (26mm), *C.albicans* (24mm), *S.epidermidis* (21mm) and minimum against *S.typhi* (12mm) but no inhibition against *E.coli* was observed; B_7 showed maximum inhibition against *E.coli* with zone of inhibition of 31mm, followed by *C.albicans*(30mm), *A.fumigatus* (29mm), *B.amyloliquifaciens* (28mm), *Aspergillus* sp. (25mm) and minimum against *S.epidermidis* (24mm) but failed to show inhibition against *S.typhi*; B_{11} showed maximum inhibition against *E.coli* with zone of inhibition of 28mm, followed by *Aspergillus* sp. (26mm), *A.fumigatus* and *B.amyloliquifaciens* (24mm), *S.epidermidis* (23mm) and minimum against *S.typhi*

(14mm) but no activity was observed against *C.albicans;* and B_{12} showed maximum zone of inhibition against *Aspergillus* sp. and *C.albicans* of 24mm, followed by *S.epidermidis* (20mm), *E.coli* (18mm), *B.amyloliquifaciens* (17mm) and minimum against *S.typhi* (12mm) but failed to show inhibition against *A.fumigatus* as shown in Fig 2. Similar work was performed by Tambekar *et al.* (2010), they tested the antibacterial properties of isolated LAB species from milk samples against *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Salmonella typhi* and *Shigella flexneri*. The zone of inhibition of isolated LAB species was 19mm for *Escherichia coli* and 25mm for *Salmonella typhi* whereas in present studies the zone of inhibition against *Escherichia coli* was 30mm and against *Salmonella typhi* was 15mm on average. In present study the isolated lactic acid bacterial cultures inhibited tested fungal strains more than bacterial strains except B_{11} and B_{12} which inhibited tested bacterial strains more than fungal strains. Out of tested bacterial strains gram positive bacteria were more sensitive than gram negative bacteria to lactic acid bacterial isolates and on other side out of tested fungal strains moulds were inhibited more than yeast by lactic acid bacterial cultures except B_7 and B_{12} which showed results differently as shown in table 1. The antifungal activity of lactic acid bacterial cultures was may be due to the production of organic acids, reuterin, hydrogen peroxide, proteinaceous compounds, hydroxyl fatty acids and phenolic compounds (Dalie *et al.*, 2010). The antibacterial activity was may be due to the production of acetic and lactic acids that lowered the pH of the medium or competition for nutrients, or due to production of bacteriocin or antibacterial compounds (Bezkorvainy, 2001; Tambekar *et al*, 2009).

Table . 1: Antimicrobial activity of LAB isolated from food samples

S.No.	Isolated LAB cultures	Tested bacterial strains (with ZOI in mm)				Tested fungal strains (with ZOI in mm)		
		Gram positive		Gram negative		Moulds		Yeast
		B.amyloliquifaciens	***S.epidermidis***	***E.coli***	***S. enterica***	***Aspergillus* sp.**	***A.fumigatus***	***C.albicans***
1	B_3	22	19	30	12	23	31	27
2	B_4	26	20	28	NA	25	28	23
3	B_5	29	22	30	12	24	28	24
4	B_6	30	21	NA	12	26	27	24
5	B_7	28	24	31	NA	25	29	30
6	B_8	NA	18	28	NA	NA	24	29
7	B_{10}	16	18	16	NA	20	NA	20
8	B_{11}	24	23	28	14	26	24	NA
9	B_{12}	17	20	18	12	24	NA	24
10	B_{13}	NA	NA	NA	NA	NA	15	NA
11	B_{14}	17	18	15	15	24	26	18
12	B_{16}	NA	NA	NA	11	36	28	NA
13	B_{17}	NA	NA	NA	NA	NA	NA	NA
14	B_{18}	13	11	15	NA	NA	13	NA
15	B_{19}	NA	NA	NA	NA	NA	NA	NA
16	B_{20}	NA	14	NA	NA	12	NA	11
17	B_{21}	12	NA	13	16	NA	NA	NA
18	B_{22}	NA	NA	NA	NA	NA	10	NA
19	B_{23}	NA	11	15	NA	NA	NA	NA
20	B_{24}	NA	NA	23	NA	NA	NA	NA
21	B_{25}	NA	NA	NA	NA	11	NA	11
22	B_{26}	NA	NA	NA	NA	NA	NA	NA
23	B_{27}	13	NA	NA	NA	10	12	NA
24	B_{28}	12	NA	14	NA	12	16	NA
25	B_{29}	NA	NA	NA	NA	11	NA	NA
26	B_{30}	NA	NA	NA	NA	NA	NA	13

'NA': No activity

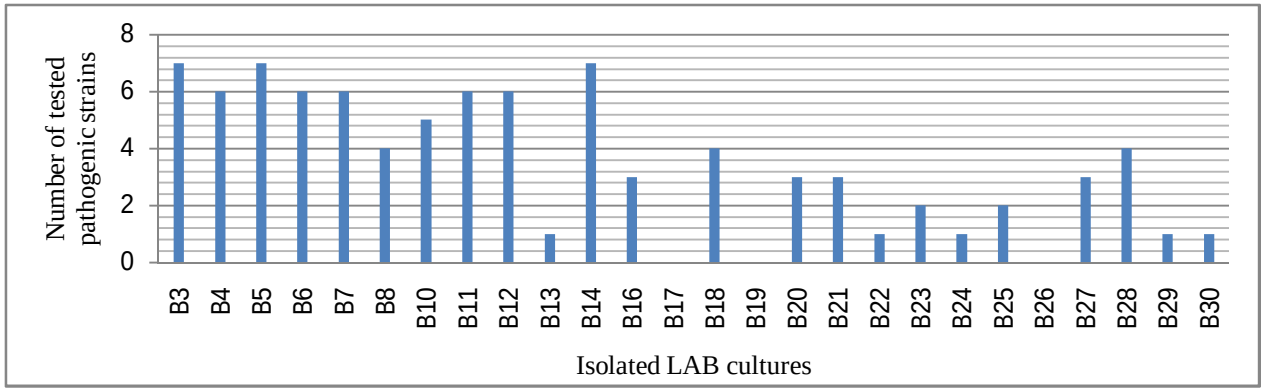

Fig. 1: Number of tested pathogenic strains inhibited by isolated LAB cultures.

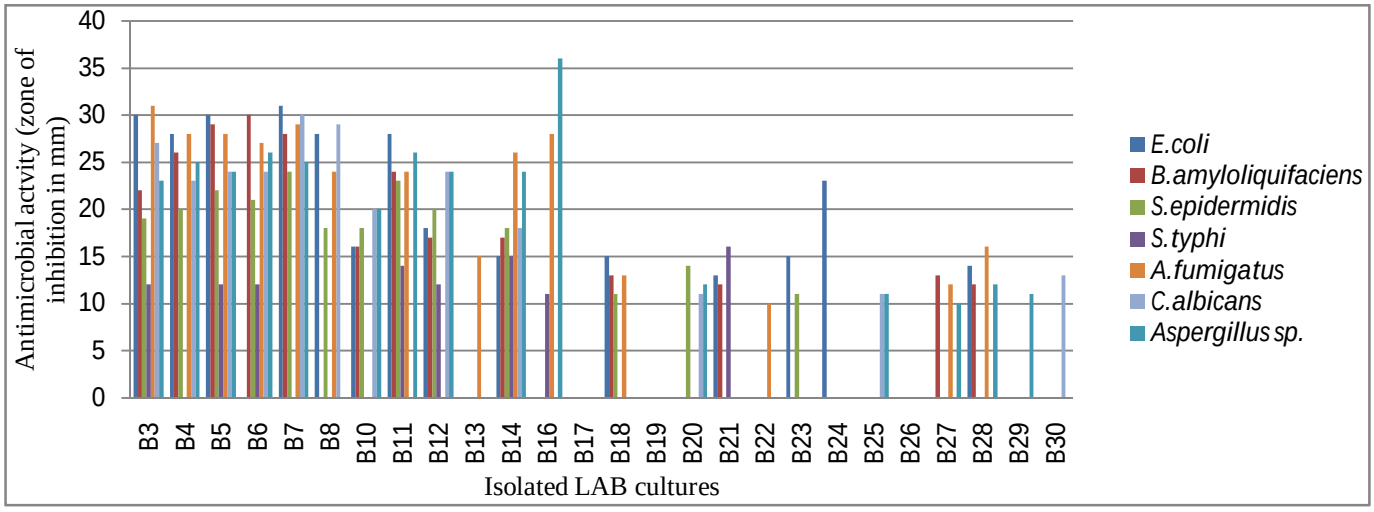

Fig. 2: Antimicrobial activity of isolated LAB cultures against pathogenic strains by agar well diffusion method.

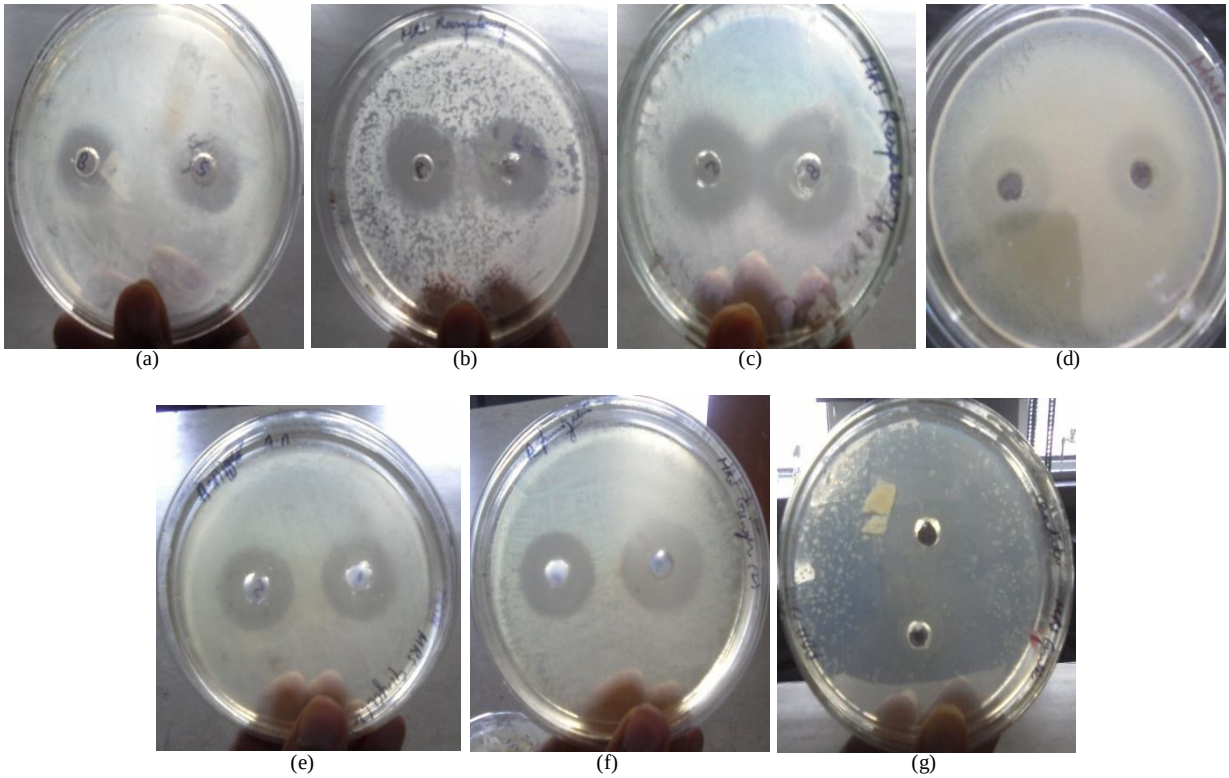

Fig. 3: Antimicrobial activity of isolate B_5 against pathogenic strains by agar well diffusion method (a) *S. epidermidis*, (b) *B. amyloliquifaciens*, (c) *E. coli*, (d) *S. enterica* (e) *Aspergillus* sp. (f) *A. fumigatus*, (g) *C. albicans*.

Table. 2: pH and bile salt tolerance of selected LAB isolates.

S.No.	Selected LAB isolates	Varying pH									Varying Bile Salt Concentration (in %)			
		7.0	6.0	5.0	4.0	3.7	3.5	3.0	2.0	1.0	2.0	1.5	1.0	0.5
1	B_3	+	+	+	+	+	+	-	-	-	+	+	+	+
2	B_4	+	+	+	+	+	+	-	-	-	+	+	+	+
3	B_5	+	+	+	+	+	+	-	-	-	+	+	+	+
4	B_6	+	+	+	+	+	+	-	-	-	+	+	+	+
5	B_7	+	+	+	+	+	+	-	-	-	+	+	+	+
6	B_{11}	+	+	+	+	+	+	-	-	-	+	+	+	+
7	B_{12}	+	+	+	+	+	+	-	-	-	+	+	+	+
8	B_{14}	+	+	+	+	+	+	-	-	-	+	+	+	+

'+' : Growth and '-': No growth

Probiotic potential of selected lactic acid bacteria cultures pH and Bile salt tolerance

In present research, the selected LAB isolates were able to grow in pH 7.0, 6.0, 5.0, 4.0, 3.7 and 3.5 but were unable to grow at pH 3.0, 2.0 and 1.0 and were also able to survive in 0.5, 1.0, 1.5 and 2.0 % bile salt concentrations as shown in Table 2. On other hand lactic acid bacteria isolated from baobab (maari) fermented seeds were able to survive at pH 2.5 but could tolerate bile salt concentration of 0.3% only (Kabore *et al.*, 2012). Tambekar (2010) reported that the three isolated excellent probiotic acid tolerance at pH 2.0 and bile salt tolerance at 2.0%. Tolerance to bile salts is a prerequisite for colonization and metabolic activity of bacteria in the small intestine of the host (Havenaar *et a.l*, 1992). This will help *Lactobacilli* to reach the small intestine and colon and contribute in balancing the intestinal microflora (Tambekar and Bhutada, 2010).

Temperatures, NaCl tolerance and Lactose utilization

In present study, all the selected LAB isolates were able to survive at temperature 25, 30, 37 and 40^0C. The temperature is an important factor which can dramatically affect the bacterial growth. The reason for choosing this temperature range was to detect whether the isolated cultures were able to grow within range of normal body temperature or not. As if the isolates were not able to survive within the selected temperature range then they would not have been able to survive in the human gut, which is an essential factor of probiotics to show their effectiveness. The results obtained were positive for growth at chosen temperature range.

The entire selected LAB isolates were grown in fermentation medium supplemented with lactose and were observed for change in colour from red to yellow which indicates the production of lactic acid. It was observed that every selected LAB isolate was able to produce lactic acid from lactose as shown in Table 3.

Lactose utilisation of LAB isolated from camel milk was assessed by Ahmed and Kanwal (2004). Lactose intolerant people cannot metabolize lactose due to the lack of essential enzyme β-galactosidase. When they consume milk or lactose-containing products, symptoms including abdominal pain, cramping and diarrhoea arise. If lactose passes through from the small intestine, it is converted to gas and acid in the large intestine by the colonic microflora. Also the presence of breath hydrogen is a signal for lactose maldigestion. The studies provide that the addition of certain starter cultures to milk products, allows the lactose intolerant people to consume those products without the usual rise of breath hydrogen or associated symptoms (Lin *et al.*, 1991; Scheinbach 1998; Fooks *et al*., 1999).

In present study the lactic acid bacterial isolates were able to tolerate 1-6.5% NaCl concentration as shown in Table 3. NaCl is an inhibitory substance which may inhibit growth of certain types of bacteria. If the lactic acid bacteria was sensitive to NaCl then it would not be able to show it's activity in presence of NaCl so it was essential to test the NaCl tolerance of lactic acid bacterial isolates, whereas Hoque *et al*. (2010) observed the NaCl (1-9%) tolerance of their *Lactobacillus* sp. isolated from yoghurts. The present experimental results were similar to the work done by Adebayo-tayo and Onilude (2008).

Antibiotic susceptibility pattern of selected LAB isolates

Antibiotic susceptibility pattern of selected LAB isolates was observed by using Kirby-Bauer disc diffusion method the results as shown in Table 4. Isolate B_3 was sensitive to drug Amoxyclav (11mm), Erythromycin (25mm), Penicillin G (30mm), Oxacilli (10mm), Cephalothin (26mm), Clindamycin (35mm); B_4 showed sensitivity for Erythromycin (35mm), Penicillin G (30mm), Cephalothin (30mm), Clindamycin (35mm) but was resistant to Amoxyclav and Oxacillin; B_5 isolate was resistant to only Oxacillin but was sensitive to Amoxyclav (15mm), Erythromycin (35mm), Penicillin G (30mm), Cephalothin (25mm), Clindamycin (35mm); B_6 was resistant to Amoxyclav and was sensitive to Erythromycin (26mm), Penicillin G (20mm), Oxacillin (12mm), Cephalothin (15mm), Clindamycin (36mm); B_7 was sensitive to Amoxyclav (12mm), Erythromycin (30mm), Penicillin G (36mm), Cephalothin (25mm), Clindamycin (34mm) and was resistant to Oxacillin; B_{11} showed resistant to Amoxyclav and Oxacillin but was sensitive to Erythromycin (35mm), Penicillin G (30mm), Cephalothin (18mm), Clindamycin (36mm); B_{12} showed sensitivity for Erythromycin (34mm), Penicillin G (31mm), Cephalothin (30mm), Clindamycin (34mm) but was resistant to Amoxyclav and Oxacillin; B_{14} showed resistant to Amoxyclav and Oxacillin but was sensitive to Erythromycin (25mm), Penicillin G (30mm), Cephalothin (20mm), Clindamycin (35mm). Such resistance to a wide spectrum of antibiotics indicated that if isolated probiotics induced in patients treated with antibiotic therapy may be helpful in faster recovery of the patients due to rapid establishment of desirable microbial flora. Resistance of the probiotic strains to some antibiotics could be used for both preventive and therapeutic purposes in controlling intestinal infections (EI-Naggar, 2004).

The antibiotic resistance of isolated LAB was assessed using antibiotic discs (Hi media Laboratories Pvt. Ltd. Mumbai, India) on MRS agar plates against Ampicillin (1μg), Cephalothin (30μg), Co-Trimoxazole (25μg), Gentamicin (10μg), Nalidixic acid (30μg), Nitrofurantoin (300μg), Norfloxacin (10μg) and Tetracycline (25μg). The isolates were found to be resistance against almost all above drugs used. In present study isolates were sensitive for Cephalothin but contrary to present work, the work done by Tambekar and Bhutada (2010) the isolates were resistant to Cephalothin. This may vary from strain to strain or type of strain.

Identification of selected Lactic acid bacterial cultures

The LAB isolates were identified by cultural, morphological and biochemical characteristics. The cultural, morphological and biochemical characteristics of LAB isolates are presented in the Table 5. The characteristics of LAB belonging to genus *Lactobacillus* should be gram positive, rod shaped, non

endospore forming, catalase negative, must be acid producing and gas formation may or may not be there from sugars and moreover LAB should also be pH, temperature, NaCl, bile salt tolerant and lactic acid producers from sugar. In present study all the isolated bacterial cultures match the characteristics of genus *Lactobacillus* as they are Gram positive, mostly rod shaped cells, non endospore forming, catalase negative, acid producing but non gas forming and were able to withstand varying range of pH, temperature, NaCl, bile salt tolerant and lactic acid producers from sugar . So, this was confirmed that isolates B_3, B_4, B_5, B_6, B_7, B_{11}, B_{12} and B_{14} resembled the characteristics of genus *Lactobacillus* as described by Holt *et al.* (1994). In current study, the gelatin hydrolysis was also performed as was performed by Bukola (2008) for identification of LAB. B_{11} was the only isolate which successfully hydrolysis the gelatin rest of the isolates were unable to do so as shown in Table 5.

Table. 3: Temperatures, NaCl tolerance and lactic acid production from lactose of selected LAB isolates

S.No.	Sample	Varying Temperature (in ^{0}C)				NaCl concentration (in %)							Lactic acid production
		40	37	30	25	1	2	3	4	5	6	6.5	
1	B_3	+	+	+	+	+	+	+	+	+	+	+	+
2	B_4	+	+	+	+	+	+	+	+	+	+	+	+
3	B_5	+	+	+	+	+	+	+	+	+	+	+	+
4	B_6	+	+	+	+	+	+	+	+	+	+	+	+
5	B_7	+	+	+	+	+	+	+	+	+	+	+	+
6	B_{11}	+	+	+	+	+	+	+	+	+	+	+	+
7	B_{12}	+	+	+	+	+	+	+	+	+	+	+	+
8	B_{14}	+	+	+	+	+	+	+	+	+	+	+	+

'+': Growth

Table. 4: Antibiotic susceptibility pattern of selected LAB isolates .

S.No.	Drug			Selected LAB isolates (ZOI in mm)							
	Name	Code	Conc.	B_3	B_4	B_5	B_6	B_7	B_{11}	B_{12}	B_{14}
1	Amoxyclav	Ac	30 µg	11	R	15	R	12	R	R	R
2	Erythromycin	E	15 µg	25	35	35	26	30	35	34	25
3	Penicillin G	P	10 units	30	30	30	20	26	30	31	30
4	Oxacillin	Ox	1 µg	10	R	R	12	R	R	R	R
5	Cephalothin	Ch	30 µg	26	30	25	15	25	18	30	20
6	Clindamycin	Cd	2 µg	35	35	30	36	34	36	34	35

'ZOI': Zone of inhibition, 'R': Resistance.

Table. 5: Morphological and biochemical characteristics of isolated lactic acid bacteria.

S.No.	Selected LAB isolates	Cultural characteristics	Morphological characteristics	Gram's staining	Endospore staining	Catalase test	Acid and Gas formation			Gelatin hydrolysis	pH tolerance (3.5-7.0)	Bile salt tolerance	Temperature tolerance	NaCl tolerance	Lactic acid production
							Glucose	Lactose	Sucrose						
1	B_3	Colonies round, creamy, smooth, opaque, flat and entire	Purple, rod, chain	+ve	-	-	A^+G^-	A^+G^-	A^+G^-	-	+	+	+	+	+
2	B_4	Colonies round, creamy, moist, smooth, flat and entire	Purple, cocobacillary, mostly single	+ve	-	-	A^+G^-	A^+G^-	A^+G^-	-	+	+	+	+	+
3	B_5	Colonies round, creamy, smooth, viscous, opaque, raised and entire	Purple, irregular	+ve	-	-	A^+G^-	A^+G^-	A^+G^-	-	+	+	+	+	+
4	B_6	Colonies round, yellow, moist, flat and wavy	Purple, rod	+ve	-	-	A^+G^-	A^+G^-	A^+G^-	-	+	+	+	+	+
5	B_7	Colonies round, creamy, rough, opaque, raised and curled	Purple, rod, chain	+ve	-	-	A^+G^-	A^+G^-	A^+G^-	-	+	+	+	+	+
6	B_{11}	Colonies round, yellow, smooth, moist, raised and entire	Purple, rod, single, chain	+ve	-	-	A^+G^-	A^+G^-	A^+G^-	+	+	+	+	+	+
7	B_{12}	Colonies irregular, creamy, rough, dry, opaque, raised and wavy	Purple, rod, single	+ve	-	-	A^+G^-	A^+G^-	A^+G^-	-	+	+	+	+	+
8	B_{14}	Colonies irregular, yellow, smooth, moist, flat and curled	Purple, rod	+ve	-	-	A^+G^-	A^+G^-	A^+G^-	-	+	+	+	+	+

'+ve': Gram positive; '-': Negative; 'A+': Acid production; 'G-': NO gas formation; '+': Positive

CONCLUSION

LAB are typically involved in a large number of spontaneous food fermentations but they are also closely associated with the human environment.Further work can also be required to identify the isolates at species level and bioactive antimicrobial compounds before commercialization.

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