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ORIGINAL ARTICLE

In vitro assessment of the Probiotic properties and Bacteriocinogenic potential of Lactic acid bacteria from buffalo milk and curd in Haryana

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ABSTRACT
The present investigations have been aimed for isolation, identification and screening of lactic acid bacteria from milk and curd samples of different locations in Haryana (India) against gram positive and gram negative test organisms. All in all, 52 isolates were obtained from milk and curd samples. On the bases of screening, 8 potent isolates were chosen and were characterized morphologically, physiologically, and biochemically. These selected potent strains were further tested against food borne pathogenic bacteria (Bacillus cereus, Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa) using agar well diffusion assay. The antibacterial potential of the strains was checked in terms of zone of inhibition (mm). Strain BMH38 was found to be the most effective against Bacillus cereus. On the basis of molecular characterization, BMH4 and BMH38 were identified as Lactobacillus plantarum, BMH10 and BMH20 as Lactobacillus acidophilus and isolates namely BMH14, BMH27 and BMH30 were identified as Lactobacillus casei and strain BMH22 as Lactobacillus fermentum. The findings of the current study indicate that these baceriocenogenic probiotic strains of lactobacilli and the bacteriocins produced by them may be used as biopreservatives in food industry to inhibit/control the growth of food spoilage and pathogenic bacteria in near future.
Keywords: Antibacterial activity, bacteriocins, food borne pathogens, Lactic acid bacteria, probiotic

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INTRODUCTION

During recent years, researchers are discovering good bacteria and bad bacteria according to bacterial effects on animal health, pharmaceutical production, food production, preservation and/or in any other ways that are beneficial for animals. These discoveries led us to a new term "Probiotics". Probiotics originated from '*probios'* means 'for life'. Ellie Metchnikoff was the first who coined the term Probiotics. Probiotics are the bacteria which are beneficial for human when consumed in adequate quantity. Probiotics may be either a single culture of microorganism or a mixed culture of live microorganisms, which are administrated to animals and human to get good health [1, 2]. Probiotic bacteria mainly belongs to lactic acid bacteria [3], *Saccharomyces* [4], enterics [5], Bifidobacteria [6] and streptococci [7]. Milk contains a variety of lactic acid probiotic bacteria. Probiotics are being used in the industries for different applications which are useful for human and animal health. A lot of probiotic products are available comprising of various enzymes, capsules or tablets, vitamins, and many fermented foods which may have one or many probiotic species. Generally products which are manufactured for human are produced as fermented products or given in powdery forms or tablets. Probiotics products are not being used in medicinal applications but these are only used for health maintaining purposes. The oral consumption of probiotic microbes produces a protective effect on the gut flora. Many probiotic products are being used for diarrhoea, showing a positive therapeutic effect [8, 1]. Lactic Acid Bacteria act as a crucial biodefense factor against many pathogenic bacteria to stop their growth in the intestine [9, 10].

For a microbe, to be considered as probiotic, it should adapt the condition of other normal micro flora of the intestine and able to adhere and survive the gastrointestinal passage [11].

Lactic acid bacteria (LAB) have been abundantly found in dairy products, plants, soil, water, manure, sewage and silage. They are gram positive facultative anaerobic bacteria having mainly rod or cocci shape. LAB grow normally at a temperature range of 30 ºC to 40 ºC, some strains are also found to grow below 5 ºC or above 45 ºC. The major end product of LAB's intermediary metabolism is Lactic acid [12, 13]. Bacteriocin produced by LAB is harmful for pathogenic bacteria. LAB are generally regarded as safe (GRAS) by FDA as they are safe for their use in any forms. They produce many antimicrobial substances, such as bacteriocins, bacteriocins-like substances, organic acids, antibiotic like products and H_2O_2, which help to enhance shelf life of food products [14, 15]. LAB play an important role in the variety of food fermentations to extend shelf life of fermented food products. LAB also show beneficial effects on nutritional and sensory qualities [16]. Isolation, screening and characterization of new isolated strains from raw milk and milk products is utterly interesting due to the presence of the micro flora having great technological functions and potential applications in the dairy and food industries [17]. The bacteriocin produced by LAB may be used to increase the shelf life and can be used to enhance the microbial safety of foods[18]. Bacteriocins produce antagonistic effects against many food borne pathogens which confirm their use in biopreservation of food products.

Many bacteria produce protein like structure during their growth, possessing antimicrobial activities, called bacteriocin. Bacteriocins restrict their activity to strains of species that produce them and particularly to strains of the same species. On the other hand, antibiotics have a broad spectrum and even if their activity is restricted this does not show any preferential effect on closely related strains thus bacteriocins are not considered as antibiotics. Bacteriocins are proteinaceous in nature and classified in to three different classes on the bases of their molecular weight [14]. Nisin was the first lanthibiotic bacteriocin produced by *L. lactis* [19]. The involvement of bacteriocin-producing LAB as starter culture in the production of fermented food helps in achieving great quality and reasonable alternative to the addition of purified bacteriocins. LAB producing bacteriocins have been experimentally tested in the production of some varieties of cheese [20-22] and many other fermented food products [23]. Aroma and texture of the food might be able to enhance with the addition of bacteriocins [24, 25].

Milk and curd are the good source of LAB, consumed as daily food in Northern India. Nowadays people are getting aware for their food that should not contain chemical preservatives and should be beneficial to their health. This leads to development of novel approaches for less use of processing and wide use of bacteriocins for biopreservation. So these days many probiotic products are available in market. More and more study is required in this regard for the improvement of these useful products from LAB, hence the present investigations have been performed.

MATERIAL AND METHODS

Chemicals used

All the chemicals used were of analytical grade. The chemicals/ingredients and readymade culture media were purchased from HIMEDIA laboratories Pvt.

Collection of samples

Raw samples of milk and curd of buffalo (*Bubalus bubalis*) were collected from different regions of Haryana (27°39' to 30°35' N latitude and between 74°28' and 77°36' E longitude) state (India) in sterile tubes, and these were processed in lab under aseptic conditions and stored under refrigeration for further use.

Test bacterial cultures

The test cultures (*Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*) were procured from departmental Microbial Culture Collection (Lab No. 14).

Isolation and purification of Probiotic LAB

The samples were serially (10^{-1}– 10^{-6}) diluted with sterile physiological saline (0.85%; w/v) solution. The respective dilution was streaked over the agar surface of MRS agar medium and the plates were incubated at 37 ºC for 24h to obtain LAB isolates. Colonies were picked randomly and purified by transfers onto MRS medium. Then the isolates were checked for purity. The pure cultures were subcultured twice in MRS broth for further use. Every time the freshly grown pure cultures were used.

Screening of LAB isolates for antimicrobial activity

The overnight grown culture of LAB isolates were used in MRS broth at 37 ºC and were standardized using spectrophotometer (O.D. 0.5; wavelength of 600 nm). The standardized samples were used as inocula for MRS broth and cultured at 37 ºC for 48h at 150 rpm. The cells were removed from the incubated samples by centrifugation at 10,000 rpm for 15 min at 4 ^{0}C. The cell free supernatant (CFS) was

used. The pH of the CFS was set to 6.5 using sterile 1N NaOH. The antimicrobial activity spectra were tested against pathogens using Agar well diffusion assay. Clear zones around the wells indicated the antimicrobial activity of the respective isolate.

Identification of isolates

The isolates were identified by morphological, physiological and biochemical characteristics as per the available standard methods [26, 27].

Morphological study

The color, shape and margins of colony were observed, gram staining and motility tests of freshly grown isolates were performed.

Physiological study of isolates

A) Growth at different temperatures

About 5 ml of MRS broth containing Bromocresol purple (0.004%; w/v) was transferred into tubes. Freshly grown culture (0.1 ml) was inoculated in respective tube and incubated for 5 days at 20 °C, 30 °C, 37 °C and 45 °C. The growth of isolates was recorded by the change of the colour, from purple to yellow [27].

B) Oxygen requirement of the isolates

The isolates were inoculated in MRS broth and were incubated under provided oxygen conditions (oxygenated, micro-aerophilic and anaerobic) for 24- 48h at 37 °C.

C) Optimization of pH

Isolates were inoculated in different MRS broth media adjusted to various pH (3, 5, 6 and 8). Bacterial growth was recorded on each pH.

D) Effect of NaCl conc on growth of isolates

Different conc of NaCl (2%, 4% and 6%; w/v) was used in MRS broth. The isolates were inoculated and incubation was done for 24–48h at 37 °C. Growth of the isolates was observed.

Biochemical characterization of isolates

A) Catalase test

One ml overnight broth cultures of isolates were dropped on the glass slides and then H_2O_2 (2% , v/v) solution was applied, the isolates producing bubbles were considered as catalase positive.

B) Citrate utilization test

Simmons citrate agar was used for inoculation of isolates and incubated for 24h at 37 °C. Colour of the isolates was observed, isolates showing the blue colour were recorded as positive.

C) Gas production from Glucose

About 10 ml glucose broth containing inverted Durham's tube was inoculated with respective isolate and incubated for 24 - 48h at 37 °C. It was observed for the production of bubbles or gas.

D) Indole production

Tryptone(1%, v/v) broth was inoculated with isolates and samples were tested at regular intervals by adding 2 mL of reagent (p-dimethyl amino benzaldehyde 2 g, 75% ethanol 100 ml, Ehrlich‟s reagent 1 ml). Further, concentrated HCl was added drop by drop to get a red zone between the alcohol and the peptone solution.

E) Voges-Proskauer (VP) test

The isolates were grown in VP broth (peptone 7 g; glucose 5 g; NaCl 5 g; pH 6.5). About 2 ml sample of respective culture was tested by adding 5 ml of 40% KOH and a trace of creatine, shaking vigorously and allowed to stand for 60 min. Appearance of a cherry red colour indicated positive reaction.

F) Methyl red (MR) test

To one part of the culture (grown in VP medium), one drop methyl red solution (Methyl Red 0.4 g in 100 m distilled water) was added. A magenta red colour indicated positive reaction.

G) Oxidase test

A colony of the isolate from nutrient agar plate was put on a filter paper and 2-3 drops of fresh 1% aqueous tetramethyl-p-phenylene-diamine (TMPD) dihydrochloride was added to it. The presence of oxidase was confirmed if the colony turned purple within 5-10 s.

H) Nitrate reduction test

The isolates were incubated in tubes containing trypticase nitrate broth for 24 h at 37 °C. About 0.5 ml sulphanilic acid (0.8%, in 5N - Acetic acid) and 0.5 ml α-naphthylamine (0.5%, in 5N Acetic acid) were added to the tubes. Appearance of red or pink colour showed the positive test.

Carbohydrate fermentation

A total of 12 sugars were tested to check the fermentation ability of the screened isolates. Bromocresol purple was used as indicator. Colour change from purple to yellow indicated acid production [28].

Antibacterial activity

Cell free filtrates (CFF) of the screened isolates were used to analyze their antibacterial activities against test organisms by agar well diffusion assay. Nutrient medium was allowed to solidify. The lawn of the respective test organisms was made. The borer (6 mm) was used to make wells. The bottom of the wells were sealed using soft agar. The CFF (100 µL) was poured in respective well. Plates were incubated at 37 ºC for 24 h. the antibacterial effect of the CFF was analyzed in terms of the zone of inhibition (diameter in mm) around the well.

RESULTS

Isolation, purification and screening of strains

About 52 white and creamy colonies were selected randomly and streaked on MRS agar to obtain the pure cultures. Eight isolates (BMH4, BMH10, BMH14, BMH20, BMH22, BMH27, BMH30 and BMH38) producing good zone of inhibition were selected for further studies.

Morphology and staining

The colour of the colonies varied from white to pale creamy, the shape was circular, and the size ranged from 0.5 to 4 mm in diameter All the isolates were found Gram positive, rod shaped and 0.6µm to 0.9 µm in size (Table 1).

Table 1. Characteristics of LAB isolates

Characters	Isolate(s)							
	BMH 4	BMH10	BMH14	BMH20	BMH22	BMH27	BMH30	BMH38
Gram reaction	+	+	+	+	+	+	+	+
Shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Size (µm)	0.8	0.6	0.7	0.7	0.8	0.7	0.8	0.9

+ = positive

Physiological and biochemical characterization

All the 10 screened isolates were found as non motile, catalase-negative, non endospore formers, non gas producing from glucose. The strains were found to grow best at the temp between The optimum temp for the growth of the strains was 30- 37 ^{0}C while optimal pH was 6 (Table 2 & 3).

Table (2).Physiological characteristics of the isolates

Characters (growth)		Isolate(s)							
		BMH4	BMH10	BMH14	BMH20	BMH22	BMH27	BMH30	BMH38
on MRS		++	++	++	++	+	++	++	++
at pH	3.0	±	-	±	-	-	±	±	±
	5.0	+	++	+	++	+	+	+	+
	6.0	++	++	+	++	++	++	++	++
	8.0	++	-	+	-	++	+	+	++
at temp (ºC)	20	+	±	±	±	+	+	±	+
	30	+	++	+	++	++	+	+	+
	37	++	++	+	++	++	+	+	++
	45	+	+	±	+	+	-	-	+
O_2	A	+	+	+	+	+	+	+	+
	B	+	+	+	+	+	+	+	+
	C	-	-	+	-	-	+	+	-
NaCl (%)	2	++	+	++	+	+	++	++	++
	4	+	-	+	-	-	+	+	+
	6	-	-	-	-	-	-	-	-

*(-) no growth, (±) poor growth, (+) growth and (++) huge growth; A=Aerobic; B=Microearophilic; C=Anaerobic

Table 3.Biochemical characterization of the isolates

Characteristic(s)	Isolate(s)							
	BMH4	BMH10	BMH14	BMH20	BMH22	BMH27	BMH30	BMH38
Motility	-	-	-	-	-	-	-	-
Endospore	-	-	-	-	-	-	-	-
Catalase	-	-	-	-	-	-	-	-
Citrate Utilization	-	-	+	-		+	+	-

Gas production	-	-	-	-	+	-	-	-
Nitrate Reduction	-	-	-	-	-	-	-	-
Oxidase test	-	-	-	-	-	-	-	-
Indole production	-	-		-	-			-
VP test	-	-	-	-	-	-	-	-
Methyl red test	+	-	+	-	+	-	-	+

*(-) no growth, (±) poor growth, (+) growth and (++) huge growth

Carbohydrate fermentation

The sugar fermentation patterns were studied and the results were compared with Bergey's Manual of Determinative Bacteriology and the screened isolates were tentatively identified as *L. plantarum, L. acidophilus*, *L. fermentum* and *L. casei.* Four different sp. of *Lactobacillus* were found in milk and curd (Table 4; Fig 1).

Table 4. Carbohydrate fermentation pattern of the isolates

Isolates	Sugar(s)												Isolate(s) Identified as
	C	F	G	L	M	Ma	Mn	Rf	Rh	Sr	Su	X	
BMH4, BMH38	+	+	+	+	+	+	+	+	-	+	+	-	*L. plantarum*
BMH10, BMH20	+	+	-	+	-	+	+	+	+	-	+	+	*L. acidophilus*
BMH22	+	+	+	+	+	-	+	+	-	-	+	-	*L. fermentum*
BMH14, BMH27, BMH30	+	+	+	+	+	+	+	-	-	+	+	-	*L. casei*

C = Cellobiose, F = Fructose, G = Galactose, Rh = RhamnoseL = Lactose, M = Maltose; Mn = Mannitol, Ma = Mannose, Rf = Raffinose, , Sr = Sorbitol; Su = Sucrose, X = Xylose; (+) = able to ferment sugar; (–) = not able

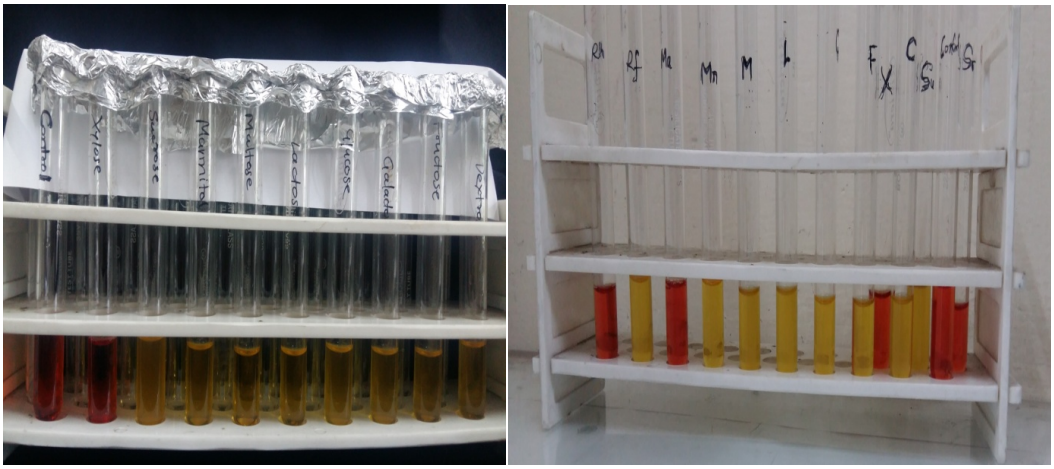
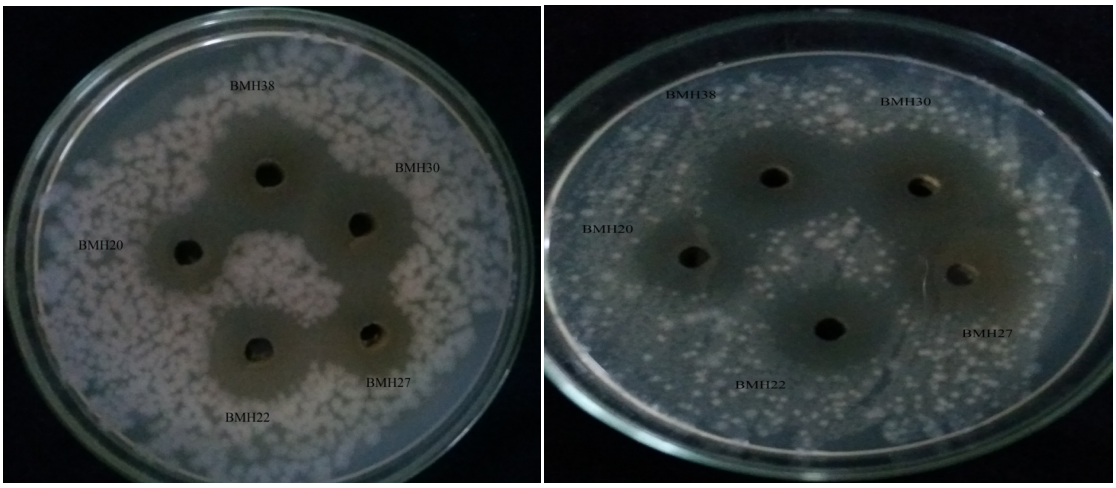


Fig 1. Carbohydrate fermentation by LAB isolates

Bioassay for bacteriocin production

The cell free filtrate (CFF) was used to check the presence of bacteriocins produced by LAB isolates. The pH of the CFF was adjusted to 6.5 with sterile 1N-NaOH. Agar well diffusion assay was used for detection of the antimicrobial activity spectra of the CFF of LAB strains against test organisms.

The bacterial strains were found to be very effective against test organisms like *Bacillus cereus*, *Escherichia Coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Strain BMH38 showed the maximum zone of inhibition (16±1.98 mm) against *B. cereus* while Strain BMH22 showed the minimum zone of inhibition (08±0.21 mm) against *Pseudomonas aeruginosa* (Table 5; fig 2).



B. cereus *E. coli*

Fig 2. Antimicrobial activity against test organisms

Table 5. Evaluation of lactic acid bacterial strains against food borne pathogens

Isolate(s)	Zone of inhibition (mm) Test organism(s)			
	B. cereus	*E. coli*	*S. aureus*	*P. aeruginosa*
BMH4	15±1.83	13±1.02	14±1.69	11±0.58
BMH10	11±2.50	12±0.30	11±1.28	10±0.21
BMH14	13±1.45	14±2.00	13±0.35	10±.35
BMH20	10±0.45	12±2.01	10±0.51	11±0.39
BMH22	13±1.67	13±1.32	12±0.60	08±0.21
BMH27	12±1.78	13±0.37	11±0.28	12±1.28
BMH30	14±2.00	12±0.59	13±1.62	09±0.02
BMH38	16±1.98	14±2.42	12±0.84	10±0.05

* including 6 mm diameter of well.

DISCUSSION

Lactic acid bacteria are being used as starter cultures in food fermentation process for many centuries. Particularly LAB which are advantageous and non-pathogenic are traditionally used in the food industry. They are assumed to play a significant role in the dairy industry because of the colossal level of human utilization of some daily life usable fermented products, especially cheese and fermented milks[29]. We have isolated, purified, identified and characterized bacteriocin producing LAB present in milk and curd of buffalo. Raw milk and curd contain a large number of bacteriocin producers. Bacteriocin activity of the isolates was confirmed after neutralizing the cell free filtrate of the obtained isolates by Agar well diffusion assay against different food borne pathogens.

The findings of the current research work are in similar lines with that of Reuben and his co-workers[30] who isolated four prominent strains of LAB which were identified as *Lactobacillus plantarum, L. paracasei*, *L. casei* and *L. fermentum*. In present investigations, All of the isolated strains were found to possess broad spectrum range of antimicrobial activity. Vanniyasingam and his co-wprkers[31] isolated the strains of LAB from cow milk. The isolated strains were characterized biochemically. The strain identified as *L. plantarum* and the strain showed broad range of antibacterial activity against *E. coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa*. The isolates in the current research work also showed the broad range of antibacterial activity. Kumar and Kumar[32] isolated lactic acid bacterial strains from milk and curd samples of Himachal Pradesh and found that out of 30 isolates, two isolates were found to show poor growth and nine isolates showed moderate and the rest 19 showed high growth rate on MRS agar media after incubation periods of 24-48h. Chauhan and Daru[33] isolated and characterize the *Lactobacillus* species from milk and curd samples. The isolated strains were tested against *Staphylococcus aureus, Salmonella typhi, Escherichia coli, Bacillus cereus* and *Pseudomonas aeruginosa* and the maximum zone of inhibition was observed against *Staphylococcus aureus* (21 mm) and minimum zone of inhibition (8 mm) against *Pseudomonas aeruginosa*. Arokiyamary and Sivakumar[34] isolated LAB from traditional milk samples and evaluated the obtained strains against *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Salmonella* sp. and *Shigella dysenteriea*, they observed the maximum zone of inhibition

(15 mm) against *Staphylococcus aureus.* They observed the best growth of isolates at the temp 37-40 ^{0}C and pH 6.0 - 6.8. Aslim and his co-workers[35] revealed that all the *Lactobacillus* strains isolated from Turkish dairy milk samples were able to produce antimicrobial substances against *Staphylococcus aureus* and *Escherichia coli*. In present study, the isolated LAB strain BMH38 was found very effective against *B. cereus* with maximum zone of inhibition (16±1.98 mm). The optimal temperature for growth of the obtained isolates was around 37 ºC and the obtained isolates were able to survive at temperature range 20 ºC to 45 ºC.

CONCLUSION

The bacteriocin produced by LAB isolated from curd and milk sample of Buffalo was partially purified. The antimicrobial activity against food borne pathogens indicated the adequacy of bacteriocin in enhancing shelf life and preservation of different food products. The results obtained indicate that LAB can be used in industrially for the production of antimicrobial peptides (Bacteriocins) and further studies can be carried out for strain improvement to enhance the production of bacteriocin. Extensive toxicological and worthiness tests ought to be performed before the item is affirmed for vast scale utilization.

REFERENCES

1. Çakır, I., (2003). Determination of some probiotic properties on *Lactobacilli* and *Bifidobacteria*, *Ph.D. Thesis*, Ankara University, Turkey.
2. Sanders, M.E., (2003).Probiotics: Considerations for human health. *Nutri. Rev,* 61, 91-99 .
3. Carr, F.J., Chill, D. and Maida, N., (2002). The lactic acid bacteria: a literature survey. *Crit. Rev. Microbiol.*, 28, 281–370.
4. Czerucka, D., Piche. T. and Rampal, P., (2007). Review article: yeast as probiotics—*Saccharomyces boulardii*. *Aliment Pharmacol. Ther*. 26, 767–778.
5. Sartor, R.B., (2003). Targeting enteric bacteria in treatment of inflammatory bowel diseases: why, how and when. *Curr. Opin. Gastroenterol.*, 19, 358–365.
6. Picard, C., Fioramonti. J., Francois, A., Robinson, T., Neant, F. and Matuchansky, C.,(2005). Review article: bifidobacteria as probiotic agents—physiological effects and clinical benefits. *Aliment Pharmacol. Ther.*, 22, 495–512.
7. Meurman J.H. and Stamatova I., (2007). Probiotics: contributions to oral health. *Oral. Dis.*, **13**, 443–451.
8. Gismondo, M.R., Drago, L. and Lombardi, A., (1999). A review of probiotics available to modify gastrointestinal flora. *Int. J. Antimicrobial Agents,* **12**, 287-292 .
9. Goldin, B.R. and Gorbach, S.L., (1992). Probiotics for humans. *Probiotics.* R. Fuller, ed. Champman & Hall, London, 355–376.
10. Naidu, A. S., Bidlack, W.R. and Clemens, R.A., (1999). Probiotic spectra of lactic acid bacteria. Crit. Rev. *Food Sci. Technol.*, **38**, 13–126.
11. Havenaar, R., Brink, B. and Huis in't Veld, J.H.J., (1992). Selection of strains for probiotic use. Probiotics. R. Fuller, ed. Chapman & Hall. London, 209–224.
12. Caplice, E. and Fitzgerald, G.F., (1999). Food fermentations: role of microorganisms in food production and preservation. *Int. J. Food Microbiol.*, **50**, 131-149.
13. Makarova, K., Slesarev, A., Wolf, Y., Sorokin, A., Sirkin, B. and Koonin, E.,(2006). Comparative genomics of the lactic acid bacteria. *Proc. National Acad. Sci.*, **103** (42), 15611- 15616.
14. Jack, R.W., Tagg, J.R. and Ray, B., (1995).Bacteriocins of gram-positive bacteria. *Microbiological Reviews*, **59**, 171–200.
15. Montville, T.J. and Kaiser, A.L., (1993). Antimicrobial proteins: classification, nomenclature, diversity and relationship to bacteriocins. *Bacteriocins of lactic acid bacteria*, **12,** 1–22.
16. De Vuyst, L. and Leroy, F., (2007). Bacteriocins from lactic acid bacteria: production, purification, and food applications. *J. Molecular Microbiol. Biotechnol.*, **13**, 194–199.
17. El-Ghaish, S., Dalgalarrondo, M., Choiset, Y., Sitohy, M., Ivanova, I., Haertle, T. and Chobert, J., (2010). Screening of strains of lactococci isolated from Egyptian dairy products for their proteolytic activity. *Food Chem.***, 120**, 758-764.
18. Olasupo N.A., Olukoya D.K. and Odunfa S.A., (1997). Assessment of a bacteriocin-producing *Lactobacillus* strain in the control of spoilage of a cereal – based African fermented food. *Folia Microbiologica*, **42**, 31-34.
19. Mattick, A.T.R. and Hirsch, A., (1947). Further observation of an inhibitory substance (nisin) from *lactic streptococci*. *Lancet*, **2**, 5–12.
20. Garde, S., Ávila, M., Gaya, P., Medina, M. and Nunez, M., (2006). Proteolysis of Hispanico cheese manufactured using lacticin 481-producing *Lactococcus lactis ssp. lactis* INIA 639. *J. Dairy Sci.,* **89,** 840–849.
21. O'Sullivan, L., Ross, R.P. and Hill, C., (2003). A lacticin 481-producing adjunct culture increases starter lysis while inhibiting nonstarter lactic acid bacteria proliferation during Cheddar cheese ripening. *J. Applied Microbiol.*, **95**, 1235–1241.

22. Ryan, M.P., Rea, M.C., Hill, C. and Ross, R.P., (1996). An application in cheddar cheese manufacture for a strain of *Lactococcus lactis* producing a novel broad-spectrum bacteriocin, lacticin 3147.*Applied Environ. Microbiol.***, 62,** 612–619.
23. Diop, M.B., Dubois-Dauphin, R., Destain, J., Tine, E. and Thonart, P., (2009). Use of a nisin producing starter culture of *Lactococcus lactis* subsp. lactis to improve traditional fish fermentation in Senegal. *J. Food Protection*, **72,** 1930–1934.
24. Fernández de Palencia, P., de la Plaza, M., Mohedano, M.L., Martínez-Cuesta, M.C., Requena, T., López, P. and Peláez, C., (2004). Enhancement of 2-methylbutanal formation in cheese by using a fluorescently tagged Lacticin 3147 producing Lactococcuslactis strain. *Int. J. Food Microbiol.*, **93**, 335–347.
25. Martínez-Cuesta, M.C., Requena, T. and Peláez, C., (2003). Lacticin 3147 favours isoleucine transamination by *Lactococcuslactis* IFPL359 in a cheese-model system. *Biotechnol. Letters*, **25**, 599–602.
26. Bergey, D.H., Harrison, F.C., Breed, R.S., Hammer, B.W. and Huntoon, F.M., (1989). Berge's Manual of Determinative Bacteriology.William and Wilkins Company London, **9**.
27. Harrigan, W.F. and McCance, M.E., (1990). Laboratory Methods in Food and Dairy Microbiology, *Academic Press, London*, **8,** 46-54 .
28. Tserovska, L., Stefanova, S. and Yordanova, T.,(2002). Identification of lactic acid bacteria isolated from Katyk, goats milk and Cheese. *J. Culture. Collect.*, **3**, 48-52.
29. Farkye, N. (2016). Cheese technology. *Int. J. dairy Technol*. **57**, 91-97.
30. Reuben R.C., Roy P.C., Sarkar S.L., Rubayet Ul Alam A.S.M. and Jahid I.K., (2020). Characterization and evaluation of lactic acid bacteria from indigenous raw milk for potential probiotic properties. *J. Dairy Sci.,* 103(2), 1223-1237.
31. Vanniyasingam, J, Kapilan, R and Vasantharuba, S.,(2019). Isolation and characterization of potential probiotic lactic acid bacteria isolated from cow milk and milk products. *AGRIEAST*. 13(1), 32-43.
32. Kumar, A. and Kumar, D., (2011). Isolation and characterization of bacteria from dairy samples of Solan in Himachal Pradesh for identification of *Lactobacillus spp*. *Int. J. Pharm. Sci. Rev. Res.,* **25** (2), 110-114 .
33. Chauhan, B.P. and Daru, D., (2016). Isolation and characterization of *Lactobacillus* from milk, curd and fecal sample and assigning their probiotic values. *Int. J. Pharma Bio Sci.*, 7(3):1070 – 1075.
34. Arokiyamary, A. and Sivakumar, P.K., (2011). Antibacterial activity of Bacteriocin producing *Lactobacillus sp*., isolated from traditional milk products. *Curr. Bot*. **2** (3), 05-08.
35. Aslim, B., Yuksekdag, Z.N. and Sarikaya, E., (2005). Determination of the bacteriocin-like substances produced by some lactic acid bacteria isolated from Turkish dairy products. *LWT-Food Mircorbiol*., **8**, 303-310.