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Bioprospecting of Lactic Acid Bacteria for Potentiality as Probiotics

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Abstract: Lactic acid bacteria have been used for many years as natural bio preservatives in fermented foods and are also believed to have beneficial health effects on the host. However selection of strain is an important criteria which is mainly based on evaluating the physiological properties of the strain. In this study, lactic acid bacteria were isolated from various dairy, non-dairy and human sources in MRS agar medium and were characterized based on physiological and biochemical characteristics. Based on their tolerance to bile and to extreme low acid conditions, four isolates were selected. Their activity was tested against common food and human pathogens and the isolates were also tested for their sensitivity towards twelve different antibiotics. The screened isolates were subjected to high temperature and salinity and were also exposed to hydrogen peroxide since the isolates were catalase negative. The results ended up with two isolates, which were promising for development as suitable candidates for use in functional foods.

Key words: Functional Food · Bile · Antibiotics · Pathogen · Lactic Acid Bacteria

INTRODUCTION

Probiotics are defined as "living micro-organisms, which upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition". FAO/WHO has adopted the definition of probiotics as "Live microorganisms which when administered in adequate amounts confer a health benefit on the host" [1]. Some selected strains of *Lactobacillus, Bifidobacterium, Streptococcus, Lactococcus* and *Saccharomyces* have been promoted in food products because of their reputed health benefits [2-5]. To achieve a probiotic status, microorganisms must fulfil a number of criteria related to safety, functional effects and technological properties [6].

Any functional food component should be stable to acid and bile for it to get into the intestinal environment, resistive to digestive enzymes, have adherence to intestinal epithtelium, act against intestinal pathogens by secreting antimicrobials, anti-carcinogenic and anti-mutagenic, have cholesterol-lowering effects, boost the immune system and improve bioavailability of food compounds [4]. The technological properties of probiotic include good sensorial properties, fermentative activity, good survival during freeze-drying or spray-drying, proper growth and viability in food products, phage resistance and high stability during long-term storage [7]. There is an increasing demand for probiotics and the new functional food market are looking for probiotic products with viable and stable cultures to impart real benefits. Hence there is a vast scope for identifying new probiotic cultures from several sources. With this background, the study has been conducted to identify potent lactic acid bacterial cultures with probiotic attributes to meet out current demands of the functional food industry.

MATERIALS AND METHODS

Isolation and Biochemical Characterization of Lactic Acid Bacteria: Lactic acid bacteria were isolated from several dairy and non-dairy sources which included vendor's unpasteurized milk, home-made curd, toddy flour from commercial outlet, cumbu flour, unripe mango and water extract of tulsi leaves, feaces of 50 days old infant male and from human milk. Prior to isolation, cumbu flour was soaked in water overnight and the fermented water was used for isolation. Similarly unripe mango was cut into small pieces, added with some common salt and allowed for natural fermentation for about 2 days.

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Sample dilutions up to 10^{-3} were prepared and lactic acid bacterial cultures were isolated in Man, Rogosa and Sharpe (MRS) agar medium. Changes in pH of the medium after growth of bacterial cultures were recorded since the bacteria metabolise sugars to produce lactic acid as the end product. The following biochemical tests were carried out using 48 hours old log cultures of the isolates as per standard procedures: Gram reaction; titrable acidity in terms of percent lactic acid, citrate utilization, growth in rice flour medium, production of amylase, lipase and catalase.

Tolerance to Low Acid Conditions: Cultures were inoculated in MRS broth acidified with 0.1N HCl to lowered pH levels of 4.0, 3.0 and 2.0 and observed for growth to test their survival under low acid conditions which is an important criterion for use as probiotics. Simultaneously, they were plated in MRS agar medium with pH adjusted to 4.0, 3.0 and 2.0 and observed for confirmation of colony formation in the plates.

Tolerance to Bile: The isolates were inoculated in MRS broth supplemented with 0.1 to 0.5% of oxbile (Oxbile dry powder, Himedia) along with a control for each of the isolates. The cultures were observed for growth for a period of 15-20 days. The selected positive cultures were further tested for tolerance towards fresh bile salt at 2% and 4% concentration.

Activity Against Common Pathogens: Log cultures (48h old) of the isolates were centrifuged at 7000x for 30 minutes and the culture filtrate was taken for the assay. Activity of the isolates were tested against the following human and food pathogens by agar well diffusion assay: Bacterial pathogens *viz*., *Salmonella* sp*., Klebsiella pneumoniae, E.coli, Staphylococcus aureus, Streptococcus, Vibrio cholerae and a fungal pathogen, Candida* sp. The test organism was seeded in nutrient glucose agar medium and plated. Cell free extract of the LAB isolates was added on to agar wells of 0.8cm diameter at the rate of 20µl, with sterile water as control. Area of inhibition of each of the isolate against the test pathogens was recorded.

Antibiotic Sensitivity Test: The LAB isolates tested positive for bile tolerance were seeded in MRS agar plates and plated. Antibiotic discs embedded with 12 different antibiotics as given below were placed over the solidified seeded plates and the isolates were observed for growth / inhibition zone in these plates.

Growth in Porcine Pancreas: Cultures tested positive for bile and acid tolerance were inoculated in MRS broth supplemented with porcine pancreas at concentrations from 0.1 to 0.5% and tested for the presence of growth.

Tolerance to hydrogen peroxide, high temperature and salinity The selected cultures were subjected to hydrogen peroxide at 100 ppm, 300 ppm and 500 ppm added to MRS broth and observed for growth. Similarly the log cultures were exposed to 100°C for 15 minutes and 30 minutes in water bath and then plated in MRS medium and observed for colonial growth in plates. Salinity tolerance test was carried out at 1-5% concentration of sodium chloride added to MRS medium and the isolates were tested for growth.

Identification of the Cultures: Based on all these screening protocols, lactic acid bacterial cultures from two sources *viz*., from cumbu flour and human milk were observed to be positive for all the tests. These two cultures were subjected for identification using MALDI-TOF.

RESULTS AND DISCUSSION

Isolation and Biochemical Characterization: Lactic acid bacterial isolates from all the eight different sources were obtained in a highly pure form. All the cultures excepting milk and curd showed moderate slime production. pH of broth which was 7.0 initially before inoculation, was recorded to be less than 3.5 after culture growth for all the other isolates whereas the isolate from milk recorded a highly alkaline pH. This was very unusual for lactic acid bacteria, which is known to produce lactic acid from any given carbon source resulting in reduced pH. This might be correlated with the production of excessive slime by the milk isolate. The result of pH study coincided with the results of titrable acidity in terms of lactic acid percentage which recorded the lowest acidity percentage of 0.16% with respect to milk isolate.

Microscopic observations revealed the cells to be rods for all the isolates, but those from milk, cumbu flour and human milk were coccal shaped. Biochemical studies showed that the isolates belong to lactic acid bacteria group, being gram positive, catalase negative, non-spore formers, mostly non-amylolytic, lipase positive and when tested for citrate utilization, cultures from infant feaces, tulsi, toddy and cumbu appeared to be citrate positive. Authentication of lactic acid bacteria by morphological and biochemical characterization has been reported by several workers. In a study [8], lactic acid bacteria (LAB)

Table 1: Acid and bile tolerance of LAB isolates and activity against pathogens

Tests		Milk	Mango	Curd	Cumbu	Toddy	Tulsi	IF	HM
Acid tolerance	pH 2	+	-	-	+	-	-	-	-
	pH 3	+	+	-	+	-	-	-	-
	pH 4	+	+	+	+	+	+	+	+
Bile tolerance	0.1%	-	-	-	+	+	+	-	+
	0.2%	-	-	-	+	+	+	-	+
	0.3%	-	-	-	-	+	+	-	+
	0.4%	-	-	-	-	+	+	-	+
	0.5%	-	-	-	-	+	+	-	+
Test against common pathogens (Area of inhibition in cm²)	*E.coli*	1.04	-	0.63	0.83	0.45	0.45	0.83	0.63
	Klebsiella pneumonia	1.04	-	1.04	1.04	1.27	1.04	2.64	1.27
	Salmonella	1.77	-	0.83	1.51	0.45	0.63	0.63	1.04
	Strepto coccus	1.77	-	0.63	1.51	2.04	1.04	1.04	1.27
	Staphylo coccus	1.04	-	0.63	1.04	1.27	0.63	1.04	0.63
	Vibrio	1.04	0.45	0.83	1.04	0.83	1.04	1.77	0.45
	Candida	-	-	-	-	-	-	-	-

were isolated from various samples of fresh and frozen fish and prawn. Among them, *L. plantarum* was the dominant species. The cultures were identified according to their morphological, cultural, physiological and biochemical tests including Gram reaction; production of catalase, cytochrome oxidase and hydrogen peroxide; growth at 15°C and 45°C in 1 week; acid production from carbohydrates (1% w/v) and nitrate reduction test. Lactic acid bacteria were isolated in a medium with protein and polysaccharide fractions of barley spent grain wherein the cultures were identified according to their morphological, cultural, physiological and biochemical characteristics up to genetic level [9]. A total of 15 presumptive isolates of lactic acid bacteria, from poultry raw meat, were isolated and identified based on standard physiological and biochemical tests [10]. From these findings it is evident that based on biochemical characterization, the cultures could be presumably identified to be lactic acid bacteria.

Acid and Bile Tolerance: To be used as a probiotic, a bacterial strain must have a good tolerance to the acidity of the stomach and to the bile salts present in the upper small intestine. The cultures tested for survival in acidic environment at varied pH levels in broth showed that the isolates from milk and cumbu were able to grow well even at the minimum tested pH of 2.0. The isolate from mango was able to tolerate and grow at a pH of 3.0. All the other isolates could not survive pH 2.0 and 3.0, but were able to grow well at a minimum pH of 4.0. The same results were confirmed in MRS medium adjusted to these pH levels with the formation of viable colonies when the cultures were plated (Table 1). *Lactobacillus* sp and *Bifidobacterium* sp have been reported to show a moderate tolerance to acid pH during 90 min incubation which is decreased after 2h but individual strains varied considerably [11]. Contrary to these results, study on survival of different strains of *Sporolactobacillus, Bacillus laevolacticus, Bacillus racemilacticus* and *Bacillus coagulans* grown in MRS broth under low pH conditions (2, 2.5 and 3) reported no survival at pH 2 for any of the isolate and that *Sporolactobacillus* could survive only at pH 3 [12]. In this study, we were able to obtain milk and cumbu isolates that were able to grow even at pH 2. Since lactic acid bacteria produce lactic acid during their fermentative type of metabolism, it is a known fact that they would be able to survive in acidic environment. However, the acidic pH inside the gut would be from 2-4, the organisms that could effectively survive the lowest possible pH are more preferred for use in functional foods.

Bile is a substance secreted by liver of mammals to assist chemical breakdown of fats. It is an essential criterion that the isolates to be used as functional food ingredients be tolerant to bile. When the cultures were inoculated in oxbile (which is similar in composition to human bile) at 0.1 – 0.5% concentrations, immediate growth was not observed as in case of control without addition of bile. It took almost 15-20 days for the cultures to grow in bile. Among the eight isolates, four isolates from cumbu, tulsi, toddy and human milk alone were able to tolerate bile up to the maximum tested concentration of 0.5%. Growth of other isolates was completely inhibited by bile even at the lowest concentration of 0.1%. Hence based on bile tolerance, out of the eight isolates, only these four isolates were screened for further tests. Bile concentration in humans is about 2-4% and hence the screened isolates were tested for tolerance at 2% and 4% levels. It was noticed that no isolate could grow even at

2% level with the exception of human milk culture which showed very little growth at 2% level. However, when the isolates were exposed to 2% and 4% levels of fresh bile salt, all the four isolates were able to grow well and produce colonies (Table 1). Similar to our results in 0.1-0.5% oxbile, in a study conducted by using six LAB strains isolated from Romanian fermented vegetables, all six strains showed a high resistance to a concentration of 0.3% (w/v), reaching a cell viability of 10^5 – 10^8 CFU/ml after 24 h of treatment. After 2 h of exposure to 0.5% (w/v) of bile salts, the viability of strains *L.brevis* and *L. mesenteroides* reached about 10^6 CFU/ml, a value still adequate for the use of these strains as probiotics [13]. However, our studies resulted in isolates that could tolerate even up to 0.5% of oxbile with more population count.

An interesting study that LAB were susceptible to bovine and porcine bile *in vitro* has been studied [14]. However, they were resistant to human bile which correlated with the survival in the human GIT. Pancreatic juice inhibits growth of multiresistant bacterial strains and for some probiotic bacteria. However, individual strains tolerated growth in media supplemented with pancreatic juice independent of proteolytic activity [15]. In a study, eleven lactic acid bacteria strains of importance to the dairy industry were subjected to *in vitro* analyses to determine their probiotic potential [16]. L. lactis strains were more tolerant to low pH than Lactobacillus spp.; all were tolerant to pancreatin and bile salts and showed antibacterial activity. Our study showed that all the four isolates were able to grow in porcine pancreas at all the tested levels from 0.1 – 0.5%, indicating their ability to survive bile and pancreatic juices secreted inside the body, along with the low acidic environment. The high resistance to low pH values and presence of bile salts enable the strains to survive in the stomach and intestine, to compete with other bacterial groups in this environment and to colonize the gastro-intestinal tract of the host.

Test Against Common Pathogens: Possible antagonistic effects of lactic acid-producing bacteria against pathogens have been proposed to include organic acid production, competition for nutrients, hydrogen peroxide formation and production of bacteriocins and antibiotic-like substances [17]. Generally, the addition of LAB to various foods including milk has been believed to be a bio preservation measure to inhibit and probably eliminate food spoilage and pathogenic microorganisms [18]. Various workers indicated microbial antagonism to be the basis for preservation and enhancement of microbiological safety of fermented products [19, 20]. In the present study, cell free extract of the cultures was tested for the activity against common food borne and human pathogens by agar well diffusion assay. All the isolates excepting mango isolate, produced clear inhibition zones against all the bacterial pathogens tested such as *Klebsiella, Vibrio, E.coli, Salmonella, Streptococcus* and *Staphylococcus*. Culture from mango was able to arrest the growth of only *Vibrio* and not other tested pathogens. No isolate was able to inhibit the growth of the fungal pathogen, *Candida*. Among the tested pathogens, very clear zones of inhibition were produced against *Klebsiella* and *Vibrio* sps. (Table 1) (Fig. 1 & 2). Among the isolates, more antagonistic activity was observed with the isolate from infant feaces against *Klebsiella*.

These results on antagonistic activity of lactic acid bacteria against several pathogens is in conformity with the findings of many workers. *L.acidophilus* produced an extracellular substance which inhibited several enteropathogens in vivo and in vitro, including *Salmonella enterica var. typhimurium* [21]. Faecal isolates from healthy Brazilian volunteers were screened for *in vitro* antagonistic activity against *Vibrio cholerae* [22]. Three *Pediococcus* sp. were shown to produce bacteriocins, sensitive to protease treatment but resistant to amylase and pepsin, with activity against several gram-negative and gram-positive bacteria, such as *Pseudomonas aeruginosa, B. cereus* and *S. aureus* [23].

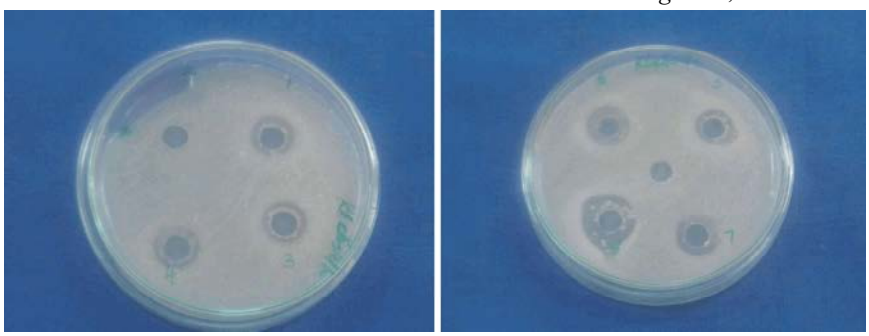


Fig. 1: Antagonistic activity of lactic acid bacterial cultures against Klebsiella pneumoniae

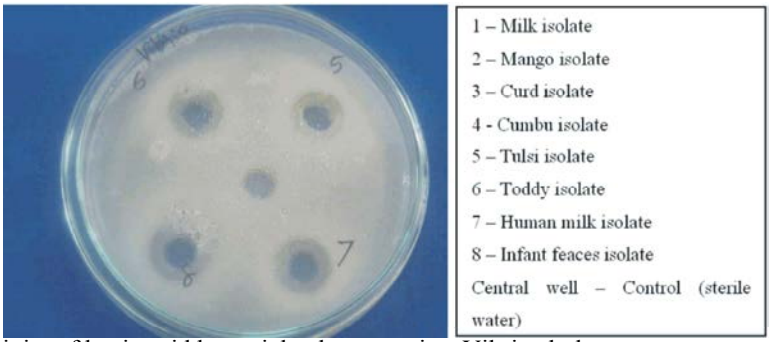


Fig. 2: Antagonistic activity of lactic acid bacterial cultures against Vibrio cholerae

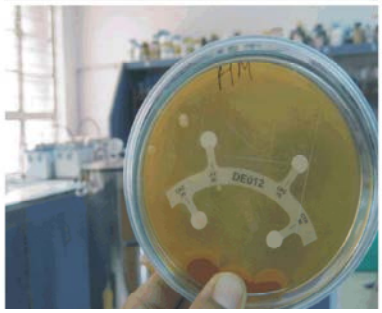
Table 2: Sensitivity of LAB isolates from different sources to antibiotics

Antibiotic	Conc.	Tulsi	Toddy	Cumbu	Human milk
Cefuroxime	30mcg	+	-	-	-
Cefactor	30mcg	+	-	-	-
Ceftriaxone	30mcg	+	-	-	+
Cefalexin	30mcg	+	-	-	-
Ceftazidime	30mcg	+	-	-	+
Ceftizoxime	30mcg	+	-	+	+
Cefadroxil	30mcg	+	-	+	+
Ampicillin / Cloxacillin	30mcg	+	-	-	+
Cefaperazone	75mcg	+	-	-	+
Cefotaxime	30mcg	+	-	-	++
Cefixime	5mcg	+	-	+	+
Cefazolin	30mcg	+	-	-	-

In a study [24], lactic acid bacteria were isolated from raw and fermented products like milk, curd, idli batter and pickle and tested against selected pathogens of both gram positive and gram-negative group. Bacteriocin-like substance of *E. faecalis* in combination with 0.1% EDTA showed the best antibacterial activity among all the isolates tested. The inhibitory property of nine pure or mixed cultures of potentially probiotic lactic acid bacteria (LAB) was tested against *Escherichia coli, Salmonella typhimurium DT104* and *Staphylococcus aureus* during fermentation and storage of borde and shamita at ambient temperatures [25]. They have reported that pure LAB cultures reduced in average the count of test pathogens by 5-6 and 4 log cycles at 24 h during fermentation of borde and shamia respectively. Mixed LAB cultures reduced the counts of pathogens by 7 and 5 log units after 24 h of fermentation in borde and shamita, respectively and strongly suggested that the isolates are possible candidates for the formulation of starter cultures that can be used to produce safe and bioprotective products. Our results are in confirmity with these findings. There was no evidence of antagonism by lactic acid cultures against *Candida* or any other fungal pathogen. In our results, we observed very clear inhibition zones against all the tested bacterial pathogens by most of the isolates.

Antibiotic Sensitivity Test: Probiotic strains should carry few, if any, mechanisms for antibiotic resistance and *preferably* no plasmids with antibiotic resistance. The screened isolates (isolates from tulsi, toddy, cumbu and human milk) were subjected to antibiotic tolerance/sensitivity tests. Tulsi culture was found to be totally sensitive to all the 12 different antibiotics tested in the study, showing clear inhibition zones around the discs. Inversely, culture from toddy was observed to be totally resistant to all the 12 tested antibiotics, with good growth of colonies all around the discs. The other two isolates from cumbu and human milk showed mixed results. Cumbu isolate survived most of the tested antibiotics excepting that it was sensitive only to ceftizoxime, ceixime and cefadroxil at the given concentration. On the other hand, isolate from human milk appeared to be sensitive to most of the antibiotics tested (Fig 3) (Table 2).

For a culture to be utilized in functional foods, different schools of thought with respect to sensitivity towards antibiotics prevail. One is that the culture should be sensitive to antibiotics as it serve as an effective indicator that the body had accepted the given antibiotic; on the other hand, the culture should be resistant to the antibiotic and remain viable and stable inside the human body and serve the purpose for which it was



Antibiotic	Symbol	Conc.
Cefuroxime	CXM	30mcg
Cefactor	CF	30mcg
Ceftriaxone	CTR	30mcg
Cefalexin	CN	30mcg
Ceftazidime	CAZ	30mcg
Ceftizoxime	CZX	30mcg
Cefadroxil	CFR	30mcg
Ampicillin / Cloxacillin	AX	30mcg
Cefaperazone	CPZ	75mcg
Cefotaxime	CTX	30mcg
Cefixime	CFM	5mcg
Cefazolin	CZ	30mcg

Fig. 3: Sensitivity of human milk isolate to the 12 different antibiotics by antibiotic disc diffusion assay (Antibiotic symbol with concentration has been indicated in the disc itself)

taken. Considering both the possibilities, from our study, the possibility of using the isolates from tulsi and toddy, which are totally sensitive or totally resistant respectively to the all the tested antibiotics, is ruled out. The other two cultures from cumbu and human milk could be possibly identified as suitable candidates. The development of resistance amongst bacteria to antimicrobials remains a serious concern. For this reason, viable micro-organisms used as the active agent(s) in feed additives should not add to the pool of antimicrobial resistance genes already present in the gut bacterial population or otherwise increase the risk of transfer of drug resistance Although it is reasonable to assume that gene transfer from viable microorganisms to other micro-organisms will occur in an open environment such as the gastrointestinal tract, intrinsic resistance is presumed to present a minimal potential for horizontal spread, whereas acquired resistance mediated by added genes is considered as having a high potential for lateral spread [26,27]. In principle, the selection of micro-organisms for use as feed additives should be oriented towards the least resistant organism whenever possible.

Tolerance to hydrogen peroxide, high temperature and salinity Since the isolates were catalase negative, their tolerance to hydrogen peroxide need to be studied. There are many reports on hydrogen peroxide production by lactic acid bacteria which is one of the cause for preservation of food fermented by them [28, 29]. When tested at 100, 300 and 500p pm, the isolates from cumbu and human milk tolerated and formed colonies even at the highest tested concentration, whereas tulsi culture could survive only up to 300ppm. Isolate from toddy did not grow even at the lower did not grow even at the minimum concentration of hydrogen peroxide.

Saline tolerance is a common character in lactic acid bacteria, since fermented foods are mostly salt based in which the organisms thrive. Moreover, addition of salt tolerant lactic acid bacteria is also known to inhibit the growth of aerobic bacteria and clostridia [30]. In the present study, when tested for salinity tolerance, cumbu, toddy and human milk cultures survived up to 5% concentration of sodium chloride, whereas tulsi culture survived 3% salt only. High temperature exposure studies in lactic acid bacteria are limited and growth studies have been undertaken up to a temperature of 32°C [31] because of the existing thermotolerant LAB strains. In our study the cultures were exposed to high temperature and observed for growth with the idea of utilising them as ingredients in millets for porridge cooking, involving a cooking time of 5-10 minutes at boiling temperature. Furthermore, for preparation of spray-dried formulations, it is essential to check their survival capacity at very high temperatures. Hence the isolates were subjected to 100°C for 15 as well as 30 minutes and plated in MRS medium. It was observed that the isolates from tulsi and toddy culture did not show any growth at all. The isolate from cumbu was able to form very few colonies at 100°C for 15 minutes alone and could not survive 30 minutes exposure

time. But the isolate from human milk exposed to 100°C even up to 30 minutes, showed very good growth in the plates, indicating a very good high temperature tolerance. All these tests clearly revealed that the cultures from cumbu flour and human milk alone have positively responded to most of the important probiotic characters tested such as tolerance to bile, acid, porcine digest, antibiotics, hydrogen peroxide, high temperature, high salinity and possess anti-microbial activity against most of the pathogens. These two cultures were identified as *Pediococcus pentosauceus* (MALDI-TOF).

CONCLUSION

From the above studies, we were able to confirm that the isolates from human milk and cumbu, satisfied most of the test criteria, to be presumably very good candidates for functional foods. Both these isolates identified as *Pediococcus pentosauceus* confirmed their usage as probiotic cultures. Hence this study on identification of the culture and the studies on key probiotic attributes is a basic investigation which would form a base for further clinical trials with the identified culture and henceforth aid in the development of value added food products.

REFERENCES

1. FAO/WHO, 2002. Guidelines for the evaluation of probiotics in food. London, Ontario, Canada, April 30 and May 1, 2002. ftp:// ftp.fao.org/ es/ esn/ food/ wgreport2.pdf).
2. Fuller, R., 1991. Probiotics in human medicine. Gut, 32: 439-442.
3. Sanders, M.E., 1998. Overview of functional foods: emphasis on probiotic bacteria. Int Dairy J., 8: 341-347.
4. Ouwehand, A.C., P.V. Kirjavainen, C. Shortt and S. Salminen, 1999. Probiotics: mechanisms and established effects. Int Dairy J., 9: 43-52.
5. Puupponen-Pimia, R., A.M. Aura, K.M. Oksman-Caldentey, P. Myllaerinen, M. Saarela, T. Mattila-Sandholm and K. Poutanen, 2002. Development of functional ingredients for gut heath. Trends Food Sci. Technol., 13: 3-11.
6. FAO/WHO, 2001. Health and nutritional properties of probiotics in food including powder milk with livelactic acid bacteria. Cordoba, Argentina, 1-4 October 2001. ftp:// ftp.fao.org/ es/ esn/ food/ probio/ report/ en.pdf
7. Saarela, M., G. Mogensen, R. Fonden, J. Mättö and T .Mattila-Sandholm, 2000. Probiotic bacteria: safety, functional and technological properties. J. Biotechnol., 84: 197-215.
8. Nair, P.S. and P.K. Surendran, 2005. Biochemical characterization of lactic acid bacteria isolated from fish and prawn. Journal Of Culture Collections, 4: 48-52.
9. Novik, G.I., J. Wawrzynczyk, O. Norrlow and E. Szwajcer-Dey, 2007. Fractions of Barley Spent Grain as Media for Growth of Probiotic Bacteria Microbiology, 76(6): 804-808.
10. Balia, R.L., I. Togoe, B.A. Tabac and M. Ludong, 2009. Isolation and identification of lactic acid bacteria from raw poultry meat Biotechnology in Animal Husbandry, 25(5-6): 1071-1077.
11. Charteris, W.P., P.M. Kelly, L. Morelli and J.K. Collins, 1998. Development and application of an *in vitro* methodology to determine transit tolerance of potentially probiotic Lactobacillus and Bifidobacterium species in the upper gastrointestinal tract. J. Appl. Microbiol., 84: 759-768.
12. Hyronimus, B., C. Le Marrec, A. Hadj Sassi and A. Deschamps, 2000. Acid and bile tolerance of spore-forming lactic acid bacteria International Journal of Food Microbiology, 61: 193-197.
13. Grosu-Tudor, S.S. and M. Zamfir, 2012. Probiotic potential of some lactic acid bacteria isolated from Romanian fermented vegetables Annals of RSCB, 17(1): 234-239.
14. Dunne, C., L. O'Mahony, L. Murphy, G. Thornton, D. Morrissey, S. O'Halloran, M. Feeney, S. Flynn, G. Fitzgerald, C. Daly, B. Kiely, G.C. O'Sullivan, F. Shanahan and K.C. Collins, 2001. *In vitro* selection criteria for probiotic bacteria of human origin: correlation with *in vivo* findings Am. J. Clin. Nutr., 73: 386S-392S.
15. Kruszewska, D., A. Ljungh, S.O. Hynes and S.G. Pierzynowski, 2004. Effect of the antibacterial activity of pig pancreatic juice on human multi-resistant bacteria. Pancreas, 128: 191-199.
16. Monteagudo-Mera, A., 2012. *In vitro* evaluation of physiological probiotic properties of different lactic acid bacteria strains of dairy and human origin Journal of Functional Foods, 4(2): 531-541.
17. Gibbs, P.A., 1987. Novel uses for lactic acid fermentation in food preservation. Journal of Applied Bacteriology-Symposium Supplement, 16: 515-85.

18. Stiles, M.E., 1996. Biopreservation by lactic acid bacteria Antonie van Leeuwenhoek, 70: 331-345.
19. Gänzle, M.G., A. Holtzel, J. Walter, G. Jung and W.P. Hammes, 2000. Characterization of reutericycline produced by Lactobacillus reuteri LTH2584. Applied and Environmental Microbiology, 66: 4325-4333.
20. Callewaert, R. and L. De Vuyst, 2000. Bacteriocin production with Lactobacillus amylovorus DCE 471 is improved and stabilized by fed-batch fermentation. Applied and Environmental Microbiology, 66: 606-613.
21. Coconnier, M.H., V. Liévin, M. Lorrot and A.L. Servin, 2000. Antagonistic activity of Lactobacillus acidophilus LB against intracellular Salmonella enterica serovar Typhimurium infecting human enterocyte-like Caco-2/TC-7 cells Appl. Environm. Microbiol., 66: 1152-1157.
22. Silva, S.H., E.C. Vieira, R.S. Dias and J.R. Nicoli, 2001. Antagonism against Vibrio cholerae by diffusible substances produced by bacterial components of the human faecal microbiota J. Med. Microbiol., 50: 161-164.
23. Jamuna, M. and K. Jeevaratnam, 2004. Isolation and partial characterization of bacteriocins from Pediococcus species Appl. Microbiol. Biotechnol., 65: 433-439.
24. Mallesha R. Shylaja, D. Selvakumar and J.H. Jagannath, 2010. Isolation and identification of lactic acid bacteria from raw and fermented products and their antibacterial activity Recent Research in Science and Technology, 2(6): 42-46.
25. Anteneh, T., M. Tetemke and A. Mogessie, 2011. Antagonism of Lactic Acid Bacteria against foodborne pathogens during fermentation and storage of borde and shamita, traditional Ethiopian fermented beverages International Food Research Journal, 18(3): 1189-1194.
26. Devirgiliis, C., S. Barile and G. Perozzi, 2011. Antibiotic resistance determinants in the interplay between food and gut microbiota Genes & Nutrition 6: 275-284.
27. Van Reenan, C.A. and L.M. Dicks, 2011. Horizontal gene transfer amongst probiotic lactic acid bacteria and other intestinal microbiota: what are the possibilities? A review. Archives of Microbiology 193(3): 157-68.
28. Whittenbury, R., 1964. Hydrogen peroxide formation and catalase activity in the lactic acid bacteria J. Gen. Microbiol., 35: 13-26.
29. Ishola, R.O. and B.C. Adebayo-Tayo, 2012. Screening of lactic acid bacteria isolated from fermented food for bio-moleculaes production Au.J.T., 15(4): 205-217.
30. Cai, Y., S. Ohmomo, M. Ogawa and S. Kumai, 1997. Effect of NaCl-tolerant lactic acid bacteria and NaCl on the fermentation characteristics and aerobic stability of silage J. Appl. Microbiol., 83: 307-313.
31. Ashe, B. and S. Paul, 2010. Isolation and characterization of lactic acid bacteria from dairy effluents Journal of Environmental Research and Development, 4(4): 983-991.