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PROBIOTIC POTENTIALS AMONG LACTIC ACID BACTERIA ISOLATED FROM CURD

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ABSTRACT
Curd is a commonly used fermented milk product in India since time immemorial. The scientific use of curd as a source of probiotic (good bacteria for health) has not been much examined. The yougurt (curd containing probiotics) is in Indian market and highly acclaimed. Therefore the status of curd as a source of probiotics is in question and requires scientific examination of its content, so the study was carried out. Probiotic potentials of two bacterial isolates from 20 different curd samples were identified as *Lactobacillus* spp. by the determination of morphological, cultural, physiological and biochemical characteristics. The antibacterial potential against diarrhoegenic bacterial pathogens was also examined. The reference strain used was *Lactobacillus acidophilus*, MTCC 447. The percentage survivability of the strains at pH 3.5, was found to be satisfactory (>90%). Bile salt resistance (0.3% sodium thioglycollate) was found to be between 80.41% and 83.2%. The pH decrease of the strains with time showed slow acidification activity. The lactic acid production of the strains ranges from 1.83 ± 0.12 to 3.93 ± 0.07 g. The strains were β-galactosidase producer and were resistant to principal antibiotics tested. But the absence of plasmids showed that they are intrinsically resistant or chromosome encoded. Strains showed maximum inhibition zone against *V. cholerae* O139 (13.67 ± 0.57 to 15.33 ± 0.57 mm) in comparison to other diarrhoeagenic bacteria. Only 10% of the examined curd samples had probiotic bacteria. Isolated strains of *Lactobacillus* spp. showed satisfactory probiotic potentials in comparison with reference strains and with antibacterial activity against diarrhoeagenic pathogens and thus maybe useful in the management of diarrhoea and also in functional food industry.
KEY WORDS: Curd, Lactic acid bacteria, Probiotics, Anti-diarrhoeal

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INTRODUCTION

The medical world has long been interested in the nutrient properties of curd. Lactic acid bacteria (LAB) are mainly associated with fermented dairy products such as cheese, buttermilk and curd. The health claims of ingesting live cells of *Lactobacilli* could be due to several possible mechanisms, which may include restoration of normal intestinal flora and human pathogens, accumulation of their metabolites including organic acids in the intestine and enhancement in the normal functioning of digestive tract[1].

Probiotic organisms for human should have demonstrable health benefits and have 'generally regarded as safe' (GRAS) status, with a proven low risk of inducing or being associated with the etiology of disease. The probiotic organisms must be able to survive and grow in *in vivo* conditions of the desired site of administration without thus must be able to tolerate low pH and bile acids.

Curd is the regular and common food component of India, with special reference to south India[2]. The online free dictionary.com define curd as the part of milk that coagulates when the milk sours or is treated with enzymes; curd is used to make cheese; or/and a coagulated liquid that resembles milk curd. The curd, as it is called in different parts of the country by different names, has little similarity with this definition. Milk is boiled and is brought to temperature around 40° C; then an inoculum, as starter culture is introduced. The preparation is then kept at room temperature (25-35 ° C), for 3-5 hours; then the curd is ready to serve. The mode of preparation is found same as yogurt. The curd-bacteria

especially lactic acid bacteria have been well accepted as GRAS (generally recognised as safe) for human consumption[3]. But the curd bacteria may not have probiotic potentials and may not be as good as it is labelled with yogurt. Therefore, a need was felt to examine the available curds microbiologically and to examine their probiotic activity to explore the possibility of their scientific use.

MATERIALS AND METHODS

Collection of samples

A total of 20 curd samples were collected aseptically from different sources like, VIT canteen, Boys' Hostel mess, and Girls' Hostel mess in VIT University, Vellore, India. After collection, sample was brought to the laboratory in cold condition and processed within an hour.

Isolation of bacterial strains and culture conditions

The isolation was performed by the standard microbiological procedure**.** A tenfold dilutions of curd samples were inoculated onto Lactobacillus de Man–Rogosa–Sharpe (MRS) agar (Himedia, India) and a loopful of the same sample was inoculated into MRS broth (Hi Media, India) for enrichment. Inoculated media were incubated at 37°C for 24 h. Isolated strains were kept in MRS broth plus 20% glycerol at –20 °C and sub cultured every six months. Working cultures were also kept on MRS agar slants at 4°C and sub cultured every 4 weeks. The reference strain used was *Lactobacillus acidophilus* (MTCC 447).The LAB isolates were designated as L001, L002, L003, respectively.

Physiological and biochemical tests

Each strain under examination was sub-cultured twice overnight in MRS broth. All strains were initially tested for Gram reaction, catalase production and spore formation[4]. Cell morphology and colony characteristics on MRS agar were also examined. Only the Gram-positive, catalase-negative isolates were further identified. Growth in the presence of 4.0 and 6.5% NaCl and arginine hydrolysis were performed. Gas production from glucose was also examined. Utilization of citrate was examined in Simmons citrate medium. Production of acetoin from glucose was determined using Voges-Proskauer test. Fermentation of different carbohydrates was determined. The ability to grow at 10˚C and 45˚C was assessed in MRS broth after 3 and 5 days of incubation respectively.

Acid tolerance test

Acid tolerant capabilities of the isolates were confirmed by viable count method[5]. One ml of the isolates grown in the MRS broth for three generations having an optical density of 0.280 at 600 nm were inoculated in 9 ml of sterile MRS broth whose pH was adjusted to 3.5 with 0.5N HCl. Samples were incubated at 37˚C for 4 h after inoculation. One ml of sample was taken out immediately after inoculation and after 4 h and was serially diluted with sterile saline solution and inoculated on MRS agar plates. The agar plates were incubated at 37˚C overnight and the colonies were counted using colony counter. The reduction in log cycle after exposure to low pH for 4 h as compared to control was considered as the criteria for acid resistance.

Bile salt tolerance test

Strains were cultivated in MRS broth enriched with 0.3% (w/v) of Sodium thioglycollate (Hi Media, India) at 37 ºC for 24 h. The viability was checked by spreading of 100µl of cultures of appropriate dilutions onto MRS agar. Percentage survivability of the strains to 0.3% sodium thioglycollate was calculated using the formula given below:

$$\% \text{ survivability} = (\log \text{cfu } 4^{th} \text{ hour} / \log \text{cfu } 0^{th} \text{ hour}) \times 100$$

Determination of acidifying activity

Acidification was measured by the change in pH (ΔpH). Five ml of MRS broth was inoculated with 1% of culture and incubated at 37°C. The pH was measured every 2 h till 24 h using a pH-meter (Orion smart check meter, Thermon Electron Corporation, Beverly). The acidification values were expressed as ΔpH, which is calculated as follows:

$$\Delta pH = pH_t - pH_0,$$

where pH_t is the pH of the culture at time t and pH_0 is the pH at zero time.

The cultures were considered as fast, medium or slow acidifying when a ΔpH of 0.4 U (pH units) was achieved after 3, 3-5 and > 5 h, respectively[6].

Determination of lactic acid production

The strains were grown in MRS broth for 48 h and supernatant was collected by centrifuging at 10,000 rpm for 15 minutes at 4˚C. To the 20 ml of supernatant, phenolphthalein was added as an indicator for titrimetrical estimation. One ml of 0.1M NaOH is equivalent to 90.08 mg of lactic acid[7].

ß-galactosidase assay

Demonstration of ß-galactosidase enzyme production was performed following the method described by Karasova et al., 2002[8]. The isolates were plated onto MRS agar containing 0.01% x-gal (Hi Media, India) and 0.1mM IPTG (Hi Media, India) as inducer and were incubated for 24 h at 37ºC.

Anti-diarrhoeal property

Antimicrobial effects of the strains on diarrhoegenic bacteria were determined by the agar diffusion method[9]. The test bacteria were obtained from National Institute of Cholera and Enteric Diseases (NICED), Kolkata, India, as listed below. Supernatants were collected from

overnight grown probiotic cultures and were neutralized with 1 N NaOH to pH 6.5.The neutralized supernatants of the strains of *Lactobacillus spp*. were checked for antibacterial activity against pathogenic bacteria inoculated onto Muller Hinton Agar (Hi Media, India). Nisin (Sigma, USA) – a bacteriocin, of concentration 10 mg/ml was used for comparison. A 50µl of cell free supernatants were filled in 5 mm diameter wells cut in the Muller Hinton Agar. Once solidified, the plates were stored for 2 h in a refrigerator. The inoculated plates were incubated for 24 h at 37˚C, and the diameter of the zone of inhibition was measured in millimetres. The experiment was repeated thrice and statistical analysis was carried out.

Antibiotic sensitivity test

Antibiotic sensitivity profiles of the strains were checked on MRS agar plates as per standard disc diffusion method[10]. The principal antibiotics used were erythromycin, vancomycin, tetracycline, cholaramphenicol and nalidixic acid. The diameters of the inhibition zones were measured using a ruler under a colony counter apparatus. The result was expressed as sensitive or resistant comparing with *E. coli* ATCC 25922 of known antibiotic resistance. The results were the average of 5 readings.

Plasmid DNA isolation

Plasmid DNA was isolated based on method described by Frere (1994)[11]. Purified DNA preparations were analyzed on 0.8% agarose gels stained with ethidium bromide and visualised using gel documentation system (Image quant 300, GE health care life sciences, USA). *E. coli* ATCC 25922 was used as the positive control.

Salt aggregation test

The salting out of bacterial cells was performed as described by Lindahl et al. (1981)[12]. Bacteria were agglutinated on a slide with various concentrations of ammonium sulphate (0.01-2M). Hydrophobic cells formed clumps promptly at lower molar concentrations of ammonium sulphate. The bacterial aggregates were examined against black background. The experiment was carried out 5 times for confirmation.

RESULTS

During primary isolation, 10 of twenty samples showed to be LAB (50.0%) and only 2 of them (L002 & L007) (10%) showed probiotic properties. These two strains were selected from two samples of 20 different samples of curd used in regular food chain for human consumption. **Table 1** show that the isolated two strains (L002 and L007) were Gram-positive, non-spore-forming, catalase-negative, mesophilic bacilli. L007 could grow at 45 ˚C. The comparative characteristics showed that these isolates belong to the genus *Lactobacillus*. Both the strains were found to be same with the variance of arabinose fermentation.

One of the important prerequisite of probiotic bacteria is tolerance to acidic pH which mimics the gastric environment. **Figure 1** describes the acid tolerance of isolated strains at the pH of 3.5. There is statistically insignificant decrease in strains over a period of 4 hours ($p<0.05$). This shows that both isolates are equally tolerant to acidic pH like the reference strain. The percentage survivability of the L002 and L007 are 96.46% and 97% respectively.

The normal level of bile salt in the intestine is around 0.3% [13]. Therefore resistance to bile salt is an important criterion for the evaluation of probiotics. **Table 2** demonstrates the viable cell count after exposure to 0.3% bile salt for 24 h. The survival percentage of L002 and L007 are 80.41% and 83.2% respectively in comparison to *L. acidophilus* (83.3%).

Figure 2 shows the slow rate of acidification of the strains and the pH of the culture dropped from 6.3 to 5.6. The Δ pH of the L002, L007 and *L. acidophilus* are 0.7U, 0.6U and 0.5U respectively at the end of 6 hours. This environment is sufficiently deleterious to the pathogenic gut bacteria like *Shigella spp.*

Table 3 shows the antimicrobial property of the organisms against diarrhoel pathogens. The comparative study with 10 different diarrhoeagenic pathogens showed that the isolated L002 and L007 were equally good as demonstrated by Nisin and the reference strain. Both the isolates showed better inhibition against *V. cholerae* 0139 (15.33 ± 0.57 mm) when compared to Nisin (10.33 ± 0.57 mm) and reference strain (13.67 ± 0.57 mm). All the pathogenic strains were considerably inhibited and killed showing the zone of inhibition between 10.33±0.57 and 15.33±0.57 (mm).

Both the strains showed acidification of the media by producing lactic acid between 1.83±0.12 and 2.38±0.08 g per 20 ml of culture as demonstrated in **Table 4**. The *L .acidophilus* produced 3.93±0.07 g of lactic acid per 20 ml culture. Figure 3 demonstrates the production of β galactosidase by the isolated and reference strains. The blue colour colonies indicate the production of β galactosidase enzyme.

Table 5 shows the antibiotic resistance profiles of the strains. Both the isolates and the reference strain were found to be resistant to all principal antibiotics. Plasmids were absent in both the strains. The strains were also found to be aggregating at 0.01M ammonium sulphate demonstrating that they are hydrophobic in nature and may participate in adherence to gut mucosa.

DISCUSSION

Dahi or Dadhi or Doi or Tahri or Perugu or Mosaru or Thayir are the acronyms for curd or yogurt in India. The Indian economy had been agriculture-based since the Vedic civilization. Cow and land were the sources of major economy. Almost all the oldest documents and mythological inscriptions were cow-centric. Cow milk and milk products are thus obviously intimately involved with Indian society. Right from the day of Lord Krishna to the modern age, milk, and thousands of milk-products like curd, cream, butter, paneer, sandesh, Rassogolla, ksheer; sreekhand etc. are in common use regularly. Specially, curd is a very essential and regular food item in India with particular reference to south India. It is one of the important components of Hindu ritual, called *panchamrita*. Rabindranath Tagore, the first Indian Noble Laureate (1913) sketched a curd- vendor in a novel, "Dakghar" meaning "Post office", who used to come from a remote village to sell out curd, ksheer etc. to other metropolis, about 100 years ago. During the same period, Eli Metchnikoff devoted the last decade of his life investigating means of increasing human longevity and advocating the consumption of lactic acid-producing bacteria. The curd is prepared in India by natural contamination and fermentation. The contaminants may be yeast or bacteria. The curd is used in several ways in different parts of India. In the eastern India, it is a *poyodhi* or *dadhi* or *misti doi*- a sweet milk product. Apparently, other part of world use it without sugar as happens in Vellore, a metropolis in south India. The necessity of knowing the microbial contents of curd and its beneficial potential to consumers urges to carry out this study.

The competition of curd versus yogurt has apparently surfaced even in India where a use of curd remains a tradition since the time immemorial. In general, yogurt contains the good bacteria but curd may or may not. However, the present study demonstrated the fact that 10 of 20 samples (50%) were lactic acid bacteria and 2 of 10 LAB had the probiotic strains of *Lactobacillus spp*. Both the strains showed several characteristics of being a good probiotic. Isolated strains were acid resistant, bile salt tolerant, antibacterial, and antagonistic by slow acidification and antimicrobial production, and adherent. A key requirement for probiotic strains is that they should not carry transmissible antibiotic resistance genes. Ingestion of bacteria carrying such genes is undesirable as horizontal gene transfer to recipient bacteria in the gut could lead to the development of new antibiotic-resistant pathogens[14]. In another sudy, we have found that only one LAB (GS3) could be isolated as a probiotic strain from curd[15], whereas other probiotic strain (GS4) was characterised as *Pedicoccus spp.* from khari- an Indian fermented food[16]. As both the isolates as well as reference strain showed multiple antibiotic resistances to most of the principal antibiotics, but they did not harbour any plasmid. The data confirm that none of the antibiotic resistant attributes of the strains tested in this study were plasmid linked. Such resistance were usually chromosomally encoded and non-transmissible[17]. Though the isolated strains were antibiotic resistant but the property appeared to be intrinsic and safe[14].

Diarrhoea is second fatal diseases in India and is a common problem of other developing countries. Cholera, shigellosis and other likely infections lead high mortality and also high mortality by stunting and slower growth to children below five years[18]. Use of curd has been found beneficial in one study in India[19] and elsewhere[20]. It has been observed in this study that most of the potential diarrhoagenic bacteria have been inhibited by dairy lactic acid bacteria. Hickson *et al.,* 2007 worked out the potentiality of LAB in successful control and management of diarrhoea[21]. The successful application of LAB-probiotics reduced the rate of morbidity as found among *Helicobacter pylori* infected children[22]. The use of probiotic in curd and likely food will enable to manage the large number of rural Indian population, where regular physicians are not available. As has been experienced among Nicobarese, the gravity of first-reported cholera outbreak and other diarrhoeal episodes would have been managed well with such Dohi[23,24]. However, the practice of consumption of curd containing probiotics has poorly been prescribed and advertised for good health and diarrhoea management as well in India. We believe, this study will carry the message of diarrhoea management successfully introducing good curd.

Another observation is there that all curd does not have probiotic bacteria; only a few of them offer the potentiality (10%). For the national interest, this event should be promoted to develop a network for making it useful. Probiotics in fermented milk or in milk products could be a very good choice for value added food products, in a country like India. This has right potentials to promote public health.

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REFERENCES

1. Fuller R. A review: Probiotics in man and animals. J Applied Bacteriol 1989; 66:365-78.
2. Prajapati JB & Nair BM. History of fermented foods. In: Ed Farnworth ER. Handbook of fermented functional foods, Boca Raton: CRC Press; 2003. p. 1-25.
3. Donohue DC & Salminen S. Safety of probiotic bacteria. Asia Pacific J Clin Nutr 1996; 5:25–28.
4. Harrigan WF & McCance ME. Laboratory Methods in Food and Dairy Microbiology, Academic Press, New York.1976.
5. Gilliland SE & Kim HS. Effect of viable starter culture bacteria in yogurt on lactose utilization in humans. J Dairy Sci 1984; 67:1-6.
6. Ayad EHE, Nashat S, El-Sadek N, Metwaly H & ElSoda M. Selection of wild lactic acid bacteria isolated from traditional Egyptian dairy products according to production and technological criteria. Food Microbiol 2004; 21**:**715-25.
7. A.O.A.C. Official Methods of Analysis, 15th edition, Association of Official Analytical Chemistry, Washington D. C.1990.
8. Karasova P, Spiwok V, Mala S, Kralova B & Russell NJ. Beta-galactosidase activity in psychotrophic micro organisms and their potential use in food industry. Czech J Food Sci 2002**;** 20:43-47.
9. Fleming HP, Etchells JL & Costilow RL. Microbial inhibition by an isolate of *Pediococcus* from cucumber brines. Applied Microbiol 1985; 30:1040-42.
10. NCCLS (National Committee for Clinical Laboratory Standards). NCCLS document M-100-S9. Performance Standards for antimicrobial susceptibility testing. 9th Information supplement. NCCLS: Wayne Pa.1999.
11. Frere J. Simple method for extracting plasmid DNA from lactic acid bacteria. Lett Applied Microbiol 1994; 18:227– 29.
12. Lindahl M, Faris, A, Wadstrom T & HjerteÂn S. A new test based on `salting out' to measure relative surface hydrophobicity of bacterial cells. Biochimica et Biophysica Acta 1981; 677:471- 76.
13. Mourad K & Nour-Eddine K. *In vitro* pre selection criteria for probiotic *lactobacillus* strains of fermented olives origin. International J Probiotics and Prebiotics 2006; 1**:**27-32.
14. Saarela M, Mogensen G, Fonden R, Matto J, Mattila-Sandholm T. Probiotic bacteria: safety, functional and technological properties. J Biotech 2000; 84:197–215.
15. Gowri S & Ghosh AR. Study of the probiotic potential of lactic acid bacteria isolated from a variety of indian fermented food. J Pharmacy Research 2010; 9:2254-2257.
16. Gowri S & Ghosh AR. *Pediococcus spp.* – a potential probiotic isolated from *Khadi* (an Indian fermented food) and identified by 16S rDNA sequence analysis. African J Food Sci 2010; 4:597-602.
17. Zhou JS, Pillidge CJ, Gopal PK & Gill HS. Antibiotic susceptibility profiles of new probiotic *Lactobacillus* and *Bifidobacterium* strains. International J Food Microbiol 2005; 98**:**211–17.
18. Ghosh AR & Sehgal SC. *Shigella* infections among children in Andaman-an archipelago of tropical islands in Bay of Bengal. Epidemiol Infect 1998; 121:43-48.
19. Saran S. Use of fermented foods to combat stunting and failure to thrive. Nutrition 2002; 18:393-396.
20. Vandenplas Y, Salvatore S, Viera M, Devreker T & Hauser B. Probiotics in infectious diarrhoea in children: are they indicated? European J Pediatr 2007; 166:1211-18.
21. Hickson M, D'Souza AL, Muthu N. Use of probiotic *Lactobacillus* preparation to prevent diarrhoea associated with antibiotics: randomised double blind placebo controlled trial. BMJ 2007; 335:80-83.
22. Lionetti E, Miniello VL, Casterllaneta SP. *Lactobacillus reuteri* therapy to reduce side-effects during anti- *Helicobacter pylori* treatment in children: A randomized, placebo controlled trail. Aliment Pharmacol Ther 2006; 24:1661-68.
23. Ghosh AR, Sugunan AP, Sehgal SC & Bharadwaj A P. Emergence of nalidixic acid-resistant *Shigella sonnei* in acute-diarrhea patients on Andaman and Nicobar Islands, India. Antimicrob Agents & Chemother 2003; 47:1483.
24. Sugunan AP, Ghosh AR, Roy S, Gupte MD & Sehgal SC. A cholera epidemic among the Nicobarese tribe of Nancowry, Andaman and Nicobar, India. American J Trop Med Hyg 2004; 71:822-27.

Table 1: Characteristics of the isolates from curd

Characteristics	Isolated strains		Reference strain
	L002	L007	*L. acidophilus*
Morphology and Gram stain reaction	Gram + rods	Gram + rods	Gram + rods
Spore formation	-	-	-
Cultural characteristics			
Size	>0.1mm	>0.1mm	>0.1mm
Shape	Circular	Circular	Circular
Colour	Milky white	Pale white	Milky white
Elevation	Concave	Concave	Concave
Density	Mucoid and glistening	Mucoid	Mucoid and glistening
Biochemical characteristics			
Catalase	-	-	-
Oxidase	-	-	-
Indole	-	-	-
MR/VP	-	-	-
Citrate	-	-	-
TSI	A/A^{+}	A/A^{++}	A/A^{+}
Physiological characteristics			
Growth in MRS broth			
with 0.5% NaCl	+	+	+
with 4.0% NaCl	-	-	-
with 6.5% NaCl	-	-	-
Growth at 10˚C	-	-	-
Growth at 37˚C	+	+	+
Growth at 45˚C	-	+	-
Arginine hydrolysis	-	-	-
Gas production from glucose	+	+	+
Fermentation of sugars			
Glucose	+	+	+
Adonitol	-	-	-
Arabinose	-	+	+
Lactose	+	+	+
Sorbitol	-	-	+
Mannitol	-	-	-
Rhamnose	-	-	+
Sucrose	+	+	+

Abbreviations: MR = Methyl Red; VP = Voges Proskauer; TSI = Triple Sugar Iron; A= acid; + = positive; - = negative.

Table 2: Bile salt resistant study (0.3% sodium thioglycollate)

Strains	0 h		24 h		% survivability
	cfu/ml	Log cfu/ml	cfu/ml	Log cfu/ml	
L002	52×10^{6}	7.71	16×10^{5}	6.20	80.41
L007	48×10^{6}	7.68	25×10^{5}	6.39	83.2
L. acidophilus	64×10^{6}	7.80	32×10^{5}	6.50	83.3

Table 3: Antagonistic activity against diarrhoeal pathogens

Strains used	Inhibition zone (mm ± sd)			
	L. acidophilus	L002	L007	Nisin (10 mg/ml)
EPEC	11.00 ± 1.00	11.33 ± 1.15	10.67 ± 0.89	-
ETEC	13.33 ± 1.15	12.67 ± 0.57	10.33 ± 0.57	-
EAEC	11.33 ± 0.57	10.33 ± 0.57	10.67 ± 1.15	-
S. sonnei	11.67 ± 0.57	10.67 ± 0.57	11.33 ± 0.57	10.33 ± 0.57
S. typhimurium	12.00 ± 1.00	11.67 ± 0.57	10.00 ± 0.00	9.67 ± 0.57
V. cholerae 01	10.33 ± 0.57	10.00 ± 0.00	11.33 ± 0.57	-
V .cholerae 0139	13.67 ± 0.57	15.33 ± 0.57	15.33 ± 0.57	10.33 ± 0.57
V. cholerae non-01,0139	11.33 ± 1.15	12.33 ± 0.57	11.33 ± 1.15	10.33 ± 0.57
V. parahaemolyticus	11.33 ± 0.57	10.00 ± 0.00	11.33 ± 0.57	12.33 ± 1.52

Abbreviations: EPEC = Enteropathogenic *E .coli*; ETEC = Enterotoxigenic *E .coli*; EAEC = Enteroadherent *E. coli*; sd = standard deviation

Table 4: Determination of lactic acid in isolated probiotic lab

Strains	**L002**	**L007**	***MTCC 447***
Lactic acid per 20ml of 48h culture (g)	1.83±0.12	2.38±0.08	3.93±0.07

Table 5: Antibiotic resistance profiles of the strains

Antibiotics	**Concentration (µg/disk)**	**L002**	**L007**	***L. acidophilus***
Erythromycin	10	R	R	R
Gentamycin	30	R	R	R
Vancomycin	30	R	R	R
Tetracycline	30	R	R	R
Chloramphenicol	30	R	R	R
Nalidixic acid	30	R	R	R

Abbreviation:R= resistant

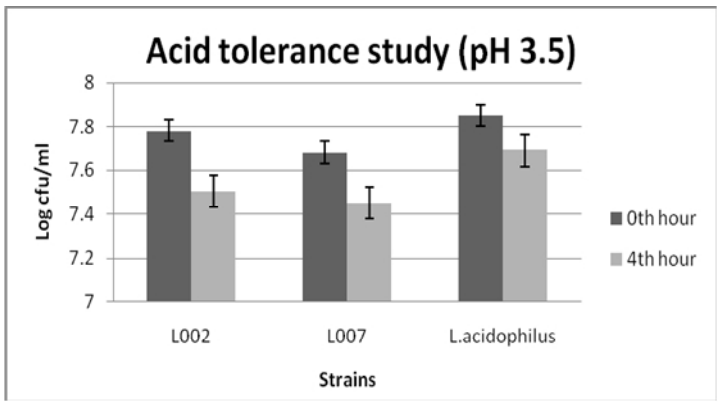

Figure 1: Acid tolerance test of LAB isolates

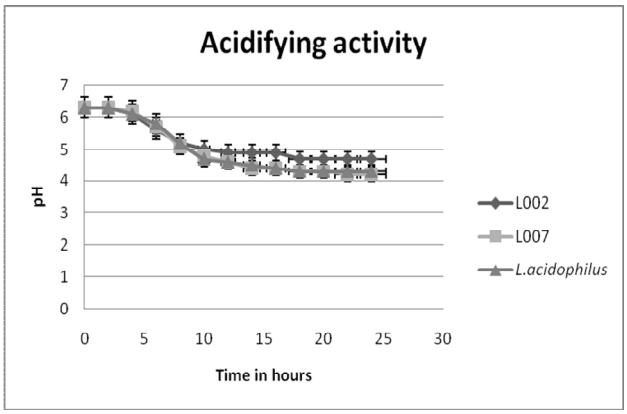

Figure 2: Acidifying activity of LAB isolates

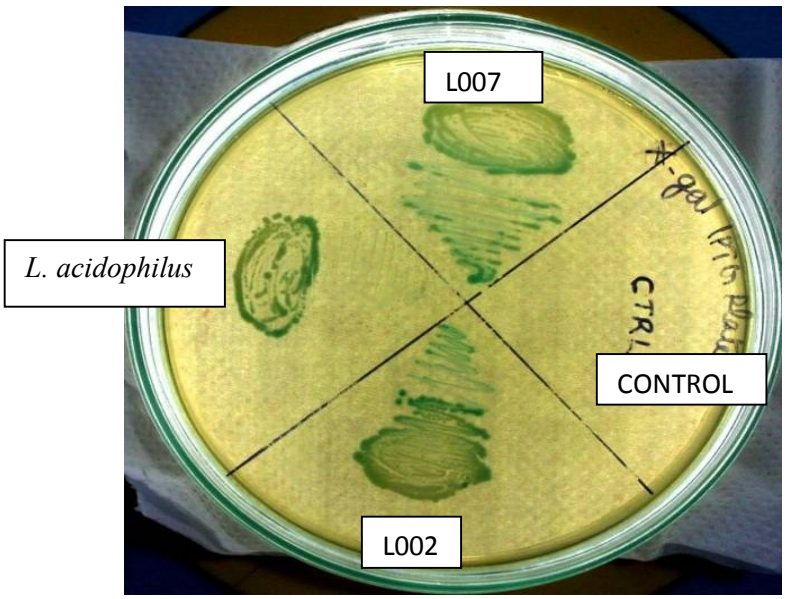

Figure 3: β – galactosidase activity of probiotic isolates

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