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Isolation and characterization of potential probiotic lactic acid bacteria isolated from cow milk and milk products

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Abstract

Probiotics refer to the living microorganisms that exhibit beneficial effect on the health of human by the intestinal microbial balance. Most widely used probiotics are lactic acid bacterial group found in milk and milk products. This study was aimed to characterize the probiotic properties of lactic acid bacterial (LAB) strains isolated from cheddar cheese, yoghurt and cow milk. Bacterial strains naturally grown in milk, cheddar cheese and yoghurt were isolated using De Mann Rogosa Sharpe (MRS) agar medium and incubated at 37°C for 48 h separately under aerobic and anaerobic conditions. There were eight strains grown under aerobic conditions and they were isolated, purified and characterization was done based on the morphological and biochemical analysis such as gram staining, catalase test and motility test. All the eight isolates were either rod or cocci shaped, gram positive, catalase negative, non-motile and non-spore formers. These eight isolated strains were identified as lactic acid bacteria. When screening of the 8 isolates was done to determine their antimicrobial activities against five human pathogenic strains such as E.coli, Klebsiella pneumoniae, Pseudomonas eaeruginosa, Salmonella sp and Staphylococcus aureus, three isolates (M6, C1 and Y1) showed wide spectrum antimicrobial activity. To determine the probiotic properties of these three isolates, different tests such as tolerance to acid, NaCl and bile, lactose utilization and antibiotic resistance were done. Though all the three isolates showed resistance to stomach pH (pH 3.0), the strain C1 showed significantly higher tolerance to stomach pH than the other strains. Though the three isolates grew well in the presence of NaCl and 0.3% bile, the isolate M6 showed significantly higher growth with NaCl and 0.3% bile than the others. Even though all the three isolates had the capacity of utilizing lactose, the isolate M6 showed prominent colour change in the lactose utilization test than the other two strains. When antibiotic susceptibility of the isolated LABs were evaluated using four antibiotics such as Ampicillin, Streptomycin, Bacitracin and Gentamycin, the strain M6 showed significantly higher resistance to Ampicillin and Bacitracin but sensitive to Streptomycin and Gentamycin. Other two isolates (C1 and Y1) were sensitive to all the four antibiotics used. Based on the antibiotic sensitivity tests and the analysis of probiotic properties, the isolated strain M6 was confirmed as a potential probiotic lactic acid bacterium. This strain was identified as Lactobacillus plantarum strain CIP 103151 through16S rDNA sequence analysis.

Keywords: Cheddar cheese, Lactic acid bacteria, Milk products, Probiotic properties

1.Introduction

Probiotics are living microorganisms which are beneficial to health when consumed. Milk and milk products are usually associated with probiotic bacteria, which provide supplements for the beneficial maintenance of the intestinal system (Tambekar and Bhutada, 2010). The main Lactic acid producing bacterial (LAB) groups are gram-positive, catalase negative organisms and they belong to genera *Lactobacillus, Bifidobacterium, Lactococcus, Pediococcus* and *Leuconostoc* (Leroy and de Vuyst, 2004). There has been an increasing attention in the use of diverse strains of LAB as probiotics, mainly *Lactobacilli* and *Bifidobacteria* that are residents of the commensal bacteria in the

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gut of human showing good therapeutic functions (Lavanya *et al*., 2011). They can produce antimicrobial substances (e.g.: - organic acid, hydrogen peroxide and bacteriocins) which can influence the growth of the possible harmful microorganisms. Enteric bacteria comprised of *Salmonella* species, *Shigella* species, *Proteus* species, *Klebsiella* species, *E. coli*, *Pseudomonas* species, *Vibrio cholera* and *S. aureus* which are major etiologic agents of enteric infection (Ballal and Shivananda, 2002). Further, probiotics are beneficial in gastrointestinal disturbances, such as diarrhoea, dysentery, typhoid etc (Tambekar and Bhutada, 2010). The antagonistic activity of such bacteria can inhibit a large number of enteric and urinary pathogenic bacteria (Hutt *et al*., 2006). They cause reduced lactose intolerance alleviation of some diarrhoea, lowered blood cholesterol, increased immune response and prevention of cancer. The selection criteria for probiotic LAB include safety, resistance to acid and bile, adherence to gut epithelial substances. The main *in vitro* selection criteria for potential probiotic strains are acid and bile resistance activities, indicating the ability of the organism to survive during the passage through the gastrointestinal tract. From good quality cheddar cheese, *Lactobacillus plantarum* and *Lactobacillus casei* were isolated (Broome *et al*., 1990). *Lactobacillus plantarum* and *Lactobacillus rhamnosus* were isolated from goat milk (Setyawardani *et al*., 2011). (Tamberkar and Bhutada, 2010) isolated *Lactobacillus acidophilus*, *Lactobacillus brevis*,*Lactobacillus plantarum*, *Lactobacillus bulgaricus*, *Lactobacillus lactis*, *Lactobacillus casei* and *Lactobacillus fermentum* from milk samples. Therefore, the objective of the study was to isolate lactic acid bacterial (LAB) strains from cheddar cheese, yoghurt and cow milk and to evaluate the probiotic properties of these strains in order to select the potential probiotic bacterial strain.

2. Materials and methods

2.1 Collection of samples

Cheddar cheese and yoghurt were obtained from Cargills super market, Jaffna. Cow milk was obtained from Kokuvil milk collection centre. Sampling was done randomly according to the studies done by Hoque (Hoque et al., 2010).

2.2 Isolation of lactic acid bacteria from milk and milk products

Lactic acid bacterial strains were isolated from milk, yoghurt and cheddar cheese by weighing 1g of the sample and serially diluted with 10 mL sterile distilled water. After homogenization, 10^{-5} serially diluted samples were spread on MRS agar medium. The plates were incubated for 48 h at 37°C. The characteristic LAB colonies growing over the incubated plates were picked up carefully and streaked on the MRS agar medium following the repeated technique, for further purification. Colonies were transferred to MRS agar slants and then maintained in the refrigerator at 4°C for further study (Pundir *et al*., 2013)

2.3 Identification of isolated bacterial cultures

The isolates were subjected to diverse biochemical and morphological studies such as colony morphology, aerobic and anaerobic growth, Gram staining, catalase test and motility test (Hanging Drop Method), in order to identify the genus of the unknown bacterial strains (C1, M6 and Y1) isolated from cheddar cheese, milk and yoghurt. (Barrow and Feltham, 1993, Theivendrarajah, 1990, Kapilan and Arasaratnam, 2010, Karuppaija *et al*., 2016).

2.4 Screening of isolated bacterial cultures for antimicrobial activity

Well diffusion assay method was used for the detection of antimicrobial activity. Antimicrobial activities of the selected isolates were evaluated against *Staphylococcus aureus, Salmonella sp, Escherichia coli, Pseudomonas*

aeruginosa,*Klebsiella* species. Isolate was grown in a nutritional broth at 37°C for 24 h in the incubator. The isolate was centrifuged at 12,000 rpm for 20 min and the supernatant of each bacterial isolate was collected in a sterilized test tube and the pellet was discarded. Then it was filtered using 0.45 µm membrane filter. A 10^8 CFU/mL (100 µL) suspension of freshly grown test organisms was mixed with 5mL of nutrient soft agar and over layered on nutrient agar. Wells of 10 mm diameter depth were cut in the nutrient agar plates with the help of sterilized cork-borer. An aliquot of 100 µL of culture supernatant of each isolate with antimicrobial potential was poured into each well. The plates were then incubated at 37°C for 24 h and zones of inhibition were measured. The isolates, which showed the strongest antagonisms against a maximum number of respective indicators with wide zones of inhibition, were selected for further studies.

2.5 Evaluation of probiotic potentials of selected *Lactobacillus*

Bacterial cultures showing wide spectrum antimicrobial activity were selected for further determination of probiotic potential as follows

2.5.1 Tolerance of inhibitory substances

Probiotic features were evaluated by checking the tolerance of the cultures to varying concentrations of acid, salt and bile salts. Tolerance to the above mentioned inhibitory substances was studied in nutrient broth with the concentration of 10^8 CFU/mL (100 µL) (Kapilan, 2015). Growth of lactic acid bacteria was monitored using spectrophotometer (Thermo scientific) at wave length of 620nm in different pH (1, 2, 3, 4, 5, 6 and 7), NaCl concentration (2, 4, 6,8and 10%), and bile salt concentrations (0.2, 0.3, 0.4 and 0.5%) at 37°C for 24 h.

2.5.2 Lactose utilization

Lactose utilization was determined using acid production by selected bacterial cultures and it 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 inoculated with different cultures and incubated at 37°C for 48 h.

2.5.3 Antibiotic susceptibility

The antibiotic resistance of isolated LAB was assessed using antibiotic discs on nutrient agar plates. A 10^8 CFU/mL (100µL) suspension of freshly grown test organisms were mixed with 5mL of nutrient soft agar and over layered on nutrient agar. The antibiotic discs were placed on the surface of the agar and the plates were incubated at 37°C for 24 h. Resistance was assessed against 10µg of Ampicilln, Gentamycin, Streptomycin and Bacitracin. The zone of inhibition was measured in millimeters.

2.6 Molecular identification

Genomic DNA from the bacterial isolate (M6) was done using the DNeasy Extraction Kit (Qiagen, USA) following the protocol provided by the manufacturer with modification (Mohanappriya and Kapilan, 2018). An overnight culture grown in MRS liquid medium was centrifuged at 5000 x g for 10 min, to harvest the cells. The pellet was washed 3 times in Tris – EDTA buffer (TE buffer). Genomic DNA was extracted from 48 h grown culture plates by using a DNeasy kit (Qiagen, CA). Amplification of the 16S rRNA genes from the genomic DNA, was amplified by Polymerase Chain Reaction (PCR) using bacterial universal primers (27F–AGAGTTTGATCCTGGCTCAG and 1492R– GGTTACCTTGTTACGACTT). The PCR amplification was done in a Techne TC-412 Thermal Cycler (Bibby Scientific Ltd, UK) in a 50 µl reactions containing 25 µl of 2 X PCR Master Mix (NorgenBiotek, Canada), 1.5 µl of template DNA (0.5 µg), 1 µl of both forward and reverse primers (2.5 µM of each) and 21.5 µl of nuclease free water in a PCR tube added in that order. PCR reaction was done at an initial denaturation step at 94°C for 2 min, followed by 30 cycles at 94°C for

30 sec, 52°C for 30 sec and 72°C for 2 min, and a final extension step at 72°C for 5 min. PCR products were separated by electrophoresis on a 1% agarose TAE gel containing ethidium bromide and visualized by UV transillumination (Fotodyne, USA). Cloning was carried out using the PCR TRAP Cloning System (GenHunter Corporation, USA), using the manufacturer's protocol. Amplicons from total bacterial community DNA were spliced into the PCR TRAP Cloning Vector using the T4 DNA ligase. Competent cells were transformed with the recombinant DNA and inoculated in Luria-Bertani (LB)-Tetracyclineagar (containing 20 µg/ml of tetracycline). The products were purified, dried, resuspended in 0.1 mM EDTA, and run on a DNA analyzer (3730 Applied Biosystems) using manufacturer's protocols. The resultant nucleotide sequences were edited by using the Sequencer program with Big Dye Terminator cycle sequencer (Macrogen, USA) and aligned. The regions of the bacterial isolate were compared with the sequences of the GenBank database using the Basic Local Alignment Search Tool (BLAST) search program at the National Centre for Biotech Information (NCBI) in order to identify the bacterial isolate.

2.7 Statistical Analysis

All experiments were carried out in triplicates. The data were presented as the mean ± standard deviation of the mean using Microsoft Excel 2013. Statistical analysis was done by two factor Completely Randomized design using the SAS-8 statistical package. Duncan's Multiple Range Test was used to compare the treatment means separation at $p<0.05$.

3. Results and Discussion

3.1 Isolation of lactic acid bacteria from milk and milk products

A total of 8 bacterial cultures were isolated from cow milk, cheddar cheese and yoghurt aerobically on MRS agar medium after 24h of incubation at 37°C and they were labeled as M1, M2, M3, M4, M5, M6, C1 and Y1. Isolates M1, M2, M3, M4, M5 and M6 were isolated from cow milk.Isolate Y1 was isolated from yoghurt. Isolate C1 was isolated from cheddar cheese (Plate 1). Sieladie *et al.* (2011) isolated one hundred and seven colonies of *Lactobacilli* from thirty-two samples of raw cow milk using MRS medium. Twelve *Lactobacillus* strains from milk using MRS medium were isolated (Chauhan and Daru, 2016).

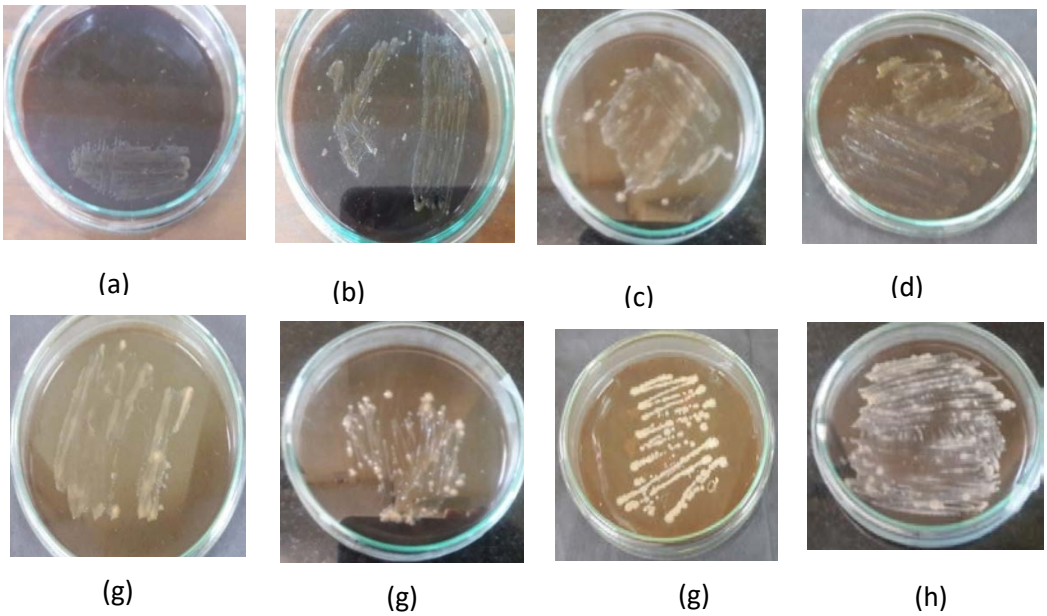

Plate 1. Isolated Lactic acid bacteria. (a) M1, (b) M2, (c) M3, (d) M4, (e) M5, (f) M6, (g) C1, (h) Y1. Strains M1, M2, M3, M4, M5 and M6 were isolated from milk, strain C1 was isolated from cheddar cheese, and strainY1 was isolated from yoghurt.

3.2 Biochemical identification of isolated bacterial cultures

The main lactic acid producing bacterial (LAB) groups are Gram positive, catalase negative and non-motile organisms (Ananthanarayan *et al*., 1997, Barrow and Feltham, 1993, Leroy and De Vuyst, 2004). Biochemical characterization of all the isolates were done and their characteristics were noted (Table 1). All the strains were found to be facultative anaerobes, as the strains grown in anaerobic condition and growth also found in presence of oxygen. All the isolates were found to be gram-positive, catalase negative and non-motile. M2, M3, M6, Y1 and C1 were identified as rod shape bacteria. M1, M4 and M5 were identified as cocci shape bacteria. According to the above findings, the isolated strains were identified as lactic acid bacteria.

Table 1. Biochemical characterization of isolates

S.No.	Isolate	Aerobic growth	Anaerobic growth	Gram's reaction	Catalase test	Shape	Motility
1	C1	Positive	Positive	Positive	Negative	Rod	non-motile
2	Y1	Positive	Positive	Positive	Negative	Rod	non-motile
3	M1	Positive	Positive	Positive	Negative	Cocci	non-motile
4	M2	Positive	Positive	Positive	Negative	Rod	non-motile
5	M3	Positive	Positive	Positive	Negative	Rod	non-motile
6	M4	Positive	Positive	Positive	Negative	Cocci	non-motile
7	M5	Positive	Positive	Positive	Negative	Cocci	non-motile
8	M6	Positive	Positive	Positive	Negative	Rod	non-motile

Strain M1, M2, M3, M4, M5 and M6 isolated from milk, strain C1 isolated from cheddar cheese and strain Y1 isolated from yoghurt.

3.3 Antimicrobial activity

Antimicrobial activity is one of the most important selection criteria forprobiotics. Antimicrobial activity tests generally target the enteric undesirables and pathogens (Klaenhammer and Kullen, 1999). Antimicrobial effects of lactic acid bacteria are formed by producing some substances such as organic acids (lactic, acetic, propionic acids), carbon dioxide, hydrogen peroxide, diacetyl, low molecular weight antimicrobial substances and bacteriocins. The results of this study are summarized in Table 2. Eight identified lactic acid bacteria were tested for their antimicrobial activity against selected pathogenic bacteria viz *E. coli, Klebsiella pneumoniae*, *Pseudomonas eaeruginosa*, *Salmonella sp* and *Staphylococcus aureus.* Antimicrobial activity of different lactic acid bacteria varied against test pathogens. Three isolates (C1, Y1 and M6) showed wide spectrum antimicrobial activity against maximum number of pathogenic organisms. Isolate M1, M3 and M5 were found to inhibit two test indicators respectively whereas; Isolate M2 and M4 were expressed inhibition against one test indicator only. Out of the 8 isolates, M6 isolate showed the maximum zone of inhibition against *Pseudomonas eaeruginosa*

followed by *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas eaeruginosa* and C1 isolate exhibited a maximum zone of inhibition against *Staphylococcus aureus*.

Table 2. Antimicrobial activity of the isolated lactic acid bacteria

S.No	Isolate	*E.coli* (mm)	*Klebsiella pneumoniae* (mm)	*Pseudomonase aeruginosa* (mm)	*Salmonella sp* (mm)	*Staphylococcus aureus* (mm)
1	C1	12.92±0.1	10.9±0.1	10.86±0.2	NA	14.92±0.1
2	Y1	NA	11.1±0.1	12.49±0.4	11.59±0.2	13.97±0.2
3	M1	NA	10.55±0.1	NA	10.78±0.1	NA
4	M2	10.80±0.2	NA	NA	NA	NA
5	M3	NA	NA	10.98±0.1	11.11±0.2	NA
6	M4	NA	11.36±0.3	NA	NA	NA
7	M5	NA	NA	11.3±0.1	10.8±0.1	NA
8	M6	12±0.3	NA	17.51±0.2	10.96±0.1	12.55±0.1

Clear zones were measured in mm. Results represent the mean ± standard deviation of three replicates. Strain M1, M2, M3, M4, M5 and M6 isolated from milk, strain C1 isolated from cheddar cheese and strain Y1 isolated from yoghurt. 'NA' –No activity.

3.4. Evaluation of the probiotic potential of selected *Lactobacillus*

3.4.1 Tolerance of inhibitory substances

When evaluating the potential of using lactic acid bacteria as effective probiotics, it is generally considered necessary to evaluate their ability to resist the effects of pH, bile and NaCl. Tolerance to acidic

condition is the most commonly used method to detect the viability and activity of probiotic bacteria in the small intestine and stomach. According to a previous study by (Azat *et al*, 2016), the survival rate at pH 3.0 is considered as optimal acid tolerance for selected probiotic strains. In present research, isolates M6, C1 and Y1 were assessed for their viabilities in the different pH (7, 6, 5, 4, 3, 2 and 1) during 24 h of incubation. Based on the Optical

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LAB isolates were able to grow in pH 7, 6, 5, 4, 3 and 2 but were unable to grow at pH 1 during 24 hours incubation (Figure 1).All three isoates able to tolerate pH 3.Therefore, they can be considered as acid tolerance LAB strains and among the three isolates, strain C1 and M6 were having better pH tolerance compared to strain Y1.(Shobharani and Agarwal, 2011) reported that *L. paramesenteroides*, a potent probiotic strain isolated from cheddar cheese which was found to survive in pH 4.0, but no growth was observed with substantially decrease in the pH. The difference in the results may be due to the acid regulatory mechanisms of the LAB have failed to maintain their intracellular pH and the internal acidification had reduced the activity of enzymes, damaged certain proteins and DNA, which leads to death and different source used for the isolation of *Lactobacillus.*

The human liver secretes bile into the small intestine each day, and thus exposure to bile is a serious challenge to probiotics. Therefore, tolerance to bile salt has often been used as the most important selection criteria. The relevant physiological concentration of human bile ranged from 0.3% to 0.5% (Dunne *et al*, 2001). In present study, the survival of isolates at different bile salts concentration (0.2%, 0.3%, 0.4% and 0.5%) was examined during 24 hours incubation. All isolates were also bile tolerant and among the three isolates, strain M6 was having better bile tolerance compared to strain C1 and Y1 (Figure 2). Similar results were obtained by chauhan and daru (2016), in an experiment carried out with 14 *Lactobacillus* isolated from milk, curd and faecal sample. All 14 *Lactobacillus* were found to show growth at bile salt 0.3% concentration. Among these isolates, B.A.2 showed maximum growth at 0.3% bile concentration and with increase in bile salts concentration, the tolerance level of isolates declined.

NaCl is an inhibitory substance which may inhibit the growth of certain types of bacteria. If the lactic acid bacteria were sensitive to NaCl than it would not be able to show its activity in presence of NaCl so it was essential to test the NaCl tolerance of lactic acid bacteria. In the present study, the isolates were able to tolerate 2-10% NaCl concentration as shown in Figure 3. Among the three isolates, strain M6 showed better tolerance compared to strain C1 and Y1. Similar results were obtained by Hoque*et al*. (2010), in an experiment carried out with *Lactobacillus sp.* isolated from yoghurts.

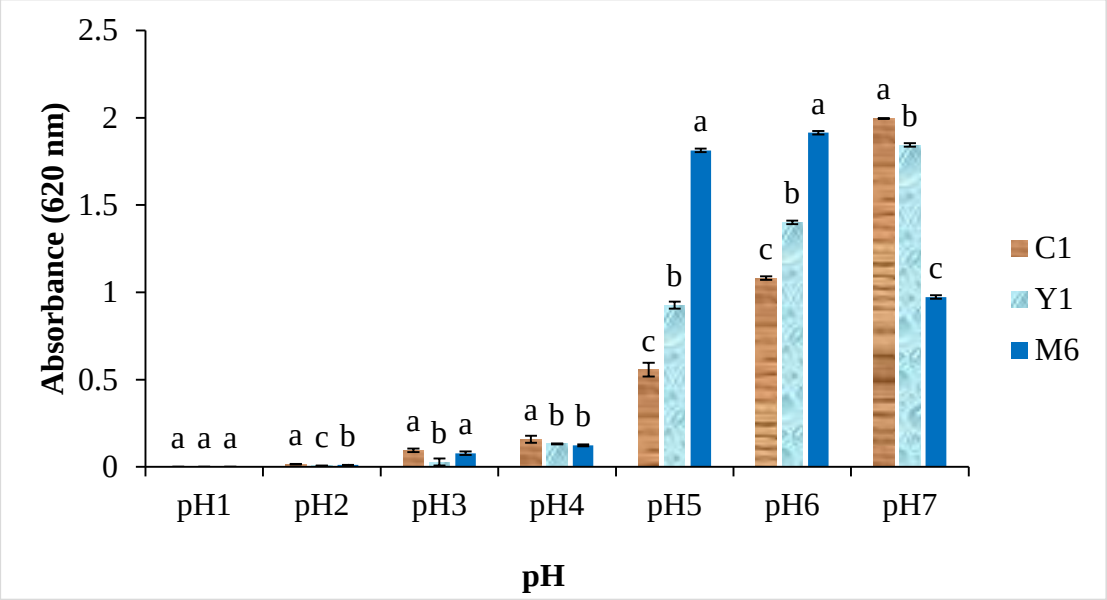

Fig.1. Effect of different pH on selected *Lactobacillus* isolates. Means with different letters within the same column indicate significant difference at $p<0.05$

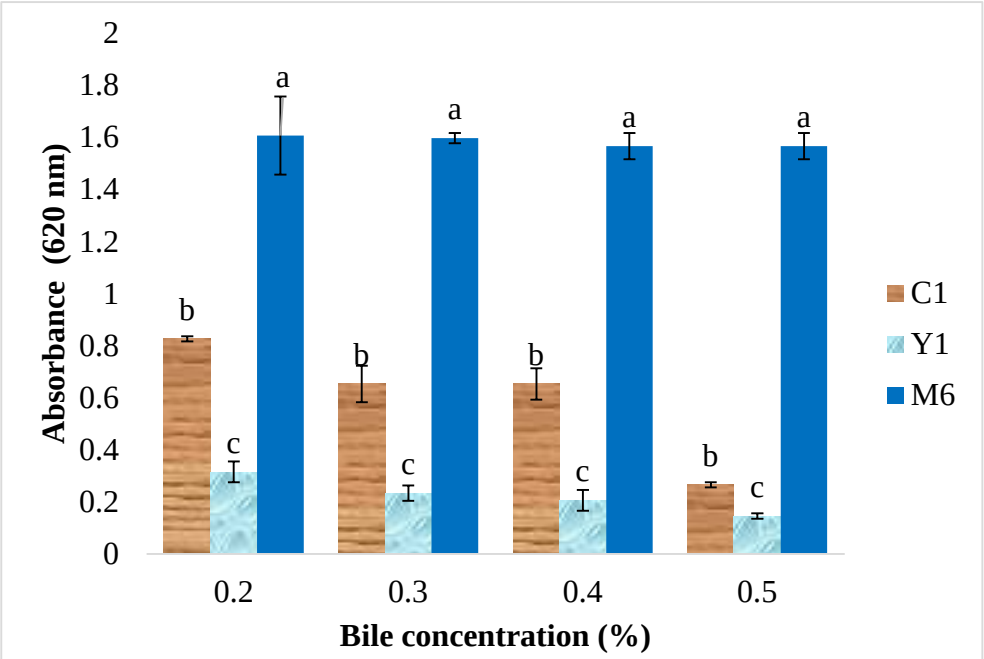

Fig.2. Effect of different concentration of bile on selected *Lactobacillus* isolates. Means with different letters within the same column indicate significant difference at $p<0.05$.

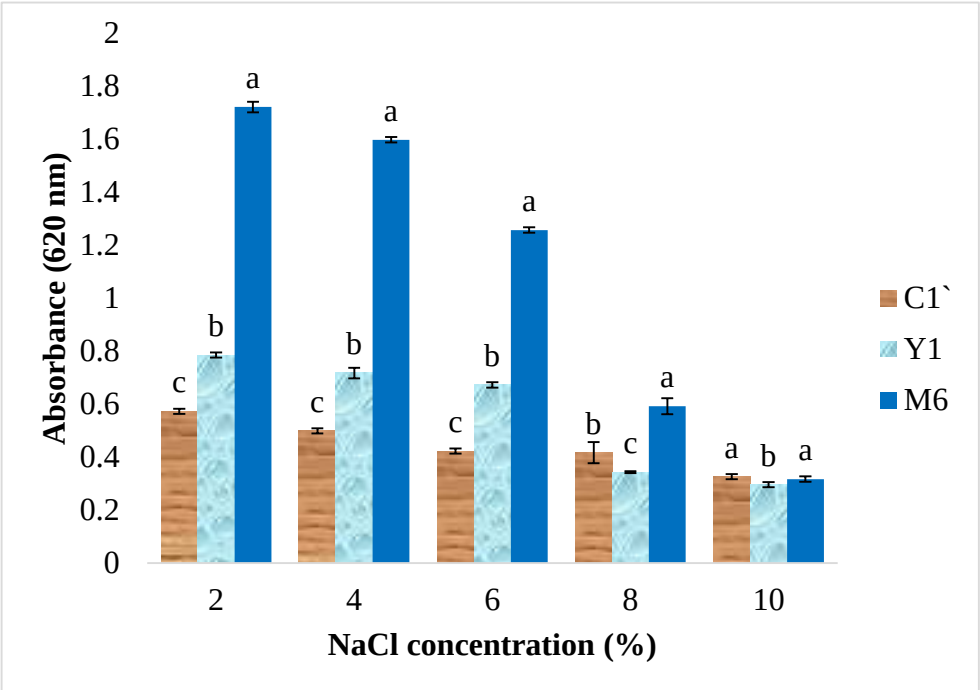

Fig.3 Effect of different concentration of NaCl on selected *Lactobacillus* isolates.Means with different letters within the same column indicate significant difference at $p<0.05$.

3.4.2 Lactose utilization

In the present study the selected LAB isolates (M6,C1 and Y1) were grown in nutrient broth medium supplemented with lactose and was observed for change in colour from red to yellow/orange which indicates the production of lactic acid. It was observed that every selected LAB isolate was able to produce lactic acid from lactose. A similar observation was noted by Pundir *et al*. (2013) with selected LAB products (Fooks*et al*., 1999) isolates using lactose medium. 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. The studies provide that the addition of certain starter cultures, allows the lactose intolerant people to consume those

Table 3. Antibiotic sensitivity patterns of the selected *Lactobacillus* isolates

	The diameter of inhibition zones(mm)			
Isolates	Ampicillin	Streptomycin	Bacitracin	Gentamycin
M6	R	17.56± 0.4	R	18.02 ± 0.1
C1	42± 0.1	12.3± 0.2	10.07±0.1	26.04 ± 0.3
Y1	14.2± 0.2	16.05± 0.3	11.7 ± 0.1	20.56 ± 0.2

Inhibition zones were measured in mm. Results represent the mean ± standard deviation of three replicates. R – Resistance

3.4.3 Antibiotic sensitivity test

Antibiotic susceptibility of lactic acid bacteria is one of the crucial criteria from the safety point of view of potential probiotics. Three potentially probiotic lactic acid bacteria isolates were subjected to antibiotic susceptibility testing using the disc diffusion method (Table 3). The strain M6 was resistant to two antibiotics such as Ampicillin and Bacitracin but sensitive to Streptomycin and Gentamycin. Other two strains (C1 and Y1) were sensitive to all the antibiotics tested (Plate 2). Tambekar and Bhutada (2010)determined the antibiotic sensitivity profile of 11 lactic acid bacteria isolated from milk of domestic animals and commercial available probiotic preparationand most of the stains were resistant to Ampicillin and Gentamycin.Shobharani and Aagarwal (2011) determined the antibiotic sensitivity profile of lactic acid bacteria isolated from cheddar cheese, *Leuconostoc paramesenteroides* was found to be resistant to Streptomycin and Gentamycin and sensitive to Ampicillin and Bacitracin.The difference in results may be due to different source used for the isolation of lactic acid bacteria and vary from strain to strain or type of strain.

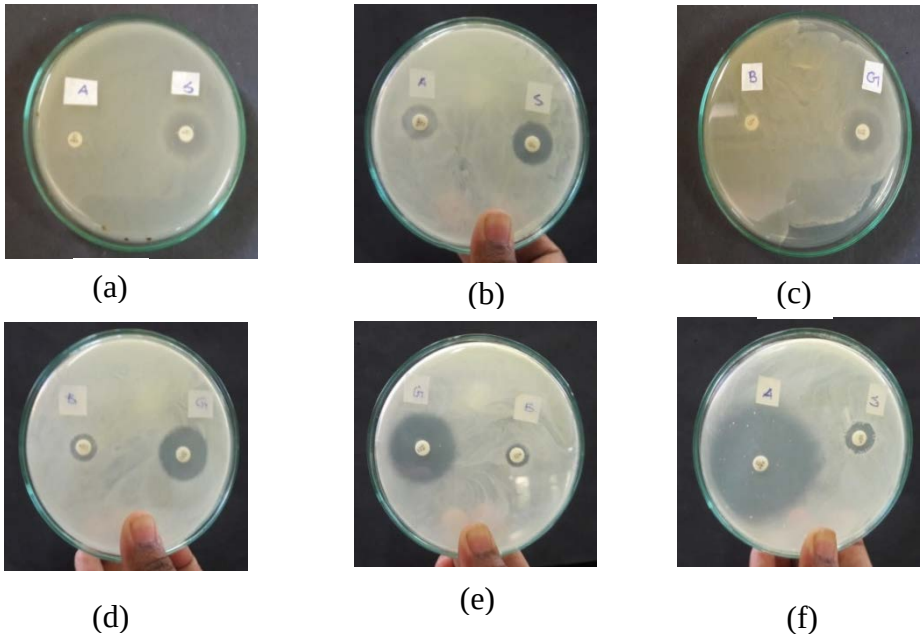

Plate 2. Resistance and sensitivity of *Lactobacillus* isolates for different antibiotics (a) Isolate M6 resistance to A and sensitivity to S(b) Isolate M6 resistance to B and sensitivity to G(c) Isolate Y1 sensitivity to A and S(d) Isolate Y1 sensitivity to B and sensitivity to G (e) Isolate C1 sensitivity to A and sensitivity to S (f) Isolate C1 sensitivity to G and B. A – Ampicillin, B – Bacitracin, S – Streptomycin, G – Gentamycin,M6 – LAB isolated from milk, C1 – LAB isolated from cheddar cheese, Y1- LAB isolated from yoghurt.

3.5 Molecular identification

Selected lactic acid bacterial isolate (M6) was identified by using the method of analysis of genus specific PCR and 16S rDNA sequence analysis and NCBI BLAST. Total 495 base pairs partial 16S rDNA sequence was retrieved in FASTA format and subjected for BLAST search in GenBank. The query sequence showed 100 % similarity with the *Lactobacillus plantarum* in NCBI BLAST suggests the relatedness of the tested organism with same and identity within the genus. The potential probiotic lactic acid bacterium (M6) was identified as *Lactobacillus plantarum* strain CIP 103151. The similar strain was obtained by Qian*et al.* (2018), in an experiment performed with the strain isolated from yak yoghurt using the methods of homology analysis of genus specific PCR,16S rDNA sequences and species specific PCR.

4. Conclusion

There are eight bacterial strains isolated from cow milk, cheddar cheese and yoghurt. Characterization by biochemical tests and the morphological study confirms that all the isolates are lactic acid bacteria. Three isolates that showed wide spectrum antimicrobial activity against five human pathogens were chosen for the determination of probiotic potential. Based on the probiotic properties, isolate M6 is concluded as promising probiotic bacteria and identified as *Lactobacillus plantarum* strain CIP 103151 based on the biochemical and 16S rDNA sequence analysis.

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References

Ananthanarayan, R. and Paniker, C. K. J. (1997). In: Text Book of Microbiology. Ed. Paniker, C. K. J, Indcom Press, Chennai. 46-49.

Azat, R., Liu, Y., Li, W., Kayir, A., Lin, D., Zhou, W. and Zheng, X. (2016). Probiotic properties of lactic acid bacteria isolated from traditionally fermented Xinjiang cheese, *Journal of Zhejiany – SCIENCE B*, 17(8):597-609

Ballal, M. and Shivananda, P.G. (2002). Rotavirus andenteric pathogens in infantile diarrhoea in Manipal South India,*Indian Journal of Pediatrics*, 69:393-396.

Barrow, G. I. and Feltham, R. K. A. (1993). Staining of Bacteria. In: Cowan and Steel's Manual for the identification of medical bacteria. Eds. Barrow, G. I. and Feltham, R. K. A. Cambridge University Press, Great Britain. ISBN 0-521-32611-07. 51-93.

Broome, M.C., Krause, D.A. and Hickney, M.W. (1990). The isolation and characterization of lactobacilli from cheddar cheese, *Australian Journal of Dairy Technology*, 45:60-66.

Chauhan, B.P., and Daru, D. (2016). Isolation and characterization of lactobacillus from milk, curd and fecal sample and assigning their probiotic values, *International Journal of Pharma and Bio Sciences*, 7(3):1070 – 1075.

Dunne, C., O'Mahony, L., Murphy, L., Thornton, G., Morrissey, D., O'Halloran, F.M., Flynn, S., Fitzgerald, G., Daly, C., Kiely, B., O'Sullian, G., Shanahan, F. and Collins, J.K. (2001). *Invitro*selection criteria for probiotic of human origin: correlation with *in vivo* findings, *American Journal of Clinical Nutrition,* 73(2): 386-392.

Fooks, L.J., Fuller, R. and Gibson, G.R. (1999). Prebiotics, probiotics and human gut microecology, *International Dairy Journal*, 9:53–61.

Hutt, P., Shchepetova, J., Loivukene, K., Kullisaar, T. and Mikelsaar, M. (2006) Antagonistic activity of probiotic *Lactobacilli* and*bifidobacteria*against entero- and uropathogens, *Journal of applied Microbiology*, 100:1324–1332**.**

Hoque, M.Z., Akter, F., Hossain, K.M., Rahman, M.S.M., Billah, M.M. and Islam, K.M.D. (2010)**.** Isolation, identification and analysis of probiotic properties of *Lactobacillus* spp. from selective regional yoghurts, *World Journal of Dairy Food Science,* 5(3):9-46

Kapilan, R. (2015). Determination of antibacterial activity of some important spices, *International Journal of Research – Granthaalayah*, 3(10):57-64.

Kapilan, R.and Arasaratnam, V. (2010). A Novel *Bacillus pumilus* strain for alkaline xylanase production at and above 40^{o}C. *Ceylon Journal of Science (*Bio Science)**,** 39(1):61-70.

Karuppaija, S. Kapilan, R and Vasantharuba, S. (2016). Characterization of best naringinase producing fungus strain isolated from palmyrah (*Borrasusflabellifer*) fruit pulp, *International Journal of Biological Research***,** 4(2):97-101.

Klaenhammer, T.R., Kullen, M.J. (1999) .Selection and design of probiotics, *International journal of food microbiology*, 50: 45-57.

Lavanya, B., Sowmiya, S., Balaji, S. and Muthuvelan, B. (2011). Screening and characterization of lactic acid bacteria from fermented milk, *British Journal of Dairy Sciences,* 2(1): 5-10.

Leroy, F. and De Vuyst, L. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry, *Trend in Food Science & Technology*, 15(2): 67-78.

Mohanappriya, S. and Kapilan, R. (2018). Isolation of efficient cellulase producing *Aspergillus unguis* UCSC324 and determination of the kinetic properties of the crude cellulase, *Agrieast, Journal of Agricultural science*, 12(2):35-48

Pundir, K.R., Rana, S., Kashyap, N. and Kaur, A. (2013). Probiotic potential of lactic acid bacteria isolated from food samples: an in vitro study, *Journal of Applied Pharmaceutical Science,* 3(3):085-093

Qian, Y., Long, X., Pan, Y., Li, G. And Zhao, X. (2018). Isolation and identification of lactic acid bacteria (*Lactobacillus plantarum* YS2) from yak yoghurt and its probiotic properties, *Biomedical research*, 29(4): 815-820

Setyawardani, T., Rahayu, W.P., Maheswari, R. And Palupi N.H.S. (2011). Identification and Characterization of Probiotic Lactic Acid Bacteria Isolated from Indigenous Goat Milk, *Animal Production*, 13(1):57-63

Shobharani, P. and Aagrawal, R. (2011). A potent probiotic strain from cheddar cheese,*Indian Journal of Microbiology*, 51(3):251-258

Sieladie, D.V., Zambou, N.F., Kaktcham, P.M., Cresci, A. and Fonteh, F. (2011). Probiotic properties of *Lactobacilli* strains isolated from raw cow milk in the Western Highlands of Cameroon, *Innovative Romanian Food Biotechnology,* 9:12-28.

Shobharani, P. and Aagrawal, R. (2011). A potent probiotic strain from cheddar cheese, *Indian Journal of Microbiology*, 51(3):251-258.

Tambekar, D.H. and Bhutada, S.A. (2010). An evaluation of probiotic potential of *Lactobacillus*sp. from milk of domestic animals and commercial available probiotic preparations inprevention of enteric bacterial infections. *Recent Research in Science and Technology*,2(10): 82-88.

Theivendrarajah, K. (1990). Microbiology Laboratory Manual: Department of Botany, University of Jaffna. University Publication. 1-33.