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Antimicrobial Effect of *Lactobacillus* spp against Gastro Intestinal Pathogens

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ABSTRACT

Milk is a rich source of protein and fats but it contains numerous number of Lactic acid Bacteria. *Lactobacillus* sp belongs to the major group of Lactic acid bacteria. Predominant gram positive isolates were obtained from the milk samples. The interesting result of the study is the isolation of micro organisms which are able to grow at low pH of 2.5. All the 5 isolates from the milk sample exhibited ɤ hemolysis which proved to be safe for consumption. The isolates were able to ferment glucose and lactose. The results of antimicrobial activity revealed that the selected isolates could inhibit only two test pathogenic bacteria, however at different inhibition levels. The isolates from the milk sample showed the most antimicrobial potency to *Staphylococcus aureus* and *Escherichia coli*. The isolates survived at acidic (pH 2.5) condition. They also exhibited tolerance to bile salt for first 3 hours. The results suggested that microorganisms isolated from milk sample have potential antimicrobial effect against human pathogens.

Keywords: Milk, *Lactobacillus*, probiotics, antimicrobial activity, gut pathogens.

INTRODUCTION

Probiotics are microorganisms that are believed to provide health benefits to the humans, when consumed. They can also be said as ingested microorganisms associated with benefits for humans and animals. They are usually safe, but may cause host- bacteria interaction and unwanted side effects in rare cases. *Lactobacillus* sp. are probiotic strains available in dairy products like Curd and Yogurt and dietary supplements. They are the genus of gram positive, facultative anaerobic or micro aerophilic, rod shaped, non-spore forming bacteria. They are major part of the lactic acid bacteria (LAB) group which converts sugars to lactic acid. In humans, they constitute a significant component of the microbiota at a number of body sites [1]. *Lactobacillus* species found in the Gastro Intestinal (GI) tract have received tremendous attention due to their health promoting properties. They are commonly used as probiotics, which are defined by the FAO/WHO as live microorganisms that when administered in adequate amounts confer health benefits on the food [2].

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Formation of organic acids and the production of antibacterial substances, termed as bacteriocins, are the anti-microbial substances in probiotics. Organic acids, in particular, acetic acid and lactic acid have a strong inhibitory effect on gram negative bacteria and they have been considered the main anti-microbial compounds responsible for the inhibitory activity of the probiotics against pathogens. The major functional properties of probiotics include its antibacterial activity against pathogens [3]

MATERIALS AND METHODS

Sample Collection

Cow's milk (Jersey Breed) samples were collected from Kumarapuram, Cuddalore. All the samples were collected in sterile containers, transported to the laboratory and subjected to microbiological analysis.

Microbiological Analysis

One milliliter of the sample was serially diluted with sterile phosphate buffer, and 0.1 ml of the aliquot was spread onto *Lactobacillus* MRS agar. The plates were incubated at 37°C for 48 hours, at micro-aerophilic condition using candle jar and the results were observed. The bacterial counts in the sample were expressed as $\log_{10}$ Colony Forming Units per ml [CFU/ml]. Representative isolates were selected and streaked on *Lactobacillus* MRS plates to obtain pure culture.

Identification of the Isolates

The presumptive isolates were identified on the basis of cell morphology, Gram staining, catalase and oxidase tests, IMViC tests, Triple sugar Iron test and Urease test by using standard procedures.

Hemolytic Activity

The isolates were inoculated on blood agar with 5 % blood and the plates were incubated at 37ºC for 24 h. Then, the plates were observed for the ability of the isolate to lyse red blood cells [4]

Antimicrobial Activity

The antimicrobial activity of the isolates was tested against *Salmonella typhimurium ATCC 14028, Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 8739* by agar well diffusion method. Test Organisms were cultured in Nutrient broth at 37° C for 24 hours. After incubation, test organisms were spread using sterile cotton swab on Muller Hinton agar plates. Fifty microlitre of the culture supernatant from selected isolates were added to the well for testing antimicrobial activity. The plates were incubated at 37°C for 24 hours. After incubation, the zone of inhibition was measured in millimeter [5].

Acid Tolerance Test

The isolates were subjected to preliminary screening intended for acid tolerance in MRS broth with slight modification [6]. Isolates were grown in MRS broth and incubated at 37°C. MRS broth was adjusted to pH 2.5, 3.5 and 4.5 with 1N HCl. Samples (0.1ml) were taken after 3 hours and 6 hours of incubation and plated on *Lactobacillus* MRS agar by pour plate method. Growth of the culture on plate was noted as acid tolerant. Number of colonies were counted on each plate.

Bile Tolerance Test

Isolates were grown in MRS broth with saturated bile solution at concentration of 0.5%. The cultures were incubated at 37°C for 3 hours and 6 hours. Samples (0.1ml) were taken after 3 hours and 6 hours of incubation and plated on *Lactobacillus* MRS agar by pour plate method. Growth of the culture on plate was noted as bile tolerant [6].

RESULTS AND DISCUSSION

Morphological and Biochemical Characterization of the isolates

In this study, 5 Gram positive bacilli were isolated from 6 samples. The results of biochemical tests for all the isolates were Catalase negative, Oxidase negative, MR negative, VP negative, Citrate negative and Urease negative. All the isolates fermented glucose, lactose and sucrose (A/A) sugars. No gas and H_2S production was observed. Based on the morphological and biochemical characterization observed, the isolates SLB- 1, SLB-2, SLB- 3, SLB-4, SLB-5 were found to be *Lactobacillus* sp (Table 1). This result has been interpretated based on the previous studies, reported by the researchers and according to the Bergey's Manual of Bacterial Classification.

Gram staining	Catalase	Oxidase	Indole	MR	VP	Citrate	Urease	TSI	Mannitol Motility Test	Organism Identified
Gram positive rods	-	-	-	-	-	-	-	A/A	-/Non-Motile	*Lactobacillus* sp

Note: + = POSITIVE; - = NEGATIVE; A-/A- = acid/ acid;

Table 1 Morphological and biochemical characteristics of the isolates

Screening of isolates for hemolytic activity

The hemolysis test is based on the ability of the organism to lyse red blood cells in blood agar. All the selected isolates (SLB-1, SLB-2, SLB-3, SLB-4 & SLB-5) exhibited ɤ hemolysis, that is, isolates were not able to lyse erythrocytes.

Antimicrobial activity of the isolates

Antimicrobial activity is an added characteristic of probiotic organisms. They may have the ability to destroy or kill pathogens which infect the gastro intestinal (GI) system. The selected isolates were checked for their antimicrobial activity against GI pathogens such as *Salmonella typhimurium* ATCC14028, *Escherichia coli* ATCC8739 *and Staphylococcus aureus* ATCC25923. Results of the study revealed that only one of the isolates, SLB-5 has antimicrobial activity against the pathogenic bacteria tested, however at different levels (Table 2). The difference in inhibition zone among the selected isolates could be due to intrinsic nature of the organism tested. Several researchers have observed that isolates which can produce antimicrobial substances are active against pathogenic bacteria. Many species of *Lactobacillus* produce bacteriocins that inhabit activity of most other organisms. Previous studies have reported antibiotic producing *Lactobacillus* [7]. The presence of Lactobacilli in GI Tract is important for the maintenance of the intestinal microbial ecosystem. The role of Lactobacilli in Gastro Intestinal tract is the suppression of the undesirable intestinal microflora [8]. The intestinal Lactobacilli play a major role in the regulation of bacterial population and control of various enteropathogens in the GI. They suppress the multiplication of the pathogenic and putrefactive bacteria that cause intestinal problems, diarrhea, nausea, vomiting, dysentry and digestive upsets [9]. The antagonistic activity of *Lactobacillus* against GI pathogens could be due to the production of inhibitory compounds such as organic acids, hydrogen peroxide, bacteriocins and antibiotic like substances [10].

Table 2: Antimicrobial activity of the isolates

Test Organism	*Staphylococcus aureus (ATCC 25923)*	*Escherichia coli (ATCC 8739)*	*Salmonella typhimurium (ATCC14028)*
Zone of Inhibition	13 mm	12 mm	2 mm

Acid tolerance Test

Acid tolerance is an essential feature of probiotic strain. The results revealed that the isolates exhibited tolerance at low pH. A probiotic bacterial strain should survive when it passes to the stomach where the pH is low. Researchers [11] have reported that HCl present in the stomach disrupt the biomolecules of cells, such as proteins, DNA and fatty acids. Low pH environment can inhibit the metabolism and reduce the growth and viability of bacteria. Some strains of *Lactobacillus* are able to survive in low pH (Fig 1). Studies by Montes and Makarova [12] reported that bacteria could survive at low pH. According to previous studies [13] the threshold point to state acid resistance was set as pH 2 and pH 3 for 3 hours incubation, as it stimulates the bacterial colonization in the stomach. There will be a strong influence on the number of viable bacteria at pH 2. Studies revealed that most of the *Lactobacillus* strains were tolerant to acid [14]. Generally, survival was low at pH 1.0 and 2.0, moderate at pH 3.0 and good at 4.0 and 5.0.

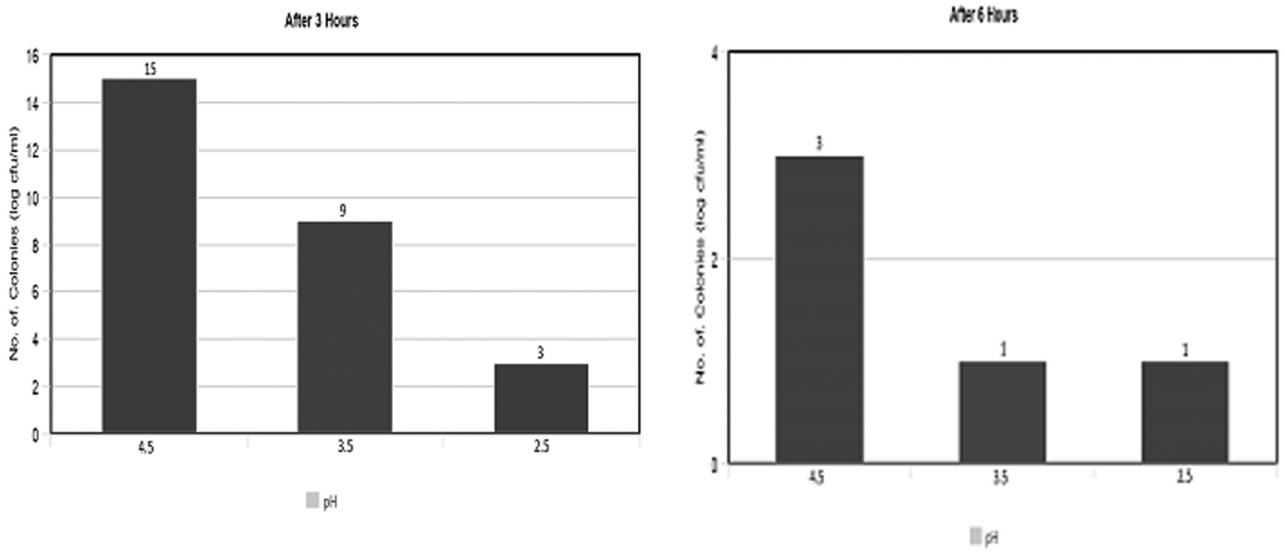

Figure 1 Acid tolerance of the isolates

Bile Tolerance Test

Bile tolerance is also an important feature of LAB. The results revealed that the selected isolate exhibited tolerance to bile salts at a concentration of 0.5% upto 3 hrs. After 6 hrs of incubation, no viable cells were observed. But there is a possibility of surviving at low bile salt concentrations [15]. Gilliland et al [16] reported that supplementation of the diet with the more bile resistant *Lactobacillus* strains like *L. acidophilus*, will increase number of the strains in the small intestine. Chateau et al [17] classified bile tolerant *Lactobacillus* into 4 groups: resistant strains (delay of growth (d) < 15 min), tolerate strains (15 min < d <40 min), weakly tolerant strains (40 mins < d < 60 min) and sensitive strains (d > 60 mins). Havenaar and Jensen [18] reported that the *Lactobacillus* strains from the caecum showed better tolerance to acid than those from the ileum.

CONCLUSION

The results of the study established that *Lactobacillus* species isolated from milk exhibited potential functional properties and showed the ability to suppress gastro intestinal pathogens.

REFERENCES

1. E. H. E. Heller, E.S. Shokery, E. Pavlova, Egypt. J. Dair Sci. 39(2011) 197-207
2. FAO/WHO (2002) Working Group Report.
3. C. Hemant, J. Harshada, Res. J. Rec. Sci. 4(2015) 173-176.
4. M.J. Lesmana, P.W. Toole, Int J Syst Evol Microbiol 58 (2001) 2945-2954.
5. L. Gonzalez, H. Sandoral, N. Sacristan, J. M. Castron, S.M. Frensno, M.E. Tornadijo, J. Food. Cont. 18 (2006) 716-722.
6. D.H. Tambekar, S.A. Bhutada, Rec. Res. Sci. Technol. 2 (2010) 94-98.
7. L. Todkar, M.C. Martini, G.L Bolweg, M.D. Levitt, D.A Savaiano, American J. Clinic. Nut. 45 (2014) 432-437.
8. S.A. Deve, Bulg. J. Agri. Sci. 12 (2006) 63-114.
9. B. Singh, R. P. Sinha, R. N. Sinha, Microbiol. Alim, Nutr 13(1995) 157-162.
10. J. R Tagg, A. S. Dajani, L. W. Wannamaker, Bacteriol. Rev. 40 (1976) 722-756
11. H. K .Chan, R.P.K. Sahadev, S.P. Leong, Int. Food Res. J 18 (2011) 1515-1522
12. R. Montes, K.S Makarova, Int. J. Food Microbiol. 41(2015) 103-125.
13. M.J. Prasad, D.C. Crowell, J. Bacteriol 9 (2009) 357-395.
14. P.L. Conway, S.L. Gorback, B.R. Goldin. J. Dair. Sci. 70 (1987) 1–12.
15. E. Both, E. Gyorgy, Z. Csaba, K. Szabo, É. Tamasa, B. Ábharam, I. Miklossy, S. Lanyi, U.P.B Sci. series B 72(2010) 37-44.
16. S.E. Gilliland, T.E. Staley, L.J. Bush, J. Dairy Sci. 67 (1984) 3045–3051.
17. N. Chateau, A.M. Deschamps, A. Hadj Sassi, Lett. Appl. Microbiol. 18 (1994) 42–44.
18. T.D. Havenaar, T.K Jensen, Peds. 124 (2002) 172-179.