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Research Article

Isolation of potential probiotic *Lactobacillus* strains from human milk

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ABSTRACT

Probiotic is a live microbial supplement which affects host's health positively by improving its intestinal microbial balance Probiotics are used for long times in food ingredients for human and also to feed the animals without any side effects. Also probiotics are acceptable because of being naturally in intestinal tract of healthy human and in foods. The Lactic acid bacteria (LAB) were isolated from fermented food products, dairy products, human intestine and feces but human breast milk is a natural source of potential *Lactobacillus* strains. Hence the present study was aimed to isolate and identify the *Lactobacillus* spp. from human milk and to test for acid tolerance, bile tolerance and antimicrobial activity. Totally 32 isolates were isolated and only two were identified as *Lactobacillus* spp. and named as LAB1 and LAB2. Results showed that a considerable variation existed among the bacterial cultures in their growth and viability in the presence of bile salt, and assimilation of cholesterol from the medium. Moreover, the strain LAB1 shown to be the most bile salt tolerant (0.5% and 1% concentration) culture and they grow well at low pH. The total plate count of LAB1 after 3 hours at pH 2 is 6.01 CFU/ml and it will decrease in pH 1. The strain LAB1 inhibit the growth of *Escherichia coli, Klebsiella pneumoniae* and *Candida albicans* and produce 9.26±0.20, 10.33±0.57 and 6.69±1.23 mm zones around the wells respectively.

Key words: Probiotics, Mother's milk, *Lactobacillus* spp, *Escherichia coli, Klebsiella pneumoniae*

INTRODUCTION

Probiotics are non-pathogenic microorganisms which confer benefits to their host when consumed in adequate amounts. The main probiotics are some lactic acid bacteria (LAB), such as *Lactobacillus* and *Bifidobacterium*, which are members of the commensal bacteria in the gastrointestinal tract of human and animals. A great number of lactic acid bacteria were isolated from various traditional naturally fermented foods (Garriga *et al*., 1998 and Bao *et al.*, 2010). After birth, breast milk is the best food for infants because it fulfills all the nutritional requirements for them during months. Also breast milk protects the new born against infectious diseases. This effect seems a result of the action of some breast milk components, like different antimicrobial compounds, immunoglobulin's, immune component cells (Martin *et al*., 2003) and also breast milk contains prebiotics substances which stimulate the growth of the beneficial bacteria neonate gut (Martin *et al*., 2004 and Martin *et al*., 2003). In a general view human milk contains fat, protein, carbohydrate, minerals and bacteria. When it comes to the microbiological point of breast milk, human milk is really an important factor in the initiation and development and of course composition of the neonatal gut microflora since it constitutes source of microorganisms to the infant gut for several weeks after birth (Martin *et al.*, 2005). It is estimated that an infant ingests$1x10^5$– $1x10^7$commensal bacteria while suckling if the infant consumes approximately 800 ml breast milk per day (Martin *et al.*, 2004, Martin *et al*., 2005 and Heikilla and Saris, 2003). There are surprisingly not so much studies on the isolation and analysis of commensal or potential probiotic bacteria from breast milk (Martin *et al.*, 2003). However, if the bacteria with the ability to provide health benefits such as protection the host from pathogenic bacteria were isolated from human milk, they would be considered attractive probiotic organisms (Martín *et al*., 2004). The main scope of this study is to isolate and identify lactobacilli and search the potential probiotic properties of these isolates using breast milk as a natural source originated from human.

MATERIALS AND METHODS

Samples were collected from healthy two month lactating mother in Gandhi gram, Dindigul District, Tamilnadu, India. The samples were collected in sterile carriers and stored on ice until delivery to the laboratory. Pour plate technique was used to isolate the organisms. Serial dilutions were plated onto de Man, Rogosa and Sharp (MRS) agar (pH 6.2) and incubated anaerobically at 37 °C for 72 hours. Totally 32 strains were isolated and only two strains were identified as *Lactobacillus* spp. based on colony morphology, catalase reaction, Gram staining, carbohydrate fermentation and growth on de Man Rogosa Sharpe (MRS) medium. The pure cultures of the isolates were preserved in MRS broth medium containing 20% (v/v) glycerol as frozen stocks at -80 °C. To determine the resistance to low pH of the two strains, medium were adjusted to stomach pH which is 3.0 (Prasad *et al*., 1998). During incubation at 37 °C, viable organisms were enumerated as CFU (colony forming unit) at the 0, 3rd and 5th hours by pour plate technique. After determination of resistance to low pH, organisms were examined for tolerance against bile salt. Because, the mean intestinal bile concentration is believed to be 0.3 % (w/v) and the staying time of food in small intestine is suggested to be 4 hours (Prasad *et al*., 1998). During the incubation period (4 h), viable colonies were enumerated at 0 and 5th hours by pour plate technique. Antimicrobial activity also tested against *Escherichia coli, Klebsiella pneumoniae* and *Candida albicans* by agar well diffusion method. Isolates were characterized according to their fermentation profiles using ability to ferment 11 different carbohydrates such as glucose, fructose, lactose, xylose, mannose, mannitol, arabinose, maltose, sorbitol, sucrose and galactose. The carbohydrate fermentation was determined by using separate medium containing Phenol red as indicator. 10 ml of the medium containing 1% different carbohydrate were inoculated with 1% inoculums and incubated for 72 hours. After the incubation period change of the color and gas production indicates the carbohydrate fermentation.

RESULTS AND DISSCUSION

The isolated lactic acid bacteria are confirmed as *Lactobacillus* spp. as described in the 8th edition of *Bergey's manual of Determinative Bacteriology*. The strains were gram-positive, catalase negative, nonsporeforming, rods and grew well at 45^oC but not at 15^0C. The strains were fermented glucose, fructose, lactose, mannose, maltose, arabinose, sucrose and galactose and none fermented mannitol, sorbitol and xylose. All isolates were detected whether they were resistant to pH 3.0 for 3 h, since the digestion process in the stomach completed in about 3 h. The LAB1 strain able to with stand the pH 3 after 3 hours and viability slightly decreased at the pH 1 there will be no growth. (Table 1) and then these isolates were screened for their ability to tolerate the bile salt. The CFU values

showed that both isolates were resistant to bile at this concentration during this period (Figure 1 and 2). Antimicrobial activity is another important criteria for selection of probiotics. The strain LAB1 shows antagonistic activity against tested pathogens. LAB1 produce 9.26±0.20, 10.33±0.57 and 6.69±1.23 mm zones against *Escherichia coli*, *Klebsiella pneumoniae* and *Candida albicans* respectively. Both isolates showed much more efficiency on *Escherichia coli*.

Table 1: Tolerance against different pH total plate count CFU/ ml

pH	0 hour		3 hour		5 hour	
	LAB1	LAB2	LAB1	LAB2	LAB1	LAB2
1	7.13	7.18	4.19	3.82	0	0
2	9.12	8.46	6.29	6.01	1.18	1.59
3	9.28	8.64	8.57	7.12	2.09	1.02

Figure 1: Tolerance against different bile concentration total plate count CFU/ ml after 0 hour

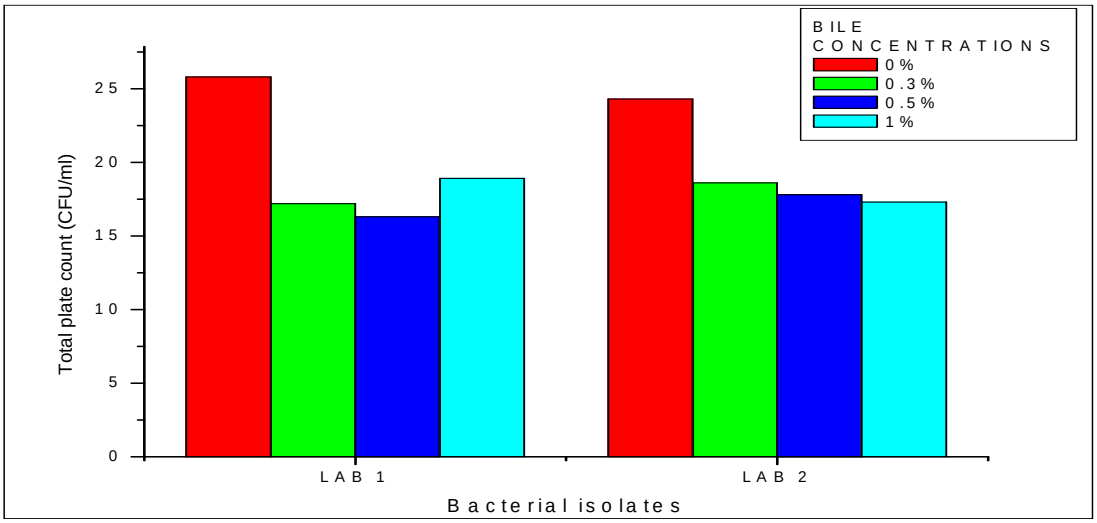

Figure 2: Tolerance against different bile concentration total plate count CFU/ ml after 3 hour

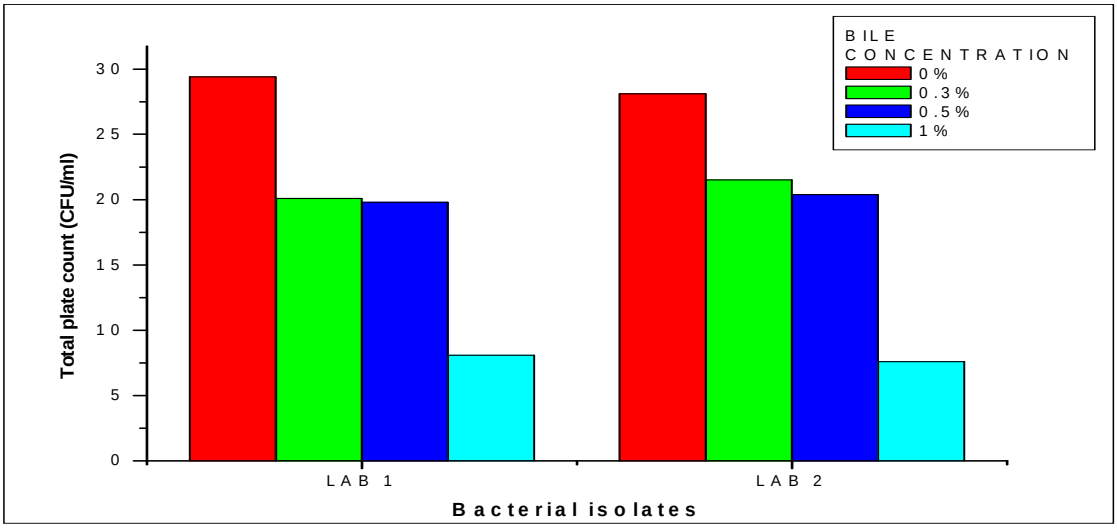

Table 2: Antimicrobial activity of LAB against test pathogens (mm)

Bacterial Isolates	TEST PATHOGENS		
	Escherichia coli	*Candida albicans*	*Klebsiella pneumoniae*
LAB 1	9.26±0.20	10.33±0.57	6.69±1.23
LAB 2	8.81±0.12	6.66±0.53	6.01±0.18

A great diversity of microorganisms has been described in the human milk; some of them originate from the skin, such as *Staphylococcus* and *Streptococcus*, while the origin of lactic acid bacteria such as *Lactobacillus*, *Lactococcus*, *Enterococcus* and even *Bifidobacterium* spp. remains unclear. Some reports suggest that these microorganisms might be brought to the lactating breast tissue through the endogenous trafficking of bacteria-loaded dendritic cells originated from the intestinal mucosa (Donnet *et al*., 2010). We searched for the lactobacilli strains which might have potential probiotic properties. There are only limited studies about the probiotic potential of lactic acid bacteria isolated from human milk. Martin *et al.*, (2004) showed that the natural microbiota of human milk contributes to prevent newborn infections. Preciously, Martin *et al.*, (2003) investigated the human milk as a source of potentially probiotic lactic acid bacteria. They isolated and identified *Lactobacillus fermentum*, predominately *Lactobacillus gasseri* and *Enterococcus faecium* among the lactic acid bacteria. These species were in use of commercial probiotic products and they were considered as probiotic bacteria. The lactobacilli strains showed variation in the study performed by Heikilla and Saris (2003). 12.5% samples contained lactic acid bacteria namely; *Lactobacillus rhamnosus*, *Lactobacillus crispatus*, *Lactococcus lactis* and *Leuconostoc mesenteroides*.

CONCLUSION

The results of this study indicate that human milk may be used as a potential natural source to isolate the effective strains of *Lactobacillus* sp. Both the isolated strains are able to grow at low pH and the presence of bile salts. This reveals that the bacteria able to grow in our intestine. And also it shows antimicrobial activity against tested pathogens this is due to the production of bacteriocins like substances. These results suggest that lactobacilli from breast milk could contribute to an anti-infective protection in neonates
and would be excellent candidates for the development of infant probiotic products.

REFERENCES

1. Y Bao ; YC Zhang ; Y Zhang 2010. Screening of potential probiotic properties of *Lactobacillus fermentum* isolated from traditional dairy products. *J. Food Control*,**2010**, *21*, 695–701.
2. A Donnet-Hughes; PF Perez; J Dore; M Leclerc; F Levenez ; J Benyacoub. Potential role of the intestinal microbiota of the mother in neonatal immune education. *Proc Nutr Soc*, **2010**, *69*, 407-15.
3. M Garriga; M Pascual; JM Monfort. Selection of *Lactobacilli* for chicken probiotic adjuncts. *Journal of Applied Microbiology*, **1998**, *84*, 125-132.
4. MP Heikkila; PEJ Saris. Inhibition of *Staphylococcus aureus* by the commensal bacteria of human milk. *Journal of Applied Microbiology*, **2003**, *95*, 471-478.
5. R Martin; S Langa; C Reviriego; E Jimenez; LM Marin; M Olivares; J Boza; J Jimenez; L Fernandez; J Xaus; JM Rodriguez. The commensal microflora of human milk: New perspectives for food bacteriotherapy and probiotics. *Trends in Food Science&Technology*, **2004**, *15*, 121-127.
6. R Martin; S Langa; C Reviriego; E Jimenez; LM Marin; J Xaus; L Fernandez; JM Rodriguez. Human milk is a source of lactic acid bacteria for infant gut. *The Journal of* Pediatrics, **2003**, 143, 754-758.
7. R Martin; M Olivares; ML Marin; L Fernandez; J Xaus; JM Rodriguez. Probiotic potential of 3 Lactobacilli strains isolated from breast milk. *Journal of Human Lactobacillus*, **2005**, 21(1), 8-17.
8. J Prasad; H Gill; J Smart; PK Gopal. Selection and Characterization of *Lactobacillus* and *Bifidobacterium* strains for use as probiotic. *International Dairy Journal*, **1998**, 8, 993-1002.