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Isolation of Lactic Acid Bacteria from Curd Sample and Screening for Probiotic Properties

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ABSTRACT

Probiotics consist of health promoting viable microorganisms, which helps by improving the intestinal microbial balance. The aim of the present study was to isolate and characterize different lactic acid bacteria with probiotic properties from the curd sample. Total twelve different lactic acid bacteria were isolated. Best probiotic *Lactobacilli* strain was selected by bile tolerance test, acid tolerant test, NaCl tolerance test, catalase activity and antibiotic resistance assay. All strains gave negative results for catalase test. Among all strains, only one strain LAB6 was able to survive up to 4% bile salt, pH value 2 and 3, salt tolerance up to 5% and also resistant to selected antibiotics. In conclusion, the present study showed that isolated organism LAB6 can be used as potential probiotic lactic acid bacteria.

KEYWORDS: Acid tolerance, Antibiotic resistance, Bile salt tolerance, Lactic acid bacteria, Probiotics.

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INTRODUCTION:

Probiotic term was derived from Greek words Pro (favour) and bios (life). Probiotics are defined as live microbial feed supplements that improve the health of human beings by its valuable secondary products[1]. LAB have been receiving considerable attention as "probiotics" because of their innate ability to exert antagonistic activity against, non-pathogenic and spoilage organisms[2]. By acknowledging curd's suitability as dietary agent of providing probiotics to intestine, the current study was designed to analyze some local curd variety for isolation of LAB equipped with probiotic action[3].

Probiotics have been administered to animals in order to prevent infectious illness, reinforcing the barrier function of the intestinal flora or increasing the immune system[4]. The stability of the GIT microflora effectively limits the capacity of invading microorganisms, including pathogens, to colonise the gut, giving rise to what has been termed 'colonisation resistance'[5]. The organisms must be able to survive the unfavorable environment of GIT, which benefits resistance to acid and bile[6]. Probiotics should be resistant to specific conditions of GIT, like it should be resistant for more than 4 hours to proteolytic enzymes, low pH values (1.8-3.2) prevailing in the stomach and to bile concentration, pancreatic juices and mucus which are the part of small intestine. Lactobacilli are catalase negative organism. Furthermore, bacterial strains to be used in probiotics are supposed to be resistant to antibiotics, lactic acid, hydrogen peroxide, bacteriocins, etc.[7, 8]. Probiotics are competing for adhesion to the intestinal brush border epithelium surface. Adherent non-pathogenic bacteria (probiotics) can prevent attachment and subsequent entry of pathogenic enteroinvasive bacteria into the epithelial cells[5]**.** *Lactobacilli* seem to adhere to gut wall with extracellular substance containing polysaccharides, protein, lipid lipotechoic acids. The objective of this study was to isolate lactic acid bacteria from native curd samples and representative isolates investigated for probiotic properties.

MATERIALS AND METHODS:

Isolation of Lactic acid bacteria from curd sample

Lactic acid bacteria were isolated from curd sample. The curd samples were suspended appropriately and diluted in sterile saline and spread plated into selective medium: De Mann, Rogosa Sharpe (MRS) [9] and incubated at 37°C for 24 h anaerobically for isolation.

Bile salt tolerance

Fresh culture of microorganisms was inoculated in MRS broth supplemented with 1.5% and 2.0% (w/v) bile salts (Oxgall, HiMedia, India) followed by incubation at 37°C. Aliquots were plated on MRS agar at 37°C for 24 hours. Bile tolerance was assessed in terms of viable colony counts after the aforesaid incubation at 37°C[10].

Acid tolerance (pH)

Acid tolerance was determined using method described by[11]. The overnight grown culture was used to inoculate MRS broth (v/v). 0.1ml culture was inoculated into 9.9 ml of sterile broth of pH such as 2 and 3. The pH of broth was adjusted with 1N HCl. Inoculated broths were incubated at 37°C for 24 hr. The results were recorded and tabulated in terms of presence and absence of growth.

NaCl tolerance

For the determination of NaCl tolerance of isolated *Lactobacillus*, 6 test tubes containing MRS broth were adjusted with different concentrations (0.5, 1, 2, 3, 4 and 5%) of NaCl. After sterilization, each test tube was inoculated with 1% (v/v) fresh overnight culture of *Lactobacillus* and incubated at 37°C for 24 hrs. After 24hrs of incubation, their growth was determined[12].

Catalase test

The catalase activity of these isolates was detected by resuspending the culture in a 3% solution of hydrogen peroxide. Catalase is an enzyme that converts hydrogen peroxide into water and oxygen. If the bubbles appear it means that this test gives positive result[13].

Antibiotics resistance

Lactic acid bacteria strains were assessed their antibiotic resistance by disc diffusion method using antibiotics discs. 1 ml of actively growing cultures was mixed with 10 ml of soft nutrient agar and poured into the base agar plate. After solidification, the antibiotic discs were placed on the solidified agar surface, and the plates were left over for 30 minutes at 4°C for diffusion of antibiotics and then anaerobically incubated at 37°C for 48 hrs[14]. Resistance was defined according to the disc diffusion method by using antibiotic discs of Chloramphenicol, Penicillin, Ampicillin, Amoxyclav, Cefotaxime, Co-Trimoxazole, Gentamycin, Tobramycin, Ceftriaxone, Ciprofloxacin, Tetracycline, Gentamycin, Linezolid, Streptomycin, Vancomycin, Clindamycin, Erythromycin, Aztreonam, Cefpodoxime, Cefpodoxime/Clavulanic acid, Ceftazidime, Cefotaxime. The diameter of zone of

inhibition was measured in millimeters and evaluated for resistance or susceptibility using the comparative standard method.

RESULT AND DISCUSSION:

Isolation of Lactic acid bacteria from curd sample

All samples harboured large number of bacteria as milk is an excellent media for growth and proliferation of bacteria. In present work, by direct method of isolation, twelve presumptive LABs were isolated on MRS media from curd sample. All isolated lactic acid bacteria named as LAB1, LAB2, LAB3, LAB4, LAB5, LAB6, LAB7, LAB8, LAB9, LAB10, LAB11 and LAB12. The morphological characteristics were also investigated and showed that most of the isolates were Gram positive rod. All isolates were catalase negative. Lactic acid bacteria (LAB) are a group of Gram-positive, non-spore forming, cocci or rod shaped, catalase-negative and fastidious organisms frequently isolated from milk and dairy products[15].

Acid tolerance

Table No. 1: Acid tolerance assay

Isolated organisms	pH		
	pH 2	pH 3	pH 7
LAB1	-	-	+
LAB2	-	-	+
LAB3	-	-	+
LAB4	-	-	+
LAB5	-	-	+
LAB6	+	+	+
LAB7	-	-	+
LAB8	-	-	+
LAB9	-	-	+
LAB10	-	-	+
LAB11	-	-	+
LAB12	+	+	+

+ = Growth - = No growth

The tolerance to acidity seems to be an important trait of probiotic strains. Generally, growth and fermentation of lactobacilli create an acidic condition and they are resistant to acidic conditions[16, 17]. The survival at low pH is important if intended to use in gastrointestinal tract for health improvement. The results of acid tolerance are shown in table no 1. Determination of acid tolerance of Lactic Acid Bacteria was investigated for pH of 2.0 and 3.0. Among all the isolates, LAB 6 and LAB 12 could grow at low pH 3. These LAB 6 and LAB 12 isolates also could grow at

pH 2 but their growth was lower as compare to growth at pH 7. It indicates that these isolates are resistance to broad pH range and able to survive at low pH and can be used in probiotic applications.

Bile salt tolerance

The probiotic microorganisms proved to exhibit an excellent quality of bile tolerance. Bile acids are amphipathic molecules with antimicrobial potential that act as a detergent and interfere with biological membranes[18]. Among five isolates, only LAB 6 isolate could grow in the range of 0.5% to 4% concentration of bile salt. Another isolates LAB7, LAB 10 and LAB 11 could able to grow up to 3% bile salt. While LAB12 isolate could able to grow up to 2%. Acidity, presence of bile salts, and pancreatic enzymes in the gastrointestinal tract (GIT) are some of the major stresses that an orally taken probiotic encountered in the GIT. It is essential that a potential probiotic strain is able to tolerate these stress conditions in order to survive in the GIT[19].

Table No. 2: Bile salt tolerance assay.

Isolated Org.	Time Hrs.	0.5%	1%	2%	3%	4%
LAB6	**24hr**	+	+	+	+	+
LAB7	**24hr**	+	+	+	+	-
LAB10	**24hr**	+	+	+	+	-
LAB11	**24hr**	+	+	+	+	-
LAB12	**24hr**	+	+	+	-	-

- = No growth occur, + = Growth occur.

NaCl Tolerance

Table No. 3: NaCl tolerance assay

Isolated Org.	NaCl Concentration					
	0.5%	1%	2%	3%	4%	5%
LAB6	+++	+++	+++	+++	+++	++
LAB7	+++	++	++	+	-	-
LAB10	++	++	+	+	-	-
LAB11	+++	++	+	+	-	-
LAB12	+++	++	+	+	+	-

- = No growth, + = Less growth, ++ = good growth, +++ = Very good growth.

The result of NaCl tolerance presented in Table no.3. Determination of NaCl tolerance of Lactic Acid Bacteria was investigated for NaCl tolerance upto 0.5% -5% in MRS broth. After 24 hrs, initially the growth was recorded in low salt concentration containing MRS broth. Among five

isolates, Only LAB6 isolate could grow up to high salt concentration of 5%. Although the growth of LAB12 was recorded less but this isolate could grow up to 4% NaCl. Isolates LAB7, LAB10 and LAB11 could grow up to 3% NaCl.

Antibiotic resistance

Resistance was defined according to the standard Kirby-Bauer disc diffusion method by using antibiotic discs of Chloramphenicol, Penicillin, Ampicillin, Amoxyclav, Cefotaxime, Co-Trimoxazole, Gentamycin, Tobramycin, Ceftriaxone, Ciprofloxacin, Tetracycline, Gentamycin, Linezolid, Streptomycin, Vancomycin, Clindamycin, Erythromycin, Aztreonam, Cefpodoxime, Cefpodoxime/Clavulanic acid, Ceftazidime, Cefotaxime. The diameter of zone of inhibition was measured in millimetres. Among two isolates, results showed that isolate LAB6 was resistant to above mentioned antibiotics. Isolated organism LAB12 was resistant to above mentioned antibiotics except CPD-Cefpodoxime and CCL- Cefpodoxime/Clavulanic acid. It indicates that isolate LAB6 was able to applicable as probiotics.

CONCLUSION:

Among 12 isolates, isolate LAB6 from curd sample gave catalase test negative, grown at pH 2 and 3, able to tolerate bile salt concentration up to 4% and NaCl concentration up to 5%. LAB6 isolate was also resistant to all antibiotics tested above. So, LAB6 isolate was considered to be suitable for probiotic applications.

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