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THE DETERMINATION OF PREDOMINANT PROBIOTIC LATIC ACID BACTERIA IN COMMERICIAL CURDS

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Abstract

The significance of Probiotics for the betterment of human health has been well understood by many especially after the pandemic due to covid19. The present study has been conducted to analyze the predominant lactic acid probiotics present in commercial curd varieties. Seven brands were selected and compared with Aavin curd and examined the differences. The selective media MRS was used and standard plate technique was employed to find out viable count that means live microorganisms that have beneficial effects on their host's health. The medical world has long been interested in nutrient properties of curd. Curd is commonly used fermented milk product in India since time immemorial. In this study lactic acid bacteria were isolated from curd and their probiotic potential was investigated. There were two predominant lactic acid fermenting bacteria and also Saccharomyces sps. were isolated and identified in Aavin. They were phenotypically characterized and identified as Lactobacillus lactis, Leuconostoc mesenteroides and the yeast Saccharomyces cerevisiae.

KeyWords:*Curd, Probiotics, Lactic Acid Bacteria,*

1. Introduction

Lactic acid bacteria one of the major probiotics have been isolated from different sources including dairy products, sewage, plants, human and animal. The health care sector has long been very much interested in the nutrient properties as well as therapeutic benefits of curd. Lactic acid fermenting bacteria (LAB) are usually associated with fermented dairy products namely curd, yoghurt, kefir, koumis, cheese, buttermilk, etc. Curd is a very common food item available in every common people and a fermented milk product which is also good source of lactic acid bacteria. The probiotics present in curd especially LAB have been greatly accepted by FDA. It has been well documented in online free dictionary.com defined curd as 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. Curd is also cheap and easily available source (Ghosh*et al.,*2011).

LAB are group of Gram-positive cocci and rods, catalase negative occurring naturally in variety of niches (Hammes and Hertel, 2006; Mohania *et al.,* 2008). Probiotics are defined as "Live microorganisms when administrated in adequateamountsconferahealthbenefitonhost"(FAO/WHO,2011).Mostprobioticsavailabletodaybelongtogenera*Lactobacillus* and *Bifidobacterium*. LAB are most important group of microorganisms used in food fermentation, they contribute to the fast and texture of fermented products and inhibit food spoilage and pathogenic bacteria by producing antimicrobial substances (lactic acid, hydrogen peroxide, bacteriocin) (Phillip *et al* ., 2012). Several mechanisms by which probiotics mediates their health benefits on the host have been suggested, and can be divided into three categories:
(i) certain probiotics have antibacterial activity and can exclude or inhibit pathogens; (ii) probiotics bacteria can enhance the intestinal epithelial barrier; (iii) probiotics bacteria are believed to modulate host immune response (Ezendam and Loveren, 2006; Marco *et al.,* 2006; Lebeer *et al.,* 2008; Lebeer *et al.,* 2010). To perform their effect in the intestineprobiotics bacteria should be capable of surviving passage through gastro intestinal tract (GIT).Thus it is essential for bacteria to have protection systems to withstand the low pH in the stomach, digestive enzymes and bile of the small intestine (Cotter and Hill, 2003; Jensen *et al.,* 2012). LAB were successfully isolated from curd samples (Ghosh *et al.,*2011).The aim of this study was to isolate lactic acid bacteria from curd and to determine their probiotics potential.

2. Materials and Methods

Isolation of Lactic Acid Bacteria from Curd

Commercial curds were procured from market and lactic acid bacteria were isolated after the ten fold dilution of the sample using sterile distilled water. After the dilution 10-3 to 10-6 were selected for the pour plate method to favour the growth of the facultative anaerobic lactic acid bacteria. The selective media Mann Rogosa Sharpe(MRS)agar was used for the pour plate method and the plates were incubatedat37°Cfor2days.Theisolatedcoloniesweretransferredto MRS broth and purified by streaking twice on MRS agar plate (Mahantesh *et al*., 2010). Gram staining was performed as described by Rakesh J. Patel (2008). Catalase test was performed by streaking MRS slant in the test tube by each of isolates. Slants were incubated for 37°C for 2 days. After incubation 3% H_2O_2 was added in that slants and slants were observed for effervescence formation to indicate evolution of oxygen. Gram-positive and catalase negative isolates were taken as lactic acid bacteria (Rasha *et al.,*2012). Gram positive and catalase negative isolates were preserved on MRS

agar slant in culture tube and stored at 4°C.Sub culturing was carried out after every15days.

Probiotic Properties of Isolates

- **Resistant to Low pH**

Being resistant to low pH is one of the major selection criteria for probiotics strains. It is often used *in vitro* assays to determine resistance to stomach pH. For this purpose active cultures were used. Cells were harvested by centrifugation. Pellets were washed once in phosphate buffer saline (PBS)(pH7.2).Then cell pellets were resuspended in PBS (pH 3) and incubated at 37°C. After 0,1,2,3 h viable inoculations was carried out in MRS broth. These MRS broths were incubated at 37°C for 48handgrowth was monitored afterincubationat$OD_{620.}$

- **Tolerance against Bile Salts**

The mean intestinal bile salt concentration is believed to be 0.3% and staying time of food is suggested to be 4 h. The experiment was applied at this concentration of bile for 4 h. For this purpose active cultures were used. Cells were harvested by centrifugation and MRS broth containing 0.3% bile salts were added to pellets. During incubation of 4 h, at every hour inoculations were carried out in to MRS broths and they were incubated at 37°C for 48 h and growth was monitored after incubation at $OD_{620.}$

- **Antibacterial Activity**

Antibacterial activity was determined against: *Escherichiacoli, Salmonellatyphi, Salmonella paratyphiA, Salmonella paratyphi B, Pseudomonas aerugenosa & Staphylococcus aureus,*

All of pathogens were incubated for 48 h at 37°C. After incubation cells were removed by centrifugation and pH of supernatants were set 6.5 and it was filtered through 0.22µm filter to obtain cell free supernatants (CFS). This CFS is used as antimicrobial agent using agar well diffusion method. Antimicrobial activity was evaluated by measuring zone of inhibition against the test organism.

Arginine Hydrolysis Test

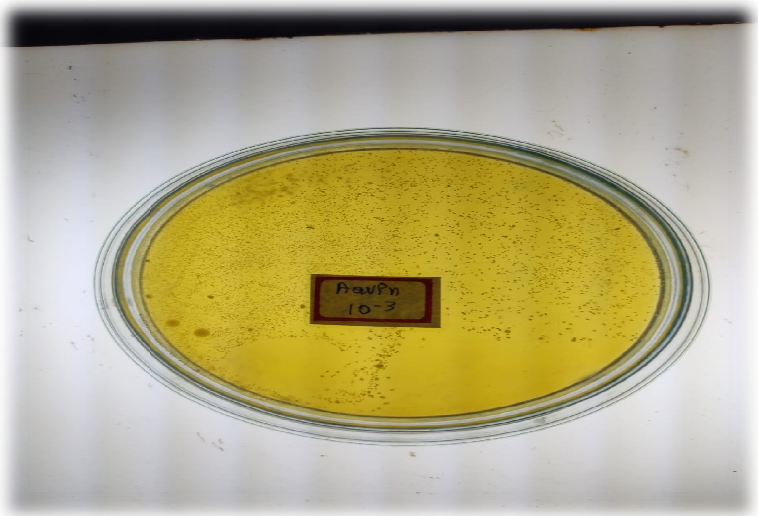
For this purpose base MRS broth without glucose and meat extract containing 0.3% arginine and 0.2% sodium citrate instead of ammonium citrate was used. This medium was transferred into tubes as 5mL and inoculated with active cultures. Tubes were incubated at 37°C for 24 h. After incubation, 100µL of inoculated culture was transferred on to white background and equal volume of Nessler's reagent was pippeted on cultures. Bright orange color indicates positive reactions while yellow indicates negative reaction.

Growth at different Temperatures and NaCl Concentrations

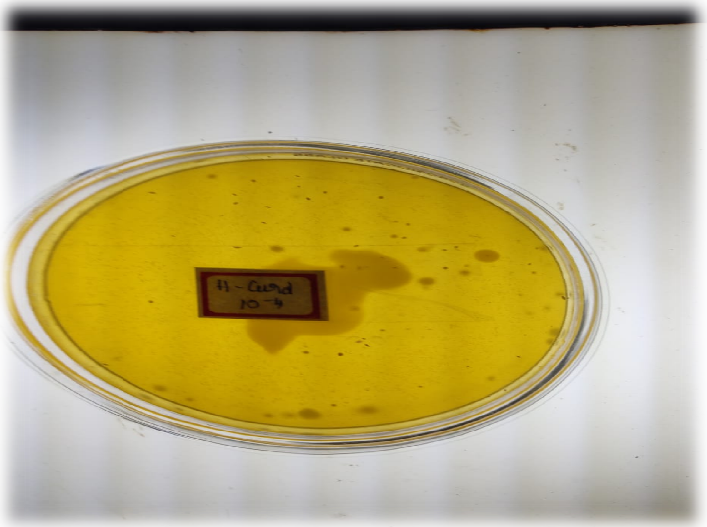
Growth at different temperature such as 10°C, 15°C, 30°C, 37°C and 45°C were checked. Growth at different NaClconcentrationssuchas2%, 3%, 4%, 5%were also checked.

3. Results And Discussion

AAVIN[10-3 DILUTION]



H-CURD[10-4 DILUTION]



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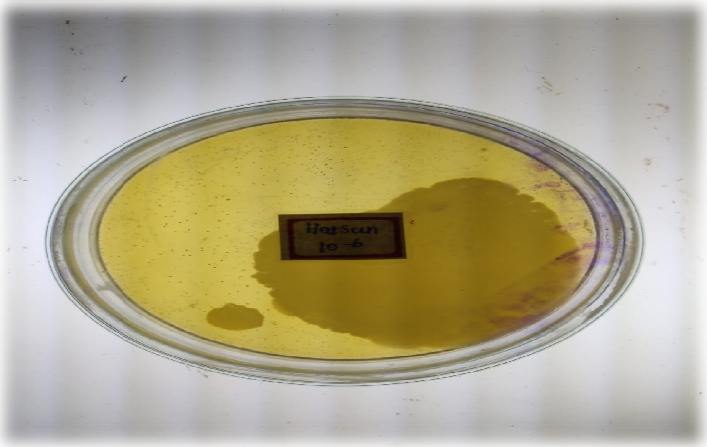

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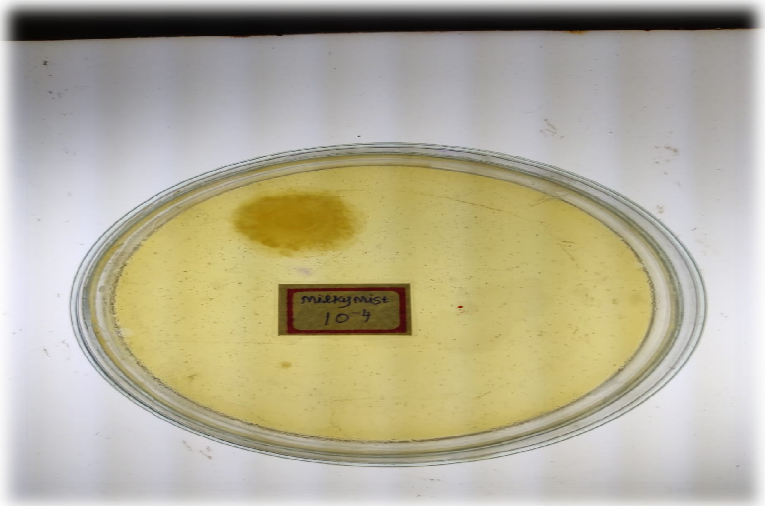
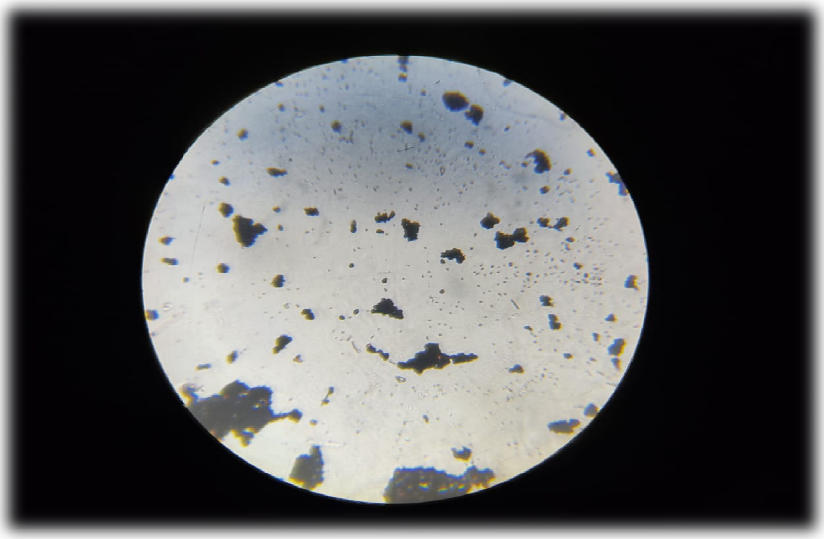
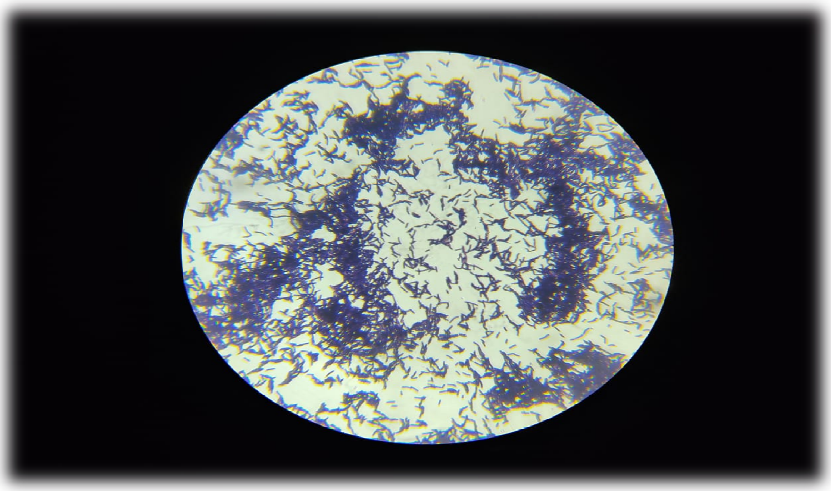
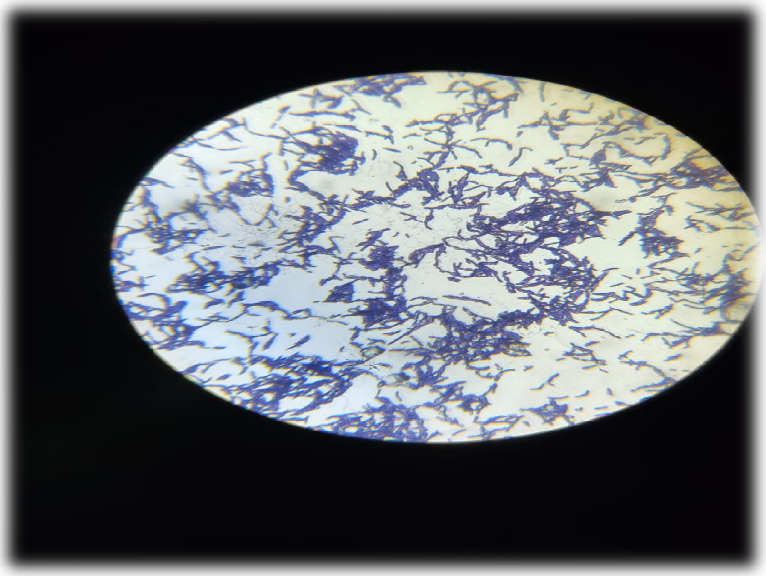
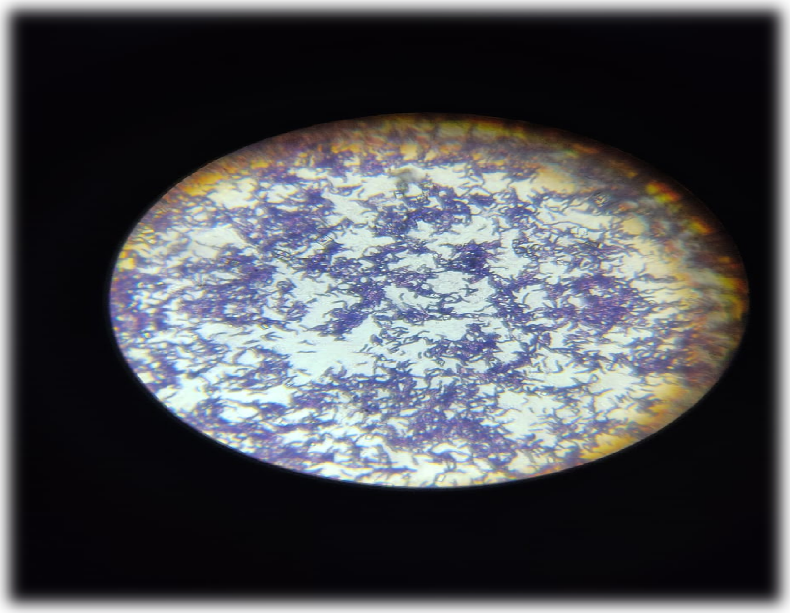
MILKYMIST[10-4 DILUTION]











Isolation of Lactic Acid Bacteria from Curd

Total 4 isolates were obtained from which only 2 were catalase negative. Both the organisms were found to be gram positive cocci. Hence two lactic acid bacteria were isolated. They were given names as strain and strain 2.

Staining results

Names of the test	Gram staining	Brand 1	Brand 2	Brand 3	Brand 4	Brand 5	Brand 6	Brand 7
Strain 1	Gram positive cocci	Absent	Absent	absent	absent	absent	absent	Present
Strain 2	Gram positive rods	Present	Present	present	present	present	present	Present

Biochemical results for Gram Positive cocci

Test	Results
Esculin hydrolysis	Positive
Acid production from raffinose	Positive
Melibiose	Positive
Arabinose	Positive
Trehalose	Positive
Growth in 6.5% Nacl	Positive
Growth in 5% sucrose	Positive
Extracellular matrix production	Positive

Basic Characteristics	**Properties (*Lactobacillus* spp.)**
Capsule	Negative (-ve)
Catalase	Negative (-ve)
Citrate	Negative (-ve)
Flagella	If present, peritrichous
Gas	Negative (-ve)
Gelatin Hydrolysis	Negative (-ve)
Gram Staining	Gram positive
H2S	Negative (-ve)
Indole	Negative (-ve)
Motility	Mostly Negative (-ve)
MR (Methyl Red)	Negative (-ve)
Nitrate Reduction	Negative (-ve)
Oxidase	Negative (-ve)

ProbioticPropertiesofIsolates

- **Resistantto LowpH**

Accordingtothisexperiment boththeisolates werefoundresistanttopH3.Resultswereshownasgraph(Figure1).

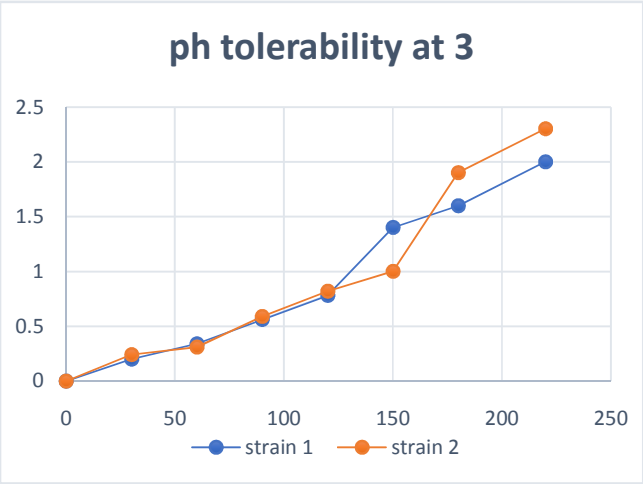


Figure 1: Survival in pH 3-OD_{620}

Values Survival rate of c1was higher than c2 at pH3.

- Tolerance against Bile Salts

According to this experiment both the isolates were found resistant to0.3%bilesaltsconcentration.Resultswereshownasgraph (Figure 2)

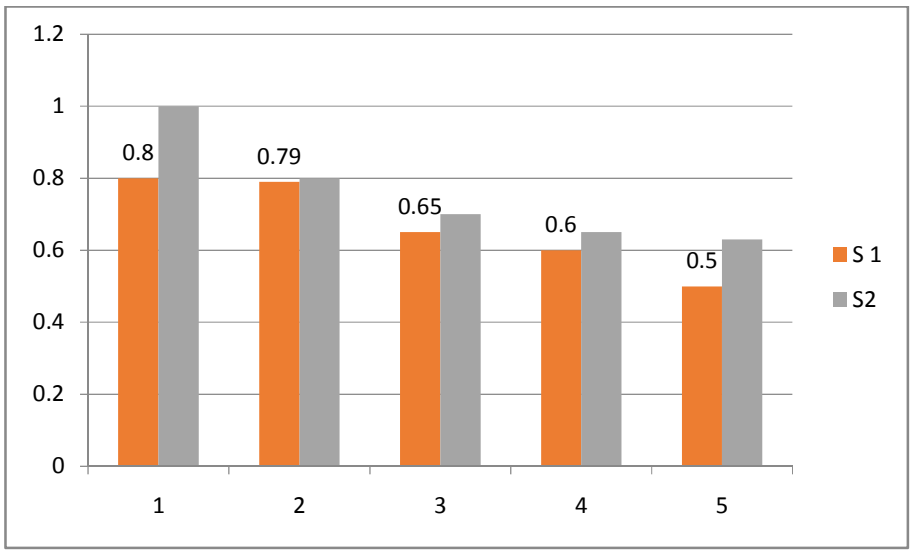


Figure 2: Survival in 0.3%Bile salts-OD_{620}

Values Survival rate of strain 1 was higher than strain 2 at 0.3% bile salts concentrations.

- **Antibacterial Activity**

Both the strains have shown activity against five strains but they did not show any activity against *Bacillus subtilis and Bacillus megaterium.*

The diameters of zone of inhibitions were measured. They are shown as follows:

Table1:Antibacterial Activity

TestOrganisms	Diameter of inhibitionzones(mm)	
	Strain1	Strain 2
Escherichiacoli	12	25
Salmonella typhi	12	12
Salmonella paratyphiA	13	11
Salmonella paratyphiB	18	19
Pseudomonasaeruginosa	16	10

Arginine Hydrolysis Test

Both the isolates were found arginine positive because they have shown bright orange color against white back ground.

Growth at different Temperatures and NaCl concentrations

Both isolates were unable to grow at 10°C and 15°C. strain 1was unable to grow at 45°C while strain 2 was growing at this temperature.

Table2:Growth at different Temperature

Temperature(°C)	10	15	30	37	45
Strain 1	-	-	+	+	-
Strain 2	-	-	+	+	+

Both the isolates were growing at 2%, 3% and 4%NaCl concentration. But strain 1 was unable to grow at 5% NaCl concentrations while strain 2 was growing at this concentration.

Table3:Growth at different NaCl concentrations

NaClconcentrations(%)	2	3	4	5
Strain 1	+	+	+	-
Strain 2	+	+	+	+

4. Conclusion And Future Perspective

Lactic acid bacteria were successfully isolated from curd. Probiotic activities of both the isolates were determined. Both of them showed resistant to low pH, tolerance against bile salts and antimicrobial activity against test microorganisms. Thus main criteria of being probiotics strains were determined. Though all the examined curd contain lactic acid bacteria, only one brand was having three kinds of probiotics such as *Lactobacillus lactis, Leuconostoc mesenteroides and Saccharomyces cerevisiae*

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