



DETERMINATION OF PROBIOTIC PROPERTIES OF LACTIC ACID BACTERIA FROM CURD

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Abstract

Probiotics mean 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. Total 2 lactic acid bacteria were isolated on the basis of catalase test and gram staining. Both the isolates were gram positive cocci and catalase negative. Both of them showed a great probiotic potential. Both the organisms were found resistant to low pH (i.e. pH 3) as well as 0.3% bile salts concentrations. It was observed that they were having antibacterial activity against eight test organisms. However, they were unable to show antibacterial activity against *B. subtilis*, and *B. megaterium*.

Key Words: Curd, Probiotics, Lactic Acid Bacteria, Bile Salts, Antibacterial Activities.

1. Introduction

Lactic acid bacteria have been isolated from different sources such as dairy products, sewage, plants, human and animal. The medical world has long been interested in the nutrient properties of curd. Lactic acid bacteria (LAB) are commonly associated with fermented dairy products such as cheese, buttermilk, curd, sreekhand etc. Curd is fermented milk product which is also good source of lactic acid bacteria. The curd bacteria especially LAB have been well accepted as GRAS (generally regarded as safe). The 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 adequate amounts confer a health benefit on host" (FAO/WHO, 2011). Most probiotics available today belong to genera *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 in to 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 intestine probiotics 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

For isolating lactic acid bacteria curd sample was suspended, appropriately diluted in sterile normal saline, spread plated on de Mann Rogosa Sharpe (MRS) agar and incubated at 37°C for 2 days. The isolated colonies were transferred to 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% H₂O₂ was added in that slants and slants were observed for gas production. 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 every 15 days.

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) (pH 7.2). 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 48 h and growth was monitored after incubation at OD₆₂₀.

- 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 OD₆₂₀.

- Antibacterial Activity

Antibacterial activity was determined against: *Escherichia coli*, *Salmonella typhi*, *Salmonella paratyphi A*, *Salmonella paratyphi B*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Micrococcus luteus*, *Serratia marcescens*, *Bacillus subtilis*, *Bacillus megaterium*.

All of LAB isolates 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 onto white background and equal volume of Nessler's reagent was pipetted 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 NaCl concentrations such as 2%, 3%, 4%, 6.5% were also checked.

3. Results And Discussion

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 2 LAB were isolated. They were given names as c1 (strain 1) and c2 (strain 2).

Probiotic Properties of Isolates

- Resistant to Low pH

According to this experiment both the isolates were found resistant to pH 3. Results were shown as graph (Figure 1).

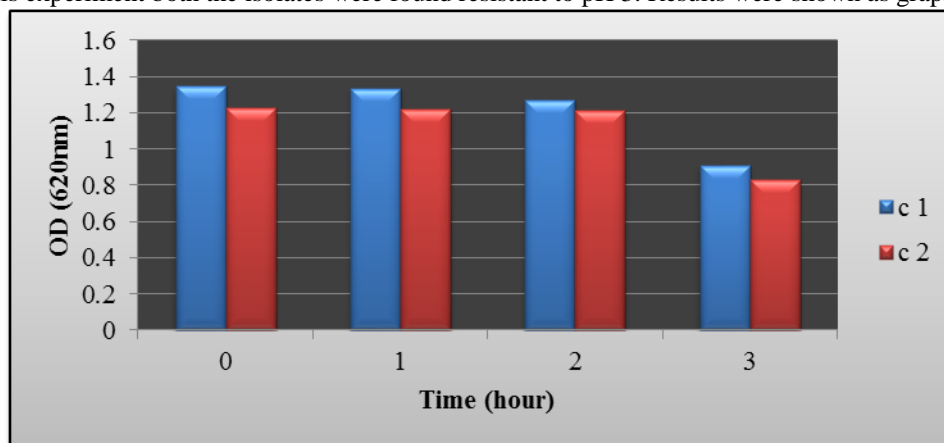
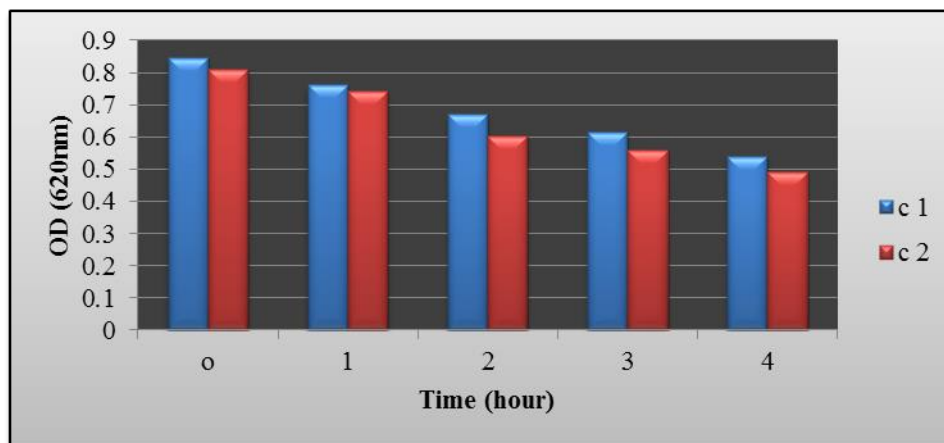


Figure 1: Survival in pH 3-OD₆₂₀ values

Survival rate of c1 was higher than c2 at pH 3.

- Tolerance against Bile Salts

According to this experiment both the isolates were found resistant to 0.3% bile salts concentration. Results were shown as graph (Figure 2).

Figure 2: Survival in 0.3% Bile salts-OD₆₂₀ values

Survival rate of c1 was higher than c2 at 0.3% bile salts concentrations.

- Antibacterial Activity

Both the strains have shown activity against eight 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:

Table 1: Antibacterial Activity

Test Organisms	Diameter of inhibition zones (mm)	
	c1	c2
<i>Escherichia coli</i>	12	25
<i>Salmonella typhi</i>	10	12
<i>Salmonella paratyphi A</i>	13	11
<i>Salmonella paratyphi B</i>	18	19
<i>Pseudomonas aeruginosa</i>	16	10
<i>Bacillus subtilis</i>	-	-
<i>Bacillus megaterium</i>	-	-
<i>Staphylococcus aureus</i>	22	13
<i>Micrococcus leuteus</i>	13	15
<i>Serratia marcescens</i>	8	15

Arginine Hydrolysis Test

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

Growth at different Temperatures and NaCl concentrations

Both isolates were unable to grow at 10°C and 15°C. c1 was unable to grow at 45°C while c2 was growing at this temperature.

Table 2: Growth at different Temperature

Temperature (°C)	10	15	30	37	45
c1	-	-	+	+	-
c2	-	-	+	+	+

Both the isolates were growing at 2%, 3% and 4% NaCl concentration. But c1 was unable to grow at 6.5% NaCl concentrations while c2 was growing at this concentration.

Table 3: Growth at different NaCl concentrations

NaCl concentrations (%)	2	3	4	6.5
c1	+	+	+	-
c2	+	+	+	+

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.

Therefore some further studies should be performed to use these isolates reliably. It would be beneficial to test following characteristics: (i) molecular identification of isolates; (ii) adhesion to mucosal surfaces; (iii) clinical studies for human health; (iv) antibiotic resistance; (v) technological properties (strain stability, viability in to products, bacteriophage resistance).

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