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Characterization of lactic cultures from dahi samples

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Abstract
Microorganisms known as starter bacteria converts milk sugars into lactic acid. They are essential to the manufacturing of fermented dairy products such cheese, yoghurt, acidophilus milk, and dahi. From these goods, various organisms are listed and separated. In current study domestic and market samples of Dahi were collected and analysed for the different isolates. The load of Lactococci ranged from 0 to 6.45 $\log_{10}$ cfu/g. The data indicates that the counts of *Streptococci* and *Leuconostoc* varied from 0 to 4.70 $\log_{10}$ cfu/ml and 4.50 $\log_{10}$ cfu/g, respectively, while the counts of Lactobacilli were found to be between 0 and 5.50 $\log_{10}$ cfu/g in the dahi samples. When total lactic acid bacteria were counted on (NRCLA) Neutral Red Chalk Lactose Agar, (YGA) Yeast Glucose Agar, (SA) Sucrose Agar and (RA) Rogosa Agar, the results ranged from 6.32 to 7.04 $\log_{10}$ cfu/g in the dahi samples D1 through D10.In this study, isolates of 62 different species were recovered; 9 Lactococci, 28 Lactobacilli, 18 *Leuconostoc*, and 7 *Streptococci* were found.

Keywords: Lactic acid bacteria, lactose, fermentation starter cultures, Dahi, yogurt, acidophilus milk and cheese

Introduction
There are microorganisms in soil, water, and the air. Given their importance, they have been divided into categories labelled as dangerous and useful. While healthy bacteria like LAB are helpful in the manufacture of different fermented foods like dahi, yoghurt, batter, sauerkraut, etc., harmful bacteria can lead to deterioration or infections.

LAB are facultative anaerobes, catalase negative, gram positive, and acid tolerant. The function of LAB is to produce lactic acid, flavour, and moderate proteolytic and lipolytic alterations that contribute to the product's flavour, body, and texture. Additionally, starter bacteria inhibit the growth of spoilage species by producing bacteriocins and acids.

These need to be enumerated in fermented milk products to know the quality of final product and its use in further product preparation. Hence the attempt was made in such view and the obtained results were discussed in this paper.

Curd is high in protein and calcium. Other advantages include that it is a great source of milk protein, which satisfies an adult's minimum daily requirement for protein, and it is easily digestible because the majority of the protein is already broken down (Sarkar *et al.*, 1996) [17]. Made from mixed milk, curd is a fermented milk product in which starter bacteria have partially broken down lactose into lactic acid. Since 60% of the lactose in curd is fermented and the protein is partially hydrolyzed, it has the same nutritional content as the milk it is made from and is more easily digested than milk (Awan and Rahman, 2002) [2].

Lactococcus lactis ssp. lactis, *Lactococcus lactis ssp.cremoris*, *Lactococcus lactis ssp. diacetylactis*, *Streptococcus thermophillus*, *Lactobacillus delbrueckii ssp. bulgaricus*, *Lactobacillus helveticus*, *Lactobacillus plantarum*, lactose fermenting yeast, and others are among the heterogeneous microflora of dahi, according to Shekar and Mariappan (2007) [8].

Certain starter bacteria species, such as *Lactobacillus acidophillus*, *Lactobacillus casei*, and *Bifidobacterium bifidum*, may have nutritional advantages. The health benefits of starter bacteria include enhanced lactose digestion, reduced intestinal infections, better nutritional content from fermented milk, prevention of colon cancer, and lower risk of cardiovascular disease by controlling serum cholesterol levels. The development and activity of starter cultures, which are used to produce cultured foods like yoghurt and dahi, is what causes these advantages. The process of making fermented milks involves using starter cultures to quickly start the acidity of heated and cooled milk (Ali, 2010) [1].

According to Khurana and Kanawjia (2007) [12], curd is a traditional fermented milk product from India that has maintained its appeal in the country's cuisine despite changes in eating habits and lifestyle.

After plating a well-diluted (105) 0.1 ml sample on de Mann Rogosa Sharpe agar (MRS) and incubating anaerobically in an anaerobic jar at 35 °C for 48 hours, starter bacteria were counted from dahi samples (Esayas *et al.*, 2003) [10]. Cheriguene *et al.* (2007) [5] stated that LAB from goat's milk were plated and cultured anaerobically using MRS agar for *Leuconostoc* and Pediococci, Rogosa agar for Lactobacilli, and M17 agar for Lactococci. By utilising Neutral Red Chalk Lactose Agar, Sucrose Agar, and Rogosa Agar in a selective plating process, the starter bacterial count from curd revealed log counts of 7.87, 3.20, and 5.45 lactobacilli per gram (Pradeep, 2007) [13].

Ten colonies were chosen at random from countable MRS agar plates after Esayas *et al.* (2003) [10] recovered LAB from dahi samples. Prior to characterization, the colonies were streaked on the proper agar media (MRS) several times to purify them. Each plate's five colonies, each with a unique morphology, were put in MRS broth, incubated for roughly 12 hours, and then kept in a refrigerator at 4 °C. After analysing the isolates' colony and cell morphologies, Gram's reaction, catalase reaction, and gas generation from glucose fermentation, they were classified as lactic acid bacteria. There is a lengthy history of using starter microorganisms in meals. Their safety is attested to by the fact that they are found in the intestinal epithelium of the human gastrointestinal tract and have long been used in dairy products and fermented meals that have a number of health benefits. Lactic acid bacteria naturally occur in milk, fruits, and vegetables, particularly in those that naturally ferment (Breidt, 2007) [4].

75 isolates of the Lactobacillus genus were identified by Subramanian and Pavan Kumarora (2007) [16] from 150 samples of buffalo milk that were gathered from various sources throughout the year. By putting these identified isolates through a battery of common physiological and biochemical testing, species level identification was achieved. Therefore, it was determined which Lactobacillus species were present, including *Lactobacillus casei* (21.05%), *Lactobacillus plantarum* (24.21%), *Lactobacillus acidophilus* (10.53%), *Lactobacillus bulgaricus* (15.79%), *Lactobacillus lactis* (12.63%), and *Lactobacillus helveticus* (15.79%). Ten raw milk samples included 65 different *Leuconostoc* species of dairy origin that were isolated. Every isolate's physiological and biochemical characteristics were examined and described. *Leuconostoc dextranicum*, *Leuconostoc mesenteroides*, *Leuconostoc paramesenteroides*, *and Leuconostoc lactis* were the species that were identified (Subramanian and Pavan kumar Ora, 2007) [16].

The lactic acid bacteria that were recovered from commercial yoghurt beverages were examined and described by Sang *et al.* (2007) [14]. Using an API 50 CHL kit, all of the isolated organisms were identified as *Lactobacillus paracasei*, *Lactobacillus helveticus*, *Lactobacillus casei ssp. casei*, and *Lactobacillus paracasei.*

Jayalalitha *et al.* (2009) [7] gathered 35 raw milk samples from various parts of Chennai. Used samples of raw milk to separate and measure the presence of lactobacilli. It was discovered that the MRS agar and LBS agar with streak plate approach worked well for separating Lactobacillus from raw milk. A total of 46.15% of isolates were found, of which 18.6% were Lactobacillus acidophilus and 27.55% were *Lactobacillus delbrueckii ssp. bulgaricus*.

35 curd samples were gathered from Rawalpindi's local market, and 69 lactic acid bacteria were found. Of the 69 isolates, 26 percent were Lactobacillus delbrueckii ssp. bulgaricus, 22 percent were *Lactobacillus thermophillus*, 16 percent were *Lactobacillus acidophillus*, and 9 percent were *Lactococcus lactis ssp lactis* (Mehmood *et al.*, 2009) [6].

Forouhandeh *et al.* (2010) [8] conducted a characterization study and found that 37 Lactobacilli isolates were found in various traditional and local cheeses and yoghurts from the Besmenj zone in Iran. According to Bergey's Manual of Systematic Bacteriology, of the 37 isolates, 69% were *Lactobacillus rhamnosus*, 15% were *Lactobacillus paracasei*, and 16% were *Lactobacillus fermentum*. Mahantesh *et al.* (2010) [9] investigated and described the isolated starter bacteria using biochemical and molecular techniques derived from the curd and cucumber samples. *Weissella*, *Pediococci*, and *Lactobacilli* were the genera to which these isolates were classified.

Materials and Methods

Enumeration and Isolation of bacteria producing lactic acid

Curd samples from the market and the home were gathered in sterile sample bottles. 11g of the sample were weighed individually under aseptic conditions on sterile aluminium foil. They were then transferred to a 99ml diluent bottle and combined while spinning on a work bench to obtain a 10-1 dilution. Using this, the necessary dilutions were made. Transferring the appropriate dilutions to four sets of Petri plates was done. Melted Neutral Red Chalk Lactose Agar kept at 50 °C was added to the first set of sterile Petri plates; Yeast Glucose Agar was added to the second set; Sucrose Agar was added to the third set; and Rogosa Agar was added to the fourth set. The mixture was well mixed and allowed to solidify. Using sterile pipettes, the first dilution was lab pasteurised at 63 °C for 30 minutes, cooled to room temperature, and then the necessary dilutions were made. One millilitre of each required dilution was then transferred to labelled Petri plates, where sterile molten YGA kept at 44–46 °C was poured, thoroughly mixed, and incubated at 37 °C. Plates of Neutral Red Chalk Lactose Agar and Sucrose Agar were incubated for 24 to 48 hours at 30 °C in an anaerobic jar. For 24 to 48 hours, yeast glucose agar and rogosa agar plates were incubated at 37 °C in an anaerobic jar. Following incubation, all of the pink-colored colonies on NRCLA plates, all of the glistening colonies on Sucrose Agar, all of the colonies on YGA plates, and all of the colonies on Rogosa Agar were counted. By multiplying the average count by the dilution factor, the counts were expressed as colony forming units per gram (cfu/g), which were subsequently translated into $\log_{10}$ cfu/g of material. Harrison's disc was used to determine which colonies to choose. The isolates were kept in Yeast Glucose Broth and Stabs containing 0.75% Agar after being streaked on YGA three times to achieve purification.

Results and Discussion

The number of LAB from Bengaluru's domestic and market curd samples was tallied, and information on the curd samples were recorded (Table 1). Neutral Red Chalk Lactose Agar for Lactococci, Sucrose Agar for *Leuconostoc*, Rogosa Agar for Lactobacilli, and Yeast Glucose Agar for Total LAB count and *Streptococci* were used to analyse and count the samples

from both the domestic and market markets.

The range of Lactococci log counts was 0 to 6.45. *Leuconostoc* live counts ranged from 0 to 4.50, and *Streptococci* log counts ranged from 0 to 4.70, while lactobacilli log counts varied from 0 to 5.50 log_{10} cfu/g of curd samples. Dahi samples D1 through D10 had total LAB viable counts that varied from 6.32 to 7.04. Additionally, Pradeep (2007) [13] discovered that when lactic acid bacteria were counted from curd, viable log counts of *Leuconostoc*, lactobacilli, and lactococci were 7.87, 3.20, and 5.45, respectively. Kahala *et al*. (2008) [11] described the aroma-producing *Lactococcus lactis* ssp diacetylactis and *Leuconostoc mesentroide* sssp. *mesenyteroidesas* starters from the Villi, as well as *Lactococcus lactis ssp. lactis* and *Lactococcus lactis ssp. cremoris*.

72 isolated, purified lactic acid bacteria were recovered from Dahi samples, and they underwent a battery of preliminary tests, including the litmus milk response, catalase test, Gram's staining, and simple staining. The 72 lactic isolates were all Gramme positive, catalase negative, and showed signs of curdling milk and reducing litmus, indicating that they were lactic isolates. The isolates were recognised to the genus level by these preliminary testing. According to Borpuzari *et al.* (2007) [3], out of the 273 lactobacilli strains found in market sour curd samples, 10 strains of Lactobacillus acidophilus were found.

Since none of the nine lactococcal isolates from Dahi samples produced CO_2 from glucose, they were all homofermentative. In order to identify the species, these isolates underwent a series of biochemical tests, including growth at 40^0 and 50 °C, growth in 4% NaCl, diacetyl in milk, and ammonia from arginine. Two of the isolates were recognised as *L. lactiss sp. cremoris*, and seven of the isolates were identified as L. lactiss sp. lactis based on the results of biochemical assays (Table 2).

The seven *S. thermophilus* isolates that were isolated from curd by selective plating and lab pasteurisation were homofermentative because, unlike heterofermentative isolates, they did not release CO_2 from glucose (Table 3). The isolates fermented lactose and glucose and grew at 45 °C.

The entire set of eighteen *Leuconostoc* isolates was chosen because they were heterofermentative and could produce CO_2 from glucose. They underwent a battery of biochemical tests, including dextran from Sucrose Agar, arabinose and sucrose fermentation, survival at 55 °C for thirty minutes, and growth in 3% and 6.5% NaCl to identify the species. Based on the results of the biochemical test, only 4 out of the 18 isolates were identified as L. lactis; the other 14 isolates remained unidentified since the results of the biochemical tests did not match the *Leuconostoc* identification key (Table 4).

Of the 38 lactobacilli isolates, 32 were heterofermentative—producing CO_2 while using glucose—while the remaining 6 isolates were homofermentative—not producing CO_2 from glucose. These isolates underwent a battery of specialised biochemical tests, including growth at 450 °C and 150 °C, ammonia extraction from arginine, and fermentation of salicin and mellibiose to identify the species. 22 isolates were recognised as *L. fermentum*, 10 isolates as *L. Hilgardii*, and the remaining 6 isolates as L. acidophilus based on the outcomes of particular biochemical assays. Soomro *et al.* (2007) [15] discovered that, out of 160 strains of lactic acid bacteria from market dahi acquired from Rawalpindi, abundant species of *L. delbrueckii* ssp. bulgaricus (28 isolates) followed by *S. thermophilus* (20 isolates).

Table 1: Enumeration of Lactococci, *Streptococcus thermophilus*, *Leuconostoc* and Lactobacilli from Domestic and Market Dahi samples

Sample	Total lactic acid bacterial count	Lactococci	*S. thermophilus*	*Leuconostoc*	Lactobacilli
Domestic sample	**Count (log_{10} cfu/g)**				
D_1	6.44	4.44	-	2.00	-
D_2	6.32	4.22	-	-	2.10
D_3	7.04	6.25	-	-	-
D_4	7.04	6.32	-	2.48	5.14
D_5	6.69	6.38	-	-	5.50
Market samples	**Count (log_{10} cfu/g)**				
D_6	6.41	5.36	-	-	3.40
D_7	6.45	6.00	-	4.50	4.66
D_8	6.70	-	-	-	3.50
D_9	6.80	5.80	4.70	-	-
D_{10}	6.50	2.00	3.00	1.50	4.10

Note: Total Lactic Acid Bacterial Count – YGA plates incubated at 37 °C/24-48hrs
Lactococci-NRCLA plates incubated at 30 °C/24-48hrs
Leuconostoc-Sucrose Agar plates incubated at 30 °C/24-48hrs
Lactobacilli-Rogosa Agar plates incubated at 37 °C/24-48hrs
Streptococcus thermophillus -I dilution was pasteurized, plated using YGA & incubated at 37 °C/24-48hrs
All the plates were incubated in anaerobic jar.

Table 2: Identification of lactococcal isolates obtained from Dahi samples

Isolate No.	Growth at		Growth in 4% NaCl	Diacetyl in milk	Ammonia from arginine	Identity
	40 °C	50 °C				
Lc1, Lc2, Lc4, Lc5, Lc6, Lc7, Lc9 (7 numbers)	+	-	+	-	+	*L .lactis ssp. lactis*
Lc3, Lc8 (2 numbers)	-	-	-	-	-	*L. lactis ssp. cremoris*

Note: All these isolates were Gram positive cocci, Catalase negative, ARC in Litmus milk, No CO_2 produced from glucose

Table 3: Identification of *Streptococcus thermophilus* isolates obtained from Dahi samples

Isolate No.	Growth at 45 °C	Growth at 6.5% salt	Fermentation of Sugar		Ammonia from arginine	Identity
			Glucose	Lactose		
St1, St2, St3, St4, St5, St6, St7 (7 numbers)	+	-	+	+	-	*S. thermophilus*

Note: All these isolates were Gram positive cocci, Catalase negative, ARC in Litmus milk, No CO_2 produced from glucose

Table 4: Identification of *Leuconostoc* isolates obtained from Dahi samples

Isolate No.	Dextran from sucrose	Acid from sugar		Survival at 55 °C/30 min	Growth in		Identity
		Arabinose	Sucrose		3% Nacl	6.5% Nacl	
Leu 1, Leu 2, Leu 3, Leu 4 (4 numbers)	-	-	+	+	+	-	*Leuconosto lactis*
Leu 5, Leu 6, Leu 7, Leu 8, Leu 9, Leu 10 (6 numbers)	-	+	-	+	+	+	Unidentified
Leu 11, Leu 12, Leu 13, Leu 14 (4 numbers)	-	-	+	-	-	-	
Leu 15, Leu 16, Leu 17, Leu1 8 (4 numbers)	-	+	-	+	+	+	

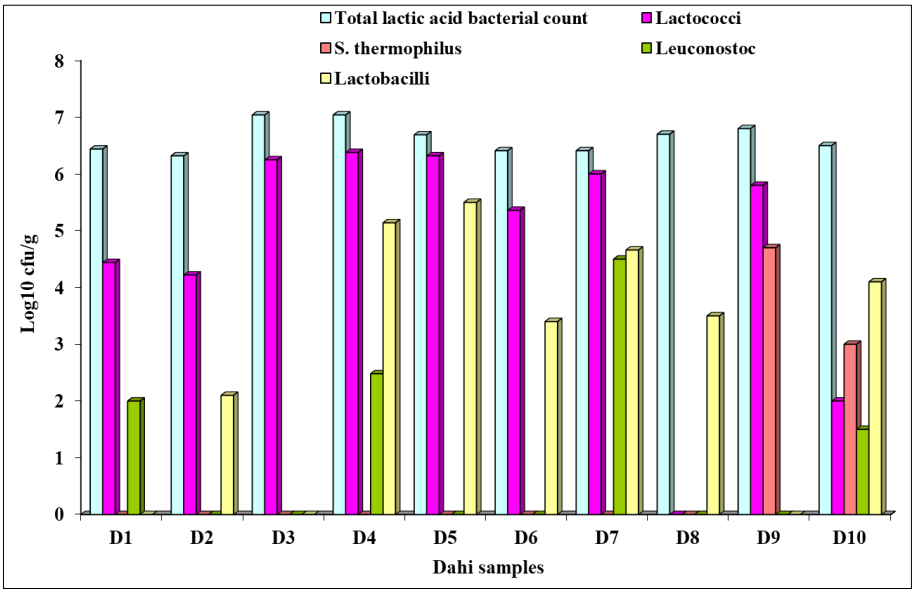

Fig 1: Enumeration of lactic acid bacteria in domestic and market Dahi samples

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