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Isolation and characterization of *Streptococcus thermophilus* from traditionally prepared *Misti dahi* samples

Amit Kumar BarmanDOI: <https://www.doi.org/10.33545/26649926.2025.v7.i2b.305>**Abstract**

Total, 14 cocci isolate of lactic acid bacteria were isolated by pour plate by plating on milk sucrose agar media with suitable dilution for 42 °C/24-48 h. Separated colonies based on colony morphology were cultured in milk sucrose media for 42 °C/8-10 h. The purity of cultures was checked by evaluating morphology by simple staining, Gram's staining property and catalase test. Cocci isolates were further confirmed by different phenotypic tests. All the cocci isolates are negative for their growth in Modified enterococcus agar. The cultures were further checked to determine the quality of *Misti dahi* in skim milk with added sugar (20 and 22.5, 25 %) and shop prepared *Misti dahi* mix in terms of acidity, body, and texture. Characterization of Lactic acid bacteria was done based on sugar fermentation pattern. According to the sugar fermentation profile the identified organism for cocci isolate was *Streptococcus thermophilus*.

Keywords: Characterization, cultures, *Misti dahi*, quality, morphology**1. Introduction**

The most frequently starter cultures used to produce fermented dairy products are thermophilic and mesophilic Lactic acid bacteria. The starter culture determines the distinctive flavor, consistency, and other characteristics of the fermented dairy products. The thermophilic lactic acid bacteria (LAB) can grow at temperatures between 37 and 45 °C, which is an important requirement for the technological method of producing fermented dairy products like yoghurt, baked fermented milk, and different types of cheese. The creation of diverse fermented dairy products depends heavily on LAB belonging to the genera *Lactococcus*, *Leuconostoc*, *Lactobacillus*, *Streptococcus*, and *Pediococcus*. *Streptococcus* and *Enterococcus* genera are typically represented by the thermophilic cocci strains that are isolated from raw milk and dairy products that spontaneously ferment. *S. thermophilus* from thermophilic LABs are "useful" examples as they can be used in starter cultures. An important issue in microbiology is the quick and precise classification of bacteria, which can be accomplished using standard approaches based on physiological, biochemical, and molecular methods. *Streptococcus thermophilus*, a *streptococcus*, gram-positive, facultative anaerobe, with non-motile and non-spore forming homo-fermentative qualities (Dewan and Tamang, 2007) [2]. These bacterial strains perform the core function of milk acidification in addition to producing secondary metabolites such as antimicrobial peptides and having additional probiotic characteristics. As the second most significant industrial dairy starter after *Lactococcus lactis*, *Streptococcus thermophilus* is crucial for the food industry since it is widely used in the production of numerous fermented dairy products. The demand for discovering novel strains of *S. thermophilus* has grown significantly along with the significant increase in cheese and yoghurt production. The heat-loving *Streptococcus thermophilus* has spherical or ovoid cells that are 0.7-1.0 µm in diameter, occur in pairs or long chains, and are chemo-organotrophic. They can grow at 45 °C, but they cannot grow at 10 °C. Fructose, glucose, lactose, mannose, and sucrose all result in the production of acid. N-acetyl galactosamine, N-acetylglucosamine, arabinose, cellobiose, glycerol, glycogen, maltose, mannitol, rhamnose, salicin, trehalose, xylose etc. do not create

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acid. Galactose, raffinose, ribose, and other sugars show a range of responses. Esculin and gelatin are not hydrolyzed by strains.

2. Materials and Methods

Fifteen (15) traditionally prepared *Misti dahi* were collected from various locations of West Bengal in multipurpose clinical sample bottle (Plate 1) kept in an insulated box with ice gel pack and temperature was maintained at (5±1) °C. The collected samples were analyzed to check the morphology of microorganism present.

2.1 Isolation of lactic acid bacteria

Well isolated colonies (yellow colour) on skim milk sucrose agar plates were picked (5-6 colonies from each sample) aseptically with the help of micropipette (0.1 ml) and transferred in skim milk sucrose medium tubes. The tubes were incubated at 42 °C for 12-15 h to get curd culture.

2.2 Evaluation of cultures for purity and activity

The culture was sub-cultured twice in skim milk sucrose medium (42 °C for 12-15 h) prior to further evaluation/analysis.

2.3 Morphology and Gram's staining

The culture was checked for purity morphologically by Gram staining. Thin smear of active skim milk sucrose medium culture was prepared on clean glass slide. The smear was heat fixed and was stained using Gram stain kit (Himedia, Mumbai, India). After drying, stained slide was observed under oil immersion objective (100X) for Gram's reaction, shape and arrangements of cells.

2.4 Catalase test

An aliquot of the active skim milk sucrose medium culture was transferred on a clean glass slide. A few drops of 3% hydrogen peroxide were poured over it and was observed for effervescence. Absence of effervescence indicated the negative for catalase production (Norris *et al.*, 1981).

2.5 Growth of cocci isolates at 10 and 45 °C

Active cocci isolates were inoculated (@1%) in skim milk sucrose medium tubes and the tubes were incubated at 10 and 45°C for 48 h. Formation of curd was indicated the positive for growth. Control tubes were kept at 42 °C.

2.6 Hetero-fermentation patterns

Active cocci isolates were inoculated (@1%) in bromocresol purple (0.004%) containing M17 broth tubes with inverted Durham's tubes. The tubes were incubated at 42 °C for 48 h. Change of medium colour from purple to yellow indicated the acid production and accumulation of gas in the Durham's tubes indicated the hetero-fermentation (Tserovskis *et al.*, 2002) [5].

2.7 Growth in presence of 4 and 6.5% NaCl

Active cocci isolates were inoculated (@1%) in bromocresol purple (0.004%) containing M 17 broth tubes with 4 and 5% added NaCl. The tubes were incubated at 42°C for 48 h. Change of colour from purple to yellow indicated the acid production and growth. Medium without added NaCl was taken as control.

2.8 Growth of cocci isolates on modified Enterococci agar

Active cocci isolates were spot-inoculated on modified Enterococci agar plates. The plates were incubated at 42 °C for 24 h. Absence of maroon colony growth was taken as negative test.

2.9 Curd setting profile of cocci isolates in skim milk with added sugar (20, 22.5, 25%) and in shop-prepared *misti dahi* mix

The skim milk (12% milk solids) with added sucrose (20, 22.5 and 25%, separately) was prepared and sterilized by autoclaving (121°C/ 15 min.). Freshly shop-prepared *misti dahi* mix was collected from a local sweet shop in sterile glass flask. Active cocci isolate culture was inoculated (@2%) and incubated at 42 °C for 12-15 h. Quality of curd formed was observed.

2.10 Preservation of culture isolates

The active culture was inoculated skim milk sucrose medium (25 ml; 3% SMP and 20% sugar) in a centrifuge tube (50 ml) and was incubated at 42 °C for 12h. The cell biomass was harvested and washed with normal saline by centrifugation (8,000 rpm, 10 min, 4 °C). Cell pellet was suspended in cryo-protective medium and was preserved by freeze drying (Lyodel, delvac Freeze dryer, India). Dried culture powder was packed in sterile cryo-vials and stored at -20 °C.

2.11 Carbohydrate fermentation

Pure potent cocci isolate identification was carried based on sugar fermentation patterns. The test was conducted in CHL broth tubes devoid of sugars containing 0.004% BCP. Sterile sugar discs (Hi Media Laboratories Ltd., Mumbai) was aseptically added to sterile CHL broth tubes and kept in incubator (37 °C) for overnight for sterility check. Active cultures were streaked on skim milk sucrose agar plates and the plates were incubated 42 °C for 24 h. Active colony was transferred in 1 ml sterile saline in eppendorf tube (2 ml) with sterile inoculating loop to get cell suspension. The cell pellet was obtained by centrifugation of eppendorf tube (10000 rpm/ 5 min). Cell pellet was washed twice with normal saline and finally was suspended in 1 ml saline for inoculation. CHL broth tubes (5 ml) with different carbohydrate discs [(Cocci isolates: Arabinose, Galactose, Glucose, Glycerol, Lactose, Maltose, Mannose, Salicin, Sucrose, Trehalose); were inoculated @1% with the cell suspension and the tubes were incubated at 42 °C and examined up to 3 days. The change of colour from purple to yellow indicated the fermentation of carbohydrate with acid production.

3. Results and Discussion

Isolation of lactic acid bacteria from traditionally prepared *misti dahi* samples. Well isolated colonies (yellow colour) on skim milk sucrose agar plates were cultured in skim milk sucrose medium. Pure cocci isolate (Plate 1) were evaluated for purity tests followed by assessing of their activity (Table 1).

3.1 Evaluation of isolates for purity and activity

All the cocci isolates were found Gram positive and catalase negative, which are the characteristics of lactic acid bacteria (Table 1). *Streptococcus thermophilus* and enterococci are

closely related species in terms of their nutritional and growth requirements. There are chances for frequent contamination of these cultures with enterococci during different steps of isolation, preservation, and maintenance. The negative growth at 10 °C and 6.5% NaCl concentration (Table 1) as well as absence of maroon colony growth on modified enterococci agar plates (Plate 2) indicated the absence of enterococci contamination (Bergey's Manual, 2007) [1] in cocci isolates. All the cocci isolates grew well at 45 °C and were homo-fermentative (Table 1). The suitability of the isolates for preparation of *misti dahi* was evaluated from curd setting profile and quality of curd in skim milk with added sugar (20, 22.5, and 25%) as well as in shop-prepared *misti dahi* mix. Fourteen cocci isolates showed clean curd formation in skim milk with added sugar and shop-prepared *misti dahi* mix. The quality of the curd from shop-prepared *misti dahi* mix was acceptable (Table 1). These cultures were preserved by freeze drying for further analysis.

3.2 Characterization of isolates

Sugar fermentation profiles of the 14 cocci isolates (C1-C14) was analyzed with different carbohydrate discs. All the

14 cocci isolate fermented glucose, lactose and sucrose. Fermentation of arabinose and aesculin hydrolysis was found negative for all cocci isolates (Plate 2). Six isolates such as C1, C5, C7, C12, and C14 were positive for galactose fermentation. For other sugars were variably fermented by different cocci isolates. Based on the sugar fermentation profile cocci isolates were identified as *Streptococcus thermophilus* (Table 2). Variability in sugar fermentation patterns may indicate the close difference among the *S. thermophilus* isolates. Zourari *et al.* (1992) [6] reported *S. thermophilus* and *Lb. delbrueckii* spp. *bulgaricus* are homo-fermentative and during their metabolism, lactose is taken up as free sugar and split with β -galactosidase.

A slight variation in fermentation patterns was observed as compared to the fermentation patterns documented in Bergey's Manual (2007) [1]. Ishola and Adebayo-Tayo (2008) reported that fermented foods are potential source for isolation of lactic acid bacteria due to prevailing of favorable conditions for growth and development of the bacteria. In present investigation, fourteen cocci isolate were collected from traditionally prepared *misti dahi* samples. All the isolates were characterized based on phenotypic and biochemical characteristics.

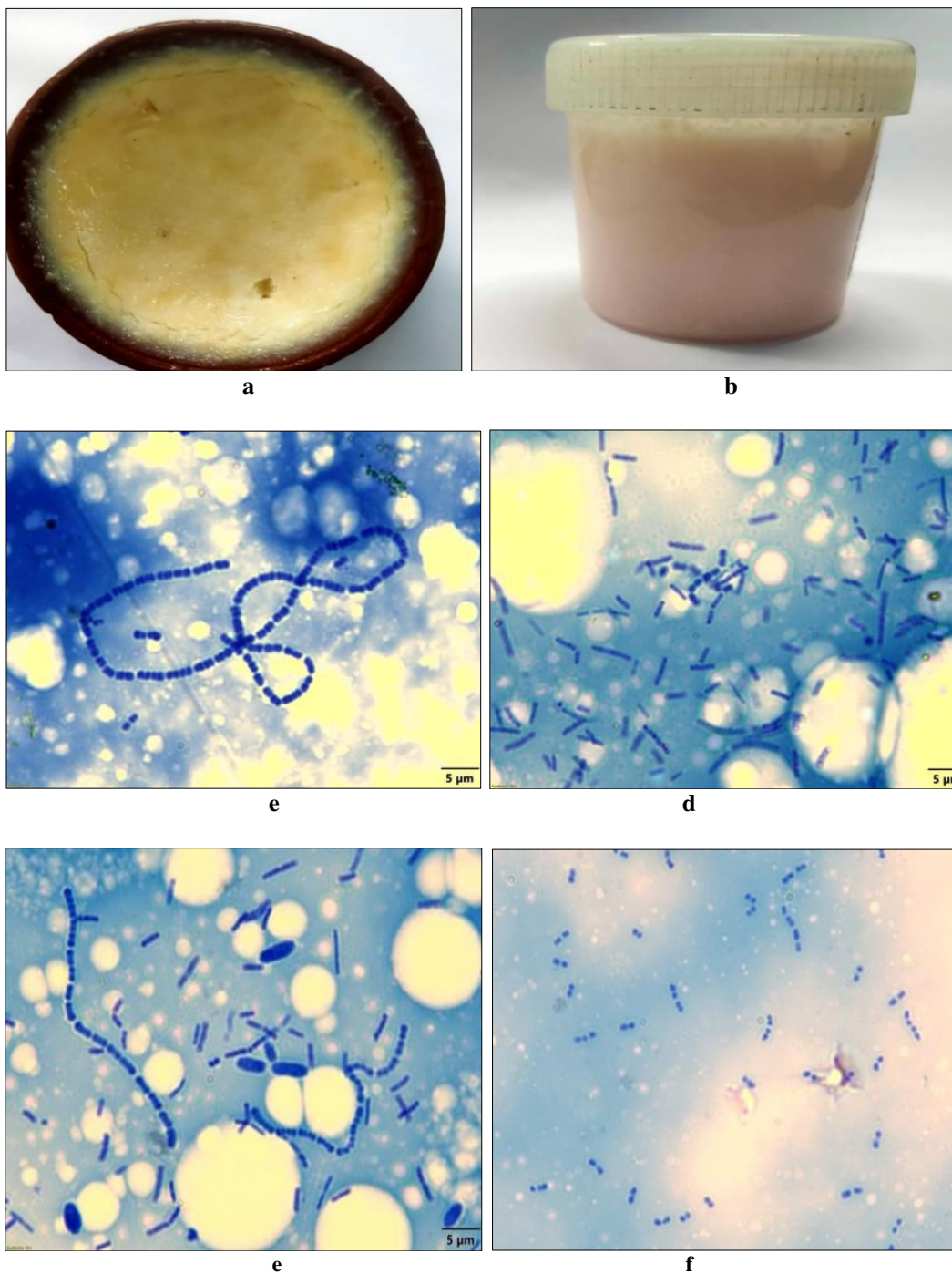
Table 1: Morphology, catalase, gram staining, growth at different temperature, salt tolerance, hetrofermentation, growth in modified enterococcus agar, quality of *Misti dahi* in skim milk with added sugar and shop prepared *Misti dahi* of cocci isolates

Sl. No.	Code	Morphology	Catalase	Gram Staining	Growth at	Growth in			Hetero fermentation	Modified Enterococcus Agar (Growth)	Curd setting			
						10 °C	45 °C	4% NaCl			6.5% NaCl	Skim milk with added sugar (%)		
					20							22.5	25	
1	C1	Cocci in chains	-	+	-	+	+	-	-	-	+	+	+	+
2	C2	Cocci in chains	-	+	-	+	+	-	-	-	+	+	+	+
3	C3	Cocci in chains	-	+	-	+	+	-	-	-	+	+	+	+
4	C4	Cocci in chains	-	+	-	+	+	-	-	-	+	+	+	+
5	C5	Cocci in chains	-	+	-	+	+	-	-	-	+	+	+	+
6	C6	Cocci in chains	-	+	-	+	+	-	-	-	+	+	+	+
7	C7	Cocci in chains	-	+	-	+	+	-	-	-	+	+	+	+
8	C8	Cocci in chains	-	+	-	+	+	-	-	-	+	+	+	+
9	C9	Cocci in chains	-	+	-	+	+	-	-	-	+	+	+	+
10	C10	Cocci in chains	-	+	-	+	+	-	-	-	+	+	+	+
11	C11	Cocci in chains	-	+	-	+	+	-	-	-	+	+	+	+
12	C12	Cocci in chains	-	+	-	+	+	-	-	-	+	+	+	+
13	C13	Cocci in chains	-	+	-	+	+	-	-	-	+	+	+	+
14	C14	Cocci in chains	-	+	-	+	+	-	-	-	+	+	+	+

Table 2: Sugar fermentation profile of cocci isolates

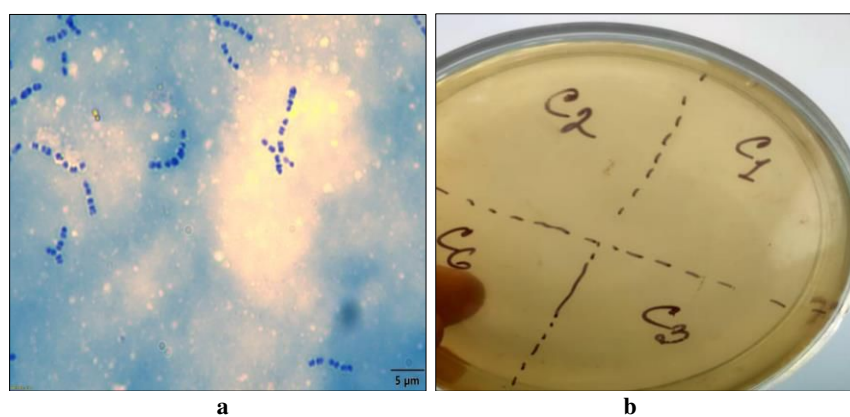
Cocciisolates	Sugar fermentation patterns										Aesculin hydrolysis	Identification
	Ara	Gal	Glu	Gly	Lac	Mal	Man	Sal	Suc	Tre		
C1	-	+	+	-	+	-	-	-	+	-	-	<i>S. thermophilus</i>
C2	-	-	+	+	+	+	-	-	+	+	-	<i>S. thermophilus</i>
C3	-	-	+	-	+	+	+	-	+	-	-	<i>S. thermophilus</i>
C4	-	-	+	-	+	+	-	-	+	-	-	<i>S. thermophilus</i>
C5	-	+	+	-	+	-	-	-	+	-	-	<i>S. thermophilus</i>
C6	-	-	+	-	+	+	-	±	+	-	-	<i>S. thermophilus</i>
C7	-	+	+	±	+	+	+	+	+	+	-	<i>S. thermophilus</i>
C8	-	±	+	-	+	+	-	-	+	-	-	<i>S. thermophilus</i>
C9	-	-	+	+	+	+	-	-	+	+	-	<i>S. thermophilus</i>
C10	-	±	+	-	+	±	-	-	+	-	-	<i>S. thermophilus</i>
C11	-	-	+	-	+	±	+	+	+	+	-	<i>S. thermophilus</i>
C12	-	+	+	+	+	+	-	-	+	-	-	<i>S. thermophilus</i>
C13	-	+	+	+	+	+	-	-	+	+	-	<i>S. thermophilus</i>
C14	-	+	+	±	+	+	+	+	+	+	-	<i>S. thermophilus</i>

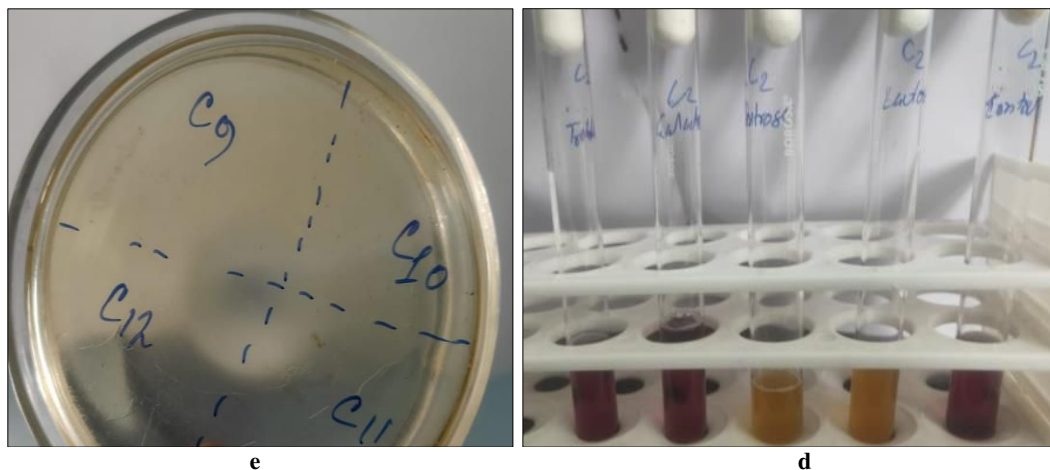
Ara - arabinose, Gal - galactose, Glu - glucose, Gly - glycerol, Lac - lactose, Mal - maltose, Man - mannose, Sal - salicin, Suc - sucrose, Tre - trehalose



Misti dahi in earthen pot (a) and collected in multipurpose clinical sample bottle (b), sample contains only cocci (c), mixture of cocci and rods (d), yeast contamination (e). Microscopic observation of C2 isolate (f)

Plate 1: Collected *misti dahi* samples and their microscopic observations.





Microscopic observation of C3 (a), negative growth of cocci isolates on modified Enterococci agar (b and c). Sugar fermentation of C2 isolate (d)

Plate 2: Microscopic observation of isolates and testing of purity

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