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RESEARCH ARTICLE

Screening of sugar tolerant fast-growing lactic acid bacteria for preparation of *Misti dahi*

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Abstract: *Misti dahi*, a popular fermented milk product in eastern India, is experiencing growing demand across the country. To meet the increasing demand, it is essential to identify sugar-tolerant starter cultures that can ensure more desirable and consistent fermentation outcomes while minimizing the risk of fermentation failure. This study aimed to isolate, screen, and identify sugar-tolerant cultures suitable for *Misti dahi* production. Out of ten isolated cultures, *S. thermophilus* MD3 exhibited remarkable tolerance to high sugar concentrations of up to 20% in milk without compromising cell viability and curdling time. Moreover, it consistently produced *Misti dahi* of superior quality in terms of physicochemical, microbiological, textural, sensory, and rheological attributes. The isolated *S. thermophilus* MD3 culture holds potential for commercialization in *Misti dahi* production.

Keywords: Lactic acid bacteria, Sugar tolerance, *Misti dahi*, Textural attributes, Rheology, Sensory properties,

Introduction

Misti dahi, also known as sweetened curd or Lal dahi, is a popular fermented milk product originating from the eastern region of India (Prajapati and Behare, 2018). Initially, the consumption of *Misti dahi* was limited to the eastern and northern parts of India, but its demand has been progressively growing throughout the country. The authentic flavour and distinctive taste of *Misti dahi* have garnered significant popularity among the Indian population. Traditionally, it was prepared on a cottage scale to meet the local demand. The customary method includes simmering milk with cane sugar in an open pan at 60-70°C for several hours, leading to moisture evaporation and distinct cooked flavors, a slightly brown color, viscosity, and other physico-chemical changes. After cooling to 30-42°C, a previous day old starter culture was added, and the mixture was then left to curdle overnight in earthenware pots (Chatterjee et al. 2022). However, a significant number of small manufacturers involved in the production of *Misti dahi* neglect the importance of ensuring the reliability and purity of the starter culture. As a result, this lack of attention leads to the production of *Misti dahi* with inconsistent quality and an increased risk of developing off-flavors.

Misti dahi is typically made using sugar-tolerant lactic acid cultures that can effectively produce the desired acid and flavor in sweetened milk, resulting in desirable attributes in the final product. *Misti dahi* starter cultures have traditionally consisted of various species such as *Lactococcus lactis*, *Lactococcus diaceytylactis*, *Lactococcus cremoris*, *Leuconostoc* spp., *Streptococcus thermophilus*, and *Lactobacillus delbreuckii* subsp *bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus plantarum* (Ghosh and Rajorhia 1990; Gupta et al. 2000; Akter et al. 2010). However, manufacturers now prioritize the use of fast acidifying starters that can produce the desired product in a shorter time. One of the challenges in the production process is the high sugar content, which can cause osmotic shock and negatively affect the viability and activity of the starter cultures (Kashket, 1987). Furthermore, the physico-chemical and sensory properties of *Misti dahi* are influenced by the use of specific starter culture. These properties, such as texture, flavor, and overall quality, are important factors in meeting consumer expectations and preferences. In unorganized sectors, undefined starter cultures are often used for making *Misti dahi*, which can lead to inconsistent quality in the end product. Therefore, it is crucial to employ pure and defined strains of osmo-tolerant cultures that can ensure uniform and predictable fermentative changes, resulting in a consistent quality of *Misti dahi*. Naturally

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fermented products that harbour a diverse range of species and strains offer potential sources for obtaining high sugar-tolerant lactic strains suitable for *Misti dahi* production. By carefully selecting and maintaining a suitable starter culture, the industry can meet the increasing demand for *Misti dahi* while ensuring consistent quality and consumer satisfaction.

In this study, we aim to isolate lactic cultures from traditional *Misti dahi* samples and identify potential strains as fast acidifying starter cultures, impacting the sensory, physico-chemical, and textural characteristics of *Misti dahi*.

Materials and methods

Collection and processing of samples

Nine *Misti dahi* samples (designated as S1 to S9) were collected from the local market of Kolkata, West Bengal. The samples were placed in an ice-box to maintain the freshness and prevent any potential microbial changes and transported to the laboratory for microbiological analysis.

Isolation of lactic acid bacteria strains

To isolate lactic cultures, the samples were diluted serially in a saline solution. Subsequently, 1 ml from each diluted sample (10^{-5}, 10^{-6}, and 10^{-7}) was aseptically transferred to sterile petri plates. Molten MRS and M17 agar media were poured onto the plates separately, and after solidification, the plates were incubated at temperatures of 30ºC, 37ºC and 42ºC for 48 to 72 hrs. The colonies displaying typical characteristics were carefully selected and transferred to MRS or M17 broth tubes. To purify the isolates, a repetitive streaking method was employed and pure isolates were subjected to further analysis.

Primary screening and identification of sugar tolerating cultures

The isolated cultures were evaluated by gram and negative staining and catalase test as per the standard protocol. Activity of the cultures was evaluated by determining titratable acidity (AOAC, 2007), pH, total lactic count in MRS or M17 agar media (ISO19344:2015) and curd setting time in 12% reconstituted skim milk (w/v) containing 15, 18 and 20% (w/v) sugar content. For the study, the sugar-tolerant *Streptococcus thermophilus* NCDC436 and the sensitive *Streptococcus thermophilus* NCDC74 reference strains were obtained from the National Collection of Dairy Cultures (NCDC), ICAR-National Dairy Research Institute, Karnal, Haryana, India, and used as positive and negative controls, respectively. Considering sugar tolerance and curd setting time as the main criteria, promising isolates were selected and subjected for genotypic evaluation.

The selected sugar tolerant isolates were identified using species-specific PCR. After growing the isolates in broth media overnight, genomic DNA was extracted according to the method of Pospiech and Neumann (1995). The genomic DNA was amplified using conserved sequences of *S. thermophilus* lacZ gene primers, F (5' CACTATGCTCAGAATACA 3') and R (5' CGAACAGCATTGATGTTA3') as suggested by Maheswari et al. (2013) in a Thermal cycler (BIO-RAD S1000 thermal cycler). The species-specific PCR protocol includes the following steps: initial denaturation at 94°C for 3 min, final denaturation at 90°C for 30 sec, annealing at 54°C for 70 sec, extension at 72°C for 30 sec, final extension at 72°C for 10 min, and a total of 35 cycles. The PCR products were separated using a 1.5% agarose gel, and visualized under a UV spectrum after staining with Ethidium bromide.

Preparation of *Misti dahi*

Buffalo milk (6.0 % fat and 9.0% SNF) was collected from Experimental Dairy, ICAR-NDRI, Karnal and *Misti dahi* was prepared according to the method described by Prajapati and Behare, (2018) with some modifications (Fig. 1). Skim milk powder (1.5%, w/v), sugar (15 %, w/v) and caramel (1% v/v) were added during preheating of milk. The mixture was then homogenised at 9000 rpm for 5 min using Ultra Turrax homogenizer. The milk mixture was then heated at 90°C for 10 min followed by cooling up to 42ºC. The sugar tolerating strains were inoculated at 2% (v/v) into the milk, distributed into cups and incubated at 42°C until it forms firm coagulum. The set product was immediately transferred to refrigerated storage. The products made by *S. thermophilus* NCDC436 and NCDC 74 were used as control.

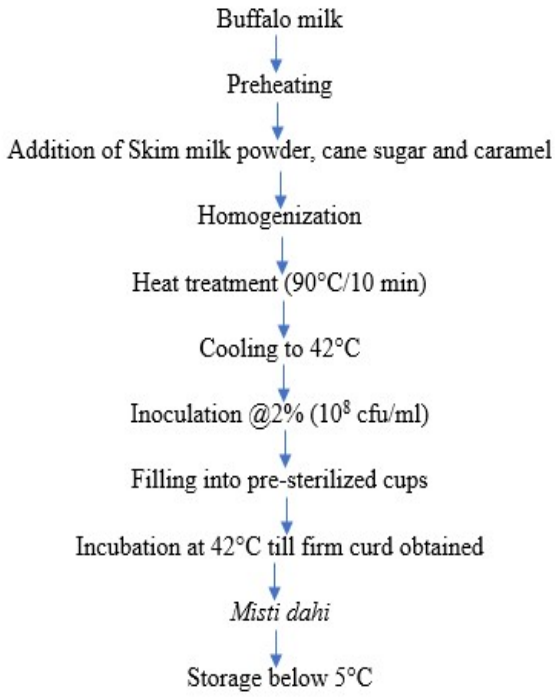


Fig.1 Process flow diagram of preparation of *Misti dahi*

Analysis of *Misti dahi*

The *Misti dahi* samples were analysed for physico-chemical, textural, rheological, Sensory and microbiological parameters. Curd setting time for *Misti dahi* was evaluated by time taken by the starter culture to form the firm curd when incubated at 42°C. Titratable acidity of products was determined by the method described by FSSAI for Dahi (AOAC, 2007) while pH by electronic pH meter.

Texture profile of the *Misti dahi* samples were analyzed by the method described by Hussain et al. (2016). Texture profiling was carried out using compression test by TA.HDplusC texture analyzer (Stable Micro Systems, UK fitted with 5 Kg load cell). *Misti dahi* samples were set in glass beakers of uniform size 150 mL having length and diameter and filled upto 100 mL mark. A cylindrical probe (P25) having diameter of 25 mm was allowed to penetrate the *Misti dahi* samples at temperature of 20°C. The textural parameters such as firmness (g), consistency (g.sec), cohesiveness (g) and work of cohesion (g.sec) were analyzed using Texture Exponent Connect Software. Firmness is defined as the value of peak force recorded during compression. Cohesiveness is regarded as the rate at which the material is disintegrated under mechanical action and it indicates the ability of the product to hold together. Consistency was measured as the area within the curve during the compression. The rheological properties of *Misti dahi* samples were studied at 20°C using rotational rheometer (Model: MCR 52, Anton Paar, Austria) fitted with a cone and plate assembly (CP-75, 75 mm diameter, 1° angle). *Misti dahi* samples were gently sampled from the cup with a spatula and placed on the pre-cooled (20°C) rheometer plate. Apparent viscosity of *Misti dahi* was measured in the shear rate range of 0.01 to 100 sec^{-1}. The apparent viscosity was recorded as Pa.s. The sensory attributes of *Misti dahi* was performed by 9 points hedonic scale using a panel of six judges having adequate knowledge about dairy products. The nine-point hedonic sensory score card comprised of the following ratings viz. like extremely (9), like very much (8), like moderately (7), like slightly (6), neither like nor dislike (5), dislike slightly (4), dislike moderately (3), dislike very much (2) and dislike extremely (1). In case of microbiological analysis total lactic count (ISO19344, 2015), coliform count (ISO 4832, 2006) and yeast and mold count (ISO 21527-1, 2008) were determined.

Statistical analysis

Three independent trials were carried out and results were expressed as mean ± standard error of mean. One way ANOVA (Analysis of variance) followed by Tukey's test was carried out in IBM-SPSS to determine significant differences among the data ($p<0.05$). Graphs were prepared in GraphPad Prism (version 8).

Results and discussions

Isolation of lactic cultures

Lactic isolates that formed typical colonies on M17 and MRS agar were randomly picked and transferred to respective broth media. Out of the 40 colonies transferred, ten pure isolates were obtained, including five cocci (MD1 to MD5) and five rods (MD6 to MD10). These isolates appeared as gram-positive, catalase-negative cocci and rods arranged in long and short chains (Table 1, Fig. 2). A total of ten lactic cultures were isolated from the nine

Table 1: Details of isolated cultures from *Misti dahi* samples

Sl. No.	Name of the culture	Source of isolation	Isolation media	Isolation temperature (°C)	Morphological characteristics
1	MD1	S-1	M17	42	Gram positive long chains of cocci
2	MD2	S-2	M17	42	Gram positive long chains of cocci
3	MD3	S-2	M17	37	Gram positive long chains of cocci
4	MD4	S-3	M17	30	Gram positive short chains of cocci
5	MD5	S-5	M17	42	Gram positive long chains of cocci
6	MD6	S-7	MRS	37	Gram positive long chains of rods
7	MD7	S-8	MRS	37	Gram positive long chains of rods
8	MD8	S-8	MRS	42	Gram positive long chains of rods
9	MD9	S-9	MRS	30	Gram positive long chains of rods
10	MD10	S-9	MRS	37	Gram positive short chains of rods

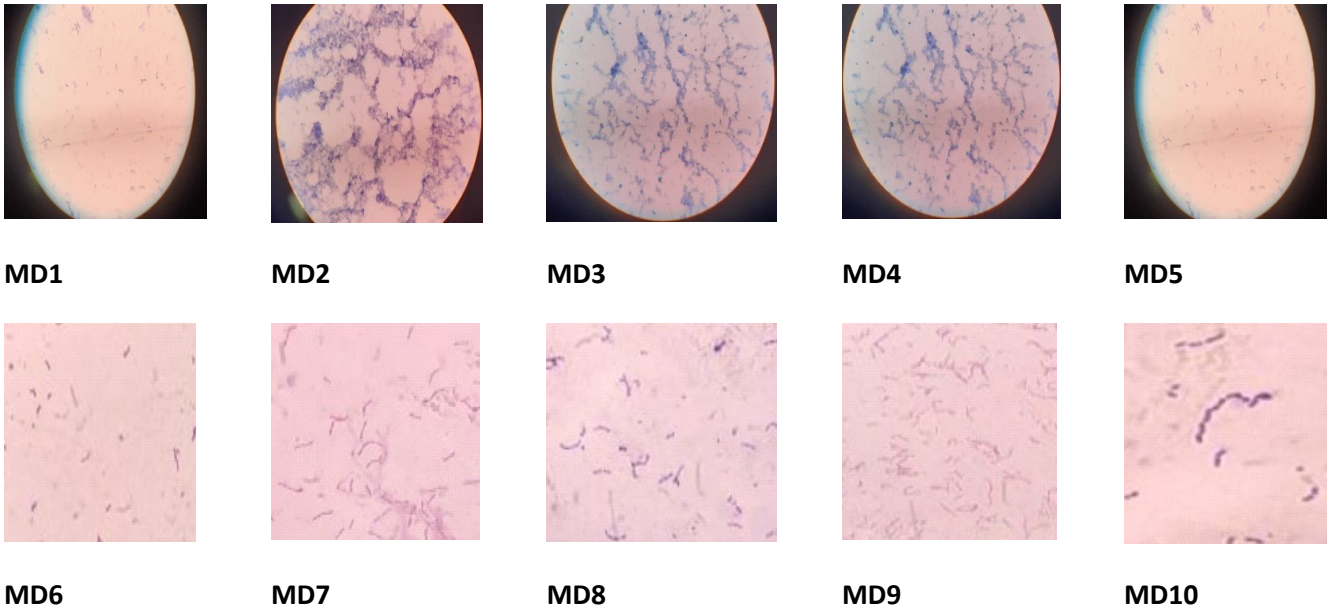


Fig. 2 Morphological evaluation of selected cultures by Gram staining

Misti dahi samples using MRS and M17 media (Table 1). They were tentatively considered as species of *Lactococcus*, *Lactobacillus* and *Streptococcus*. Out of all the isolates, only two were capable of growing at mesophilic temperatures, whereas the majority thrived in thermophilic conditions. The isolation of lactic acid bacteria from different dairy and non-dairy samples varied among the various reports. Goa et al. (2022) obtained twelve LAB isolates from three samples of Ergo collected in Jimma town, Ethiopia. These isolates comprised both mesophilic and thermophilic species such as *Lactococcus lactis* subsp. *lactis*, *Lactobacillus acidophilus*, *Lactiplantibacillus plantarum*, *Limosilactobacillus fermentum*, and *Leuconostoc lactis*. Maheswari et al. (2013) reported that 74 strains of *Streptococcus thermophilus* isolated from various plant source by polymorphic approach. On the other hands, Rashid et al. (2007) isolated 12 lactic acid bacteria strains from traditional *dahi* samples collected from the stock cultures of Animal Food Function Laboratory, The Graduate School of Natural Science and Technology, Okayama University, Japan.

Sugar tolerance of isolated cultures

In current study, osmo-stability of the isolated strains was evaluated in reconstitute skim milk added with different levels of sugar. The curdling time and metabolic activity associated with the viable cells was lower for high sugar tolerant strains (20%) (Table 2). Similarly, Sameen et al. (2010) isolated four lactic strains as starter culture which had an ablilty to ferment milk in presence of sucrose and other sugars. Sugar tolerance capability of lactic strains is considered as one of the crucial parameters for selecting starter culture for *Misti dahi*. Many bacteria do not exhibit this behaviour while few osmotolerant lactic strains have been reported to adapt these environments by displaying specific enzyme activities (Sleator and Hill., 2001). Although, lactic isolates (MD4 and MD9) obtained at mesophilic temperature curdled the milk, they took longer fermentation time as compared to thermophilic isolates. Vandna (2017) prepared mesophilic *Lactococcus* culture for *Misti dahi* which took 12-14 hrs to set the curd. In contrast, the isolated thermophilic culture was able to set the curd within four hours. Based on our experiment, four isolates MD1, MD2, MD3 and MD5 was showing significantly ($p<0.05$) higher total lactic count and titratable acidity, lower curd setting time and pH in 20% sugar containing milk and hence were further identified by species-specific PCR.

Identification of sugar tolerant lactic cultures

The size of amplified PCR products of four selected isolates was 968 bp (Fig 3.), which was corresponding to *Streptococcus thermophilus* NCDC 074 and sugar tolerating *S. thermophilus* NCDC 436. Maheswari et al. (2013) observed that the isolated *S. thermophilus* strains exhibited a species-specific PCR product size of 968 bp, which provided confirmation that the selected strains were *Streptococcus thermophilus*. This finding aligns with previous studies conducted by Lick et al. (1996) and Schroeder et al. (1991), who also reported the same size of PCR product for *S. thermophilus* strains.

Effect of sugar tolerant cultures on properties of *Misti dahi*
Physico-chemical properties

The physico-chemical properties of *Misti dahi* made by promising sugar tolerant cultures are shown in fig. 4. The titratable acidity of the samples ranged from 0.74% to 0.81% LA (Fig. 4A), falling within the limit specified by FSSAI for dahi. The pH values, as

Table 2: Preliminary technological screening of lactic strains in sugar containing milk

Isolate designation	Log of Total lactic count (cfu/ml)				Curd setting time (hr)				pH				Titratable acidity (%LA)			
	0% Sugar	15% Sugar	18% Sugar	20% Sugar	0% Sugar	15% Sugar	18% Sugar	20% Sugar	0% Sugar	15% Sugar	18% Sugar	20% Sugar	0% Sugar	15% Sugar	18% Sugar	20% Sugar
MD1	9.684±0.08^{ab}	9.629±0.05^{a}	9.509±0.04^{a}	9.203±0.08^{a}	4.25±0.35^{d}	4.5±0.00^{ef}	5.1±0.14^{bcd}	5.75±0.35^{f}	4.77±0.03^{d}	5.03±0.02^{bc}	5.04±0.00^{de}	5.10±0.01^{cd}	0.77±0.00^{b}	0.75±0.00^{b}	0.74±0.00^{ab}	0.68±0.03^{b}
MD2	9.321±0.26^{abc}	9.496±0.06^{ab}	9.381±0.09^{a}	8.985±0.27^{a}	4.25±0.07^{d}	5.1±0.14^{ef}	5.65±0.21^{cd}	5.75±0.35^{f}	4.82±0.03^{d}	5.0±0.01^{c}	5.10±0.01^{de}	5.19±0.03^{c}	0.77±0.01^{b}	0.74±0.01^{b}	0.73±0.01^{b}	0.68±0.01^{b}
MD3	9.761±0.07^{a}	9.586±0.09^{a}	9.514±0.08^{a}	9.276±0.03^{a}	3.55±0.07^{d}	3.8±0.28^{f}	4.1±0.14^{d}	4.25±0.35^{f}	4.68±0.02^{d}	4.87±0.03^{c}	4.89±0.05^{e}	4.89±0.03^{d}	0.83±0.02^{a}	0.79±0.01^{a}	0.78±0.01^{a}	0.76±0.01^{a}
MD4	9.716±0.05^{a}	9.462±0.07^{ab}	9.066±0.07^{ab}	8.915±0.05^{a}	9.1±0.14^{a}	9.25±0.35^{cd}	9.35±0.21^{bc}	10±0.00^{cd}	4.95±0.07^{cd}	5.13±0.02^{bc}	5.26±0.09^{cd}	5.5±0.07^{b}	0.68±0.01^{bc}	0.69±0.01^{c}	0.67±0.01^{cde}	0.63±0.00^{bc}
MD5	9.286±0.02^{abc}	9.298±0.07^{abc}	9.268±0.07^{a}	9.037±0.05^{a}	4.5±0.00^{cd}	4.65±0.21^{ef}	4.55±0.07^{d}	5.25±0.35^{f}	5±0.14^{bcd}	4.94±0.00^{c}	4.95±0.07^{de}	5.21±0.00^{c}	0.73±0.02^{b}	0.73±0.00^{b}	0.71±0.02^{bc}	0.68±0.00^{b}
MD6	9.245±0.03^{abcd}	9.062±0.07^{abcd}	8.656±0.16^{bc}	8.076±0.06^{b}	6.25±0.35^{bc}	6.75±0.35^{def}	8.1±0.14^{bcd}	8.75±0.35^{de}	5.26±0.02^{abc}	5.4±0.14^{ab}	5.63±0.02^{ab}	5.66±0.00^{ab}	0.68±0.01^{bc}	0.68±0.00^{cd}	0.63±0.01^{de}	0.62±0.01^{c}
MD7	8.718±0.07^{cd}	8.625±0.16^{bcd}	8.062±0.08^{d}	7.101±0.02^{c}	5.5±0.00^{bcd}	7.3±0.28^{cde}	7.85±0.07^{bcd}	8.1±0.14^{e}	5.47±0.03^{a}	5.38±0.02^{ab}	5.43±0.04^{bc}	5.53±0.02^{b}	0.67±0.01^{bc}	0.66±0.02^{cde}	0.64±0.01^{de}	0.63±0.02^{c}
MD8	9.067±0.07^{bcd}	8.46±0.33^{cd}	8.298±0.21^{cd}	7.989±0.04^{b}	9.1±0.14^{a}	10.25±0.35^{c}	10.5±0.70^{b}	11.5±0.70^{c}	5.31±0.02^{ab}	5.59±0.12^{a}	5.57±0.12^{abc}	5.60±0.07^{b}	0.64±0.00^{c}	0.65±0.01^{cde}	0.62±0.00^{de}	0.62±0.01^{c}
MD9	8.94±0.08^{cd}	8.47±0.31^{cd}	8.601±0.07^{bcd}	7.958±0.09^{b}	10.5±0.70^{a}	19±1.41^{a}	21±1.41^{a}	24±0.00^{a}	5.34±0.01^{a}	5.6±0.07^{a}	5.73±0.02^{ab}	5.72±0.06^{ab}	0.63±0.00^{c}	0.64±0.01^{de}	0.64±0.00^{de}	0.63±0.01^{bc}
MD10	8.645±0.17^{d}	8.3±0.06^{d}	8.195±0.07^{cd}	7.605±0.21^{bc}	6.5±0.70^{b}	15.5±0.70^{b}	18±0.00^{a}	18±0.00^{b}	5.55±0.07^{a}	5.7±0.07^{a}	5.83±0.02^{a}	5.83±0.02^{a}	0.62±0.01^{c}	0.63±0.01^{e}	0.61±0.02^{e}	0.60±0.01^{c}

(Different superscripts represent the significant difference ($p<0.05$) across the cultures (*Analysed by one-way ANOVA following Tukey test*).

shown in Fig. 4B, ranged from 4.58 to 4.85. The curd setting time, depicted in Fig. 4C, varied between 4 and 6 hrs. Notably, *S. thermophilus* MD3 demonstrated a curd setting time of four hours, which was significantly ($p<0.05$) lower compared to the other cultures. In contrast, the negative control exhibited an extended curd setting time of up to 11 hrs. According to Rashid et al. (2007), the pH of isolated *S. thermophilus* strains reached 3.92 after 24 hrs when incubated at 37°C in unsweetened reconstituted skim milk. Concurrently, the titratable acidity of this culture was 1.26% LA at the same time. Vandna (2017) developed freeze-dried DVS culture which could set the *Misti dahi* at 11.5, 12.5, 14 and 12 hrs by employing *Lactococcus lactis* ssp *lactis* NCDC314, *Lactococcus lactis* ssp *lactis* NCDC94, *Lactococcus* sp NCDC128 and *Lactococcus lactis* ssp *lactis* NCDC97 respectively. The spray-dried DVS cultures, in comparison, did not exhibit any significant deviation from the freeze-dried cultures. The pH of the *Misti dahi* produced using the laboratory-scale DVS culture was determined to be 4.60. In contrast, the lactic strains isolated in our study demonstrated the ability to grow rapidly, even in the presence of high sugar in the media.

Microbiological quality

Misti dahi prepared with *S. thermophilus* MD3 exhibited the highest total lactic count as shown in Fig 4D, which was comparable to the *Misti dahi* prepared using reference sugar tolerting strain *S. thermophilus* NCDC436. However, there were no significant differences ($p<0.05$) observed in *Misti dahi* made from MD1, MD2, and MD5. The absence of coliforms, yeast, and molds indicates that the product was manufactured in a hygienic environment. Vandna (2017) reported a total lactic count of 9.07 log cfu/ml in *Misti dahi* samples prepared using the DVS *Misti dahi* culture. This finding demonstrates that our culture is equally efficient in producing *Misti dahi*.

Rheological properties

Fig. 5 presents the textural properties of *Misti dahi* samples. Among the experimental *Misti dahi* samples, *S. thermophilus* MD3 obtained higher values for all the textural attributes. Textural attributes of *Misti dahi* sample prepared using MD2 were inferior when compared to others. Notably, *Misti dahi* made with reference *S. thermophilus* NCDC 436 strain demonstrated superior firmness, consistency, cohesiveness, and work of cohesion in comparison to the other samples. Textural attributes of dahi or yoghurt samples depends on various parameters viz. milk type, milk composition, heat treatment given to milk, starter culture used, and the incubation conditions. Since all other process parameters were kept similar, the differences in textural attributes of *Misti dahi* samples could be attributed to the

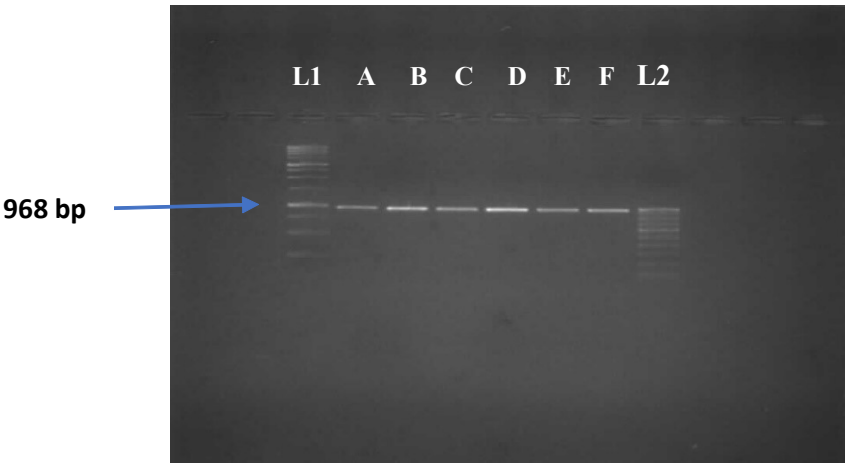


Fig. 3 Agarose gel showing PCR products obtained using *S. thermophilus* species specific primers. Lane L1: 1 kb DNA ladder, A: NCDC 074, B: NCDC 436, C:MD1, D: MD2, E: MD3, F: MD5, L2: 100 bp DNA ladder

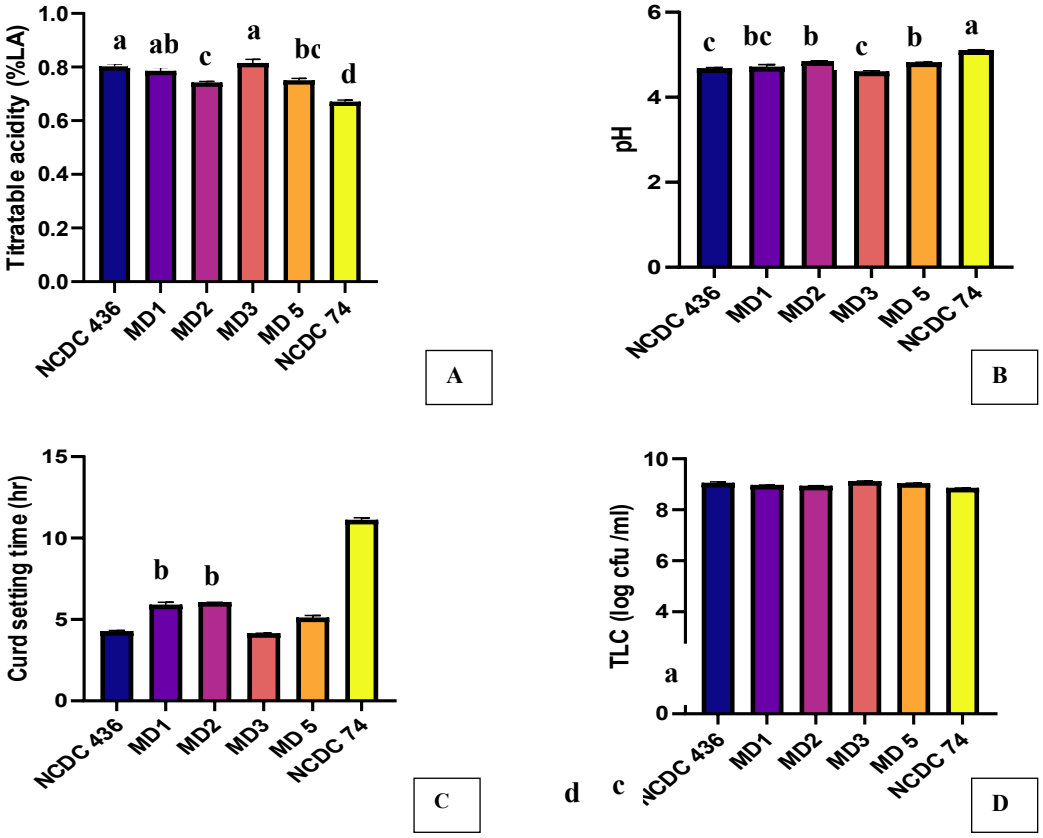


Fig. 4 Physico-chemical and microbiological properties of *Misti dahi*. A. Titratable acidity; B. pH; C. Curd setting time and D. Total lactic count

Different superscripts represent the significant difference ($p<0.05$) across the cultures (*Analysed by one-way ANOVA following Tukey test*).

use of different cultures for their preparation. Starter cultures differ in their proteolytic activity and acidification rate. Even the starter cultures belonging to same species of bacteria may have differences in their acidification rate and proteolytic activity. Amani et al. (2017) observed that yoghurts produced with starter cultures possessing distinct proteolytic activities exhibited varying textural attributes. Those starter cultures with higher proteolytic activity resulted in yoghurts with weaker textural characteristics. Similarly, Han et al. (2014) found that LAB isolates from traditional Chinese fermented foods displayed different acidification rates, leading to yoghurts with significantly diverse textural attributes. Raju and Pal (2009) also reported differences in the textural attributes of *Misti dahi* samples, which were prepared using different starter cultures.

The flow-curves of *Misti dahi* samples are given in Fig. 6. All the *Misti dahi* samples exhibited pseudoplastic (shear-thinning) behavior during the steady-shear rheological measurements. Shear thinning behaviour indicates that the viscosity of *Misti dahi* samples decreased with increasing shear. The apparent viscosity (Pa.s.) of *Misti dahi* samples at 50 s^{-1} shear rates are as follows 0.24, 0.22, 0.79, 0.26, 0.29 and 0.61 for MD1, MD2, MD3,

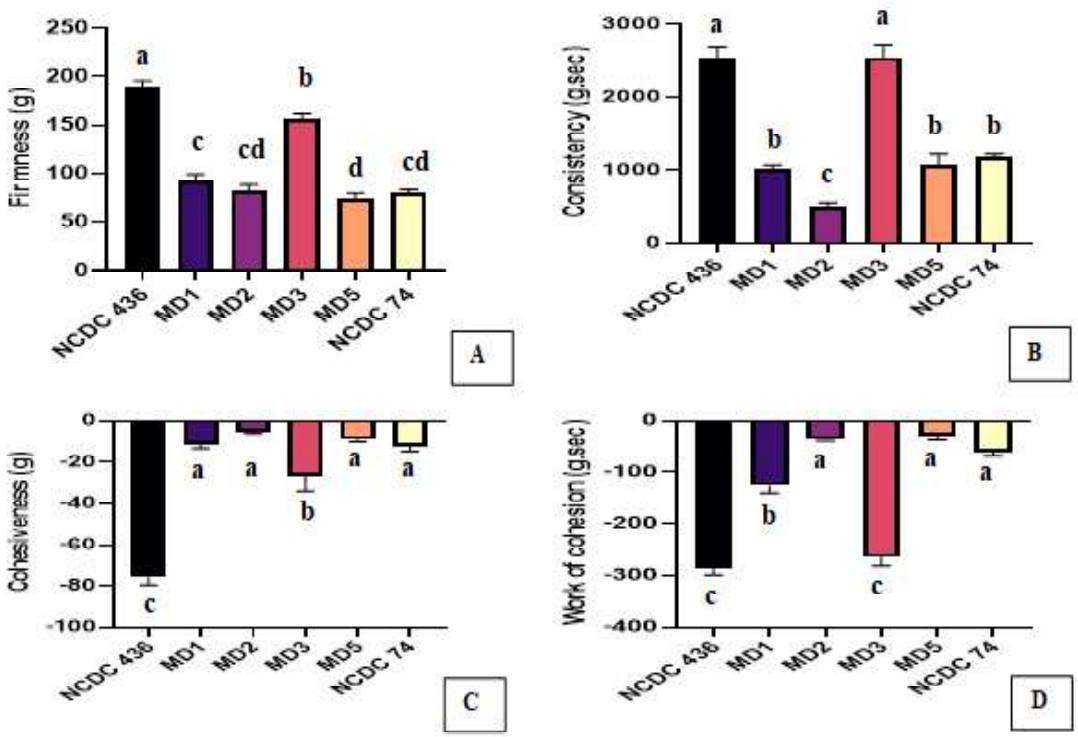


Fig. 5 Texture profile of *Misti dahi* prepared with lactic strains. A. Firmness (g) B. Consistency (g.sec) C. Cohesiveness (g) D. Work of cohesion (g.sec) Different superscripts represent the significant difference ($p<0.05$) among the strains (*Analysed by one-way ANOVA following Tukey test*).

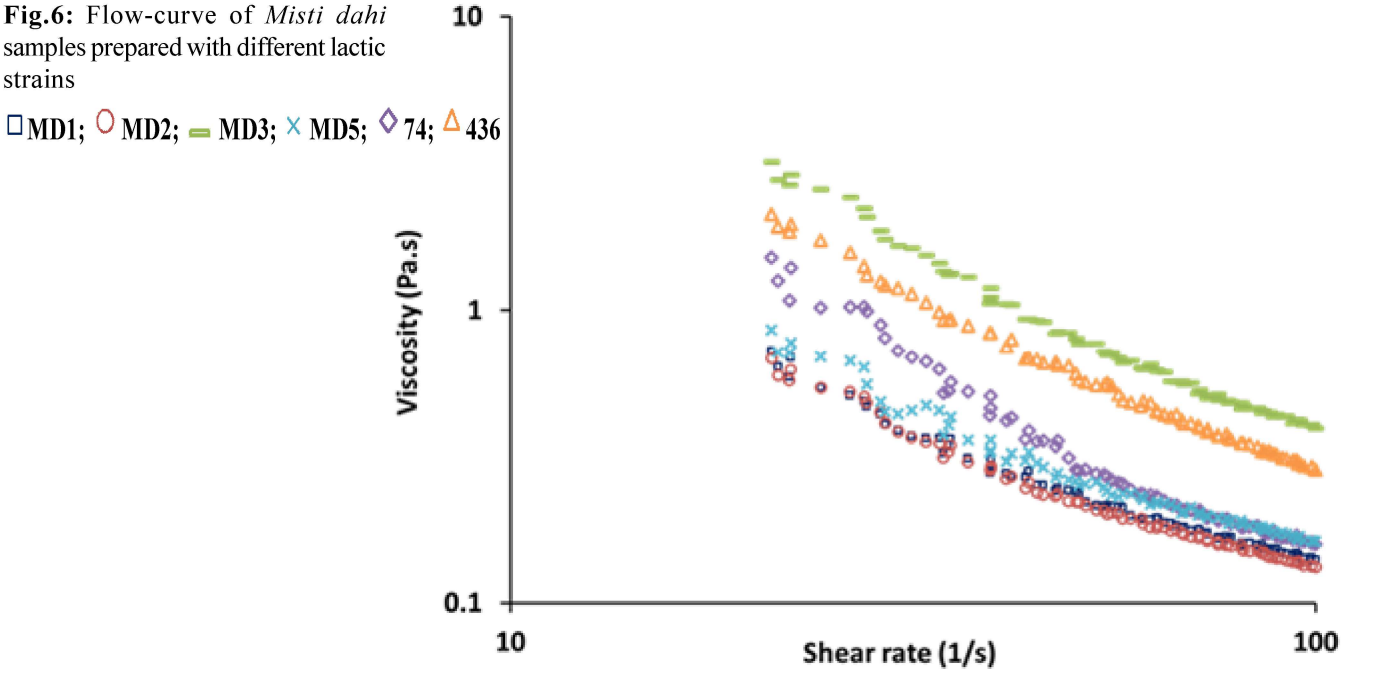


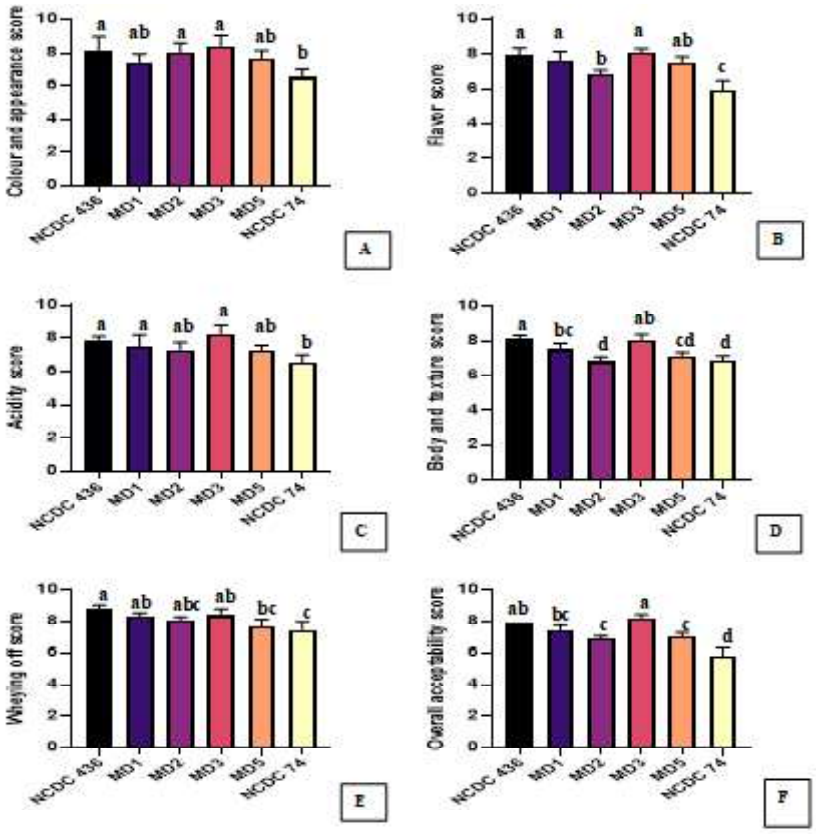
Fig.6: Flow-curve of *Misti dahi* samples prepared with different lactic strains

MD5, 74 and 436 respectively. The highest and lowest viscosity values were obtained for MD3 and MD2, respectively. Celik and Temiz (2022) reported that lactobacilli isolate from traditional yoghurts of Turkey exhibited significantly different ($p<0.05$) viscosities. The authors reported that these LAB isolates had different acidification profiles which could be a possible reason for differences in their viscosity values. Similar variations in viscosity were reported by Behare et al. (2013) in different dahi samples produced using various starter cultures. Xu et al. (2015) observed that differences in acidifying rates of yoghurt starters resulted in yoghurts with varying viscosities. Starter cultures with slow acidification rates produced yoghurts with higher viscosities. The results obtained in the current investigation were in contradiction to those mentioned by Xu et al. (2015). Our results

Fig. 7 Sensory evaluation of prepared *Misti dahi* samples

A. Colour and appearance score, B. Flavor score, C. Acidity score, D. Body and texture score E. Wheying off score, F. Overall acceptability score

Different superscripts represent the significant difference ($p<0.05$) across the cultures (*Analysed by one-way ANOVA following Tukey test*).



were supported by Ruas-Madiedo et al. (2005) who advocated that proteolytic activity of the strains did not seem to play any significant role in rheological attributes.

Sensory properties

Nine-point hedonic scale was used for sensory evaluation, and factors such flavour, body and texture, colour and appearance, acidity, wheying off, and overall acceptability were considered. The outcome depicted in Fig. 7 shows that there are discernible differences between the four distinct *Misti dahi* samples, suggesting that the product prepared with *S. thermophilus* MD3 can provide an end product of comparable quality to commercial culture i.e., *S. thermophilus* NCDC436. Whereas, in case of negative control (NCDC74), the sensory score was significantly ($p<0.05$) lower than the others. Raju and Pal (2009) found no significant differences in sensory attributes between the prepared *Misti dahi* samples and the control sample. Similarly, Akter et al. (2010) noted no significant difference in the organoleptic scores of the prepared dahi samples.

Conclusion

The present study focused on isolating and characterizing sugar-tolerant cultures to identify suitable strains for *Misti dahi* production. The selection of an ideal culture is crucial to achieve the desired attributes of the final product. Among the isolated cultures, *S. thermophilus* MD3 exhibited superior results in terms of technological, sensory, and rheological parameters of *Misti dahi*. This study confirms the suitability of *S. thermophilus* MD3 for *Misti dahi* production. Furthermore, the comprehensive evaluation of microbiological quality reaffirms the safety of the final product.

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