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RESEARCH ARTICLE

# Development of indigenous *Streptococcus thermophilus* multi-strain starter culture blend for *Dahi* production

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**Abstract:** Present study aimed to develop an indigenous *Streptococcus thermophilus* multi-strain starter culture blend for the preparation of *dahi*. A total of 89 raw milk and curd samples were collected for the isolation of *Streptococcus thermophilus* strains. Based on the morphological and genotypic methods 24 isolates were characterized as *Streptococcus thermophilus* strains. These strains were evaluated for the presence of *Als* and *eps*A gene and assessed for acid production, textural, flavour and sensory properties in 11% (w/v) Reconstituted Skimmed Milk. From the 24 *S. thermophilus* strains, 17 and 15 strains were found positive for *Als* and *epsA* gene respectively. Three strains exhibiting desirable characteristics such as reduced curd formation time, optimal pH and titratable acidity, and improved curd quality were selected for blend formulation, and their compatibility was evaluated using cross-streak and spot assay techniques. Among these, blend D1 (St1:St7:St17 in a 1:1:1 ratio) demonstrated better performance, with a curd-setting time of 3.20 ± 0.05 h, pH of 4.48 ± 0.01, titratable acidity of 0.68 ± 0.02 % LA, and a total viable lactic count of 9.87 ± 0.04 $\log_{10}$ CFU/mL. The developed blend thus offers a promising option for *dahi* manufacture and can be conveniently adopted by small and medium scale manufacturers.

**Keywords:** *Dahi*, *Streptococcus thermophilus*, Starter culture, Multi-strain blend, Technological properties

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## Introduction

*Dahi*, a traditional fermented milk product, occupies a unique and essential position in the Indian diet due to its nutritional, therapeutic and cultural importance (Mudgal and Prajapati, 2017). It is consumed across all regions of India in various forms as plain curd, in sweetened or salted preparations, and as a base for various dishes. Traditionally, *dahi* has been prepared at the household or cottage industry level to meet local consumption needs. With increasing urbanization, changes in consumer lifestyles, and the growing awareness of health benefits associated with fermented foods, its production has expanded into the organized dairy sector.

The conventional method of *dahi* preparation involves boiling milk to ensure microbial safety, cooling it to around 40°C, and inoculating it with a portion of previously set curd comprising mixtures of unknown lactic culture. The inoculated milk is then incubated until coagulation occurs, forming a soft, mildly acidic, and gel-like curd. In unorganized sectors, undefined mixed starters are commonly used, leading to inconsistency in acidity, flavour, and texture formation among the batches of production. These variations can result in product defects such as weak body, grainy texture, or whey separation, which affect consumer acceptability. In organized dairy plants, however, the use of defined starter cultures allows better control over fermentation, ensuring uniform acidification, consistent quality, and desirable sensory attributes in the final product.

Among lactic acid bacteria (LAB), *Streptococcus thermophilus* is one of the most important thermophilic starter organisms widely employed in milk and dairy fermentations. It is known for its rapid acid production, ability to enhance product texture, and compatibility with other LAB strains like *Lactobacillus delbrueckii* subsp. *bulgaricus* (González et al. 2022). In Indian dairy industry, *S. thermophilus* is often preferred for *dahi* production due to its fast curd-setting ability, high acidification rate, and adaptability to milk environment. However, when used as a single-strain starter, *S. thermophilus* has certain limitations that affect the quality of the final product. *Dahi* prepared solely with *S. thermophilus* often exhibits weak gel strength, higher whey separation (syneresis), and poor mouthfeel (Han et al. 2022;

Sarkar et al. 2022). These defects not only lower consumer acceptance but also make storage and handling more difficult. Hence, multi-strain starter cultures are preferred in commercial *dahi* production to achieve improved body, texture, and balanced flavour (Majumder et al. 2025).

Currently, there are few indigenous multi-strain *S. thermophilus* blends in the market with fast acidifying rates and better textural attributes. Thermophilic cultures are widely used and are particularly suitable for specific geographical regions because they perform well with local milk and environmental conditions (Mudgal and Prajapati, 2017). This underscores the importance of isolating novel, indigenous thermophilic LAB strains adapted to regional conditions that can also be adequately blended for optimal performance. Compatibility among strains within a blend is essential to avoid antagonistic interactions that may negatively affect the final product (Fidan et al. 2022). Blending strains with complementary traits can enhance fermentation efficiency, improve sensory attributes, and strengthen overall starter-culture performance (Chukwuma et al. 2025).

The present study focused on the isolation and characterisation of indigenous *S. thermophilus* strains and the development of multi-strain blends for good-quality industrial *dahi* production. The novelty of this work involves the integration of strains with complementary functional attributes, such as rapid acidification, EPS production, and improved textural properties, enabling the development of robust starter cultures for consistent production of industrial *dahi* suited to local milk characteristics and environmental conditions.

## Materials and Methods

### Collection of samples

A total of 89 samples comprising raw milk, *lassi* and curd were collected from households and local markets of Karnal, Kurukshetra, Panipat, Sonipat and Yamunanagar districts of Haryana, India (Supp. Table 1). The samples were immediately placed in a sterile ice-box to maintain low temperature and transported to the laboratory for further microbiological analysis.

### Isolation of *Streptococcus thermophilus* cultures

For isolation of *S. thermophilus* cultures, each sample was serially diluted in sterile saline solution (0.85% NaCl prepared in distilled water, w/v). From the suitable dilutions ($10^{-6}$ , $10^{-7}$ , and $10^{-8}$, 1 ml aliquots were aseptically transferred to sterile Petri plates, followed by the addition of molten M17 agar medium. After gentle mixing and solidification, the plates were incubated at 37°C and 42°C for 24-48 h to allow the growth of *Streptococcus thermophilus*. Colonies showing typical morphological characteristics of circular, smooth, convex, creamy-white, with glistening surface were carefully selected and subcultured in sterile skim milk and M17 broth for purification. The isolates were examined microscopically using negative staining to observe cell morphology, size, and arrangement. To confirm the absence of *Enterococcus* contamination, all isolates were streaked on Bile Esculin Azide (BEA) agar plates and incubated as per the method described by Chuard and Reller (1998). The isolates showing no blackening on BEA medium were retained as presumptive lactic strains for further characterization.

### Genetic identification and characterization

Genomic DNA of all isolates was extracted following the method of Pospiech and Neumann (1995). The 16S rRNA gene was amplified using universal primers 27F (52 -AGA GTT TGA TCM TGG CTC AG-32 ) and 1492R (52 -TAC GGY TAC CTT GTT ACG ACT T-32 ) (Frank et al. 2008). PCR was performed in a BIO-RAD S1000 thermal cycler with initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation (95°C, 30 s), annealing (59°C, 35 s), and extension (72°C, 2 min), with a final extension at 72°C for 10 min. The expected ~1500 bp product was confirmed by agarose gel electrophoresis and sequenced by Sanger sequencing (Biologia Research India Pvt. Ltd., Haryana). The obtained sequences were aligned and assembled using Chromas v2.2.6, and identity was confirmed using BLAST search against NCBI GenBank (www.ncbi.nlm.nih.gov/blast). Multiple sequence alignment was carried out using MAFFT (https://mafft.cbrc.jp/alignment/server/) with default settings (Katoh and Standley, 2013). The aligned sequences were then used to construct a phylogenetic tree using MEGA software version 12 (Kumar et al. 2018). The Maximum Likelihood (ML) method was employed with the Tamura-Nei 93 (TN93) nucleotide substitution model to estimate evolutionary differences (Tamura and Nei, 1993). Bootstrap analysis (1000 replicates) was used to assess the robustness of clustering and evolutionary relationships among isolates.

Random Amplified Polymorphic DNA (RAPD) analysis was carried out to generate unique RAPD profiles and differentiate the isolates at the strain level. DNA amplification was performed using the universal RAPD primer M-13 (5'-GAG GGT GGC GGT TCT-3'), as described by Jarocki et al. (2020) and synthesized by Eurofins Genomics, India. PCR was conducted using 2X DreamTaq™ Green PCR Master Mix (Thermo Scientific) on a BIO-RAD S1000 thermal cycler with the following program: initial denaturation step at 95 °C for 7 min, followed by 40 cycles of denaturation (95 °C for 30 s), annealing (35 °C for 30 s), and extension (72 °C for 4 min), and a final extension at 72 °C for 10 min. The amplified products were resolved on a 1.8% agarose gel and visualized under UV light after staining with ethidium bromide (EtBr).

Further, isolates were screened for *Als* (encoding á-acetolactate synthase) and *epsA* (encoding transcriptional regulator) genes. The *Als* gene was amplified using primers reported by Lo et al. (2018). Similarly, the *epsA* gene involved in exopolysaccharide

synthesis was detected using primers described by Low et al. (1998). PCR products were confirmed by agarose gel electrophoresis.

**Technological screening and compatibility assessment of *S. thermophilus* isolates**

The technological attributes of *S. thermophilus* isolates, including curd setting time (CST), pH, titratable acidity (TA), and diacetyl production, were evaluated in 11% (w/v) reconstituted skim milk (RSM) following standard procedures outlined by AOAC (2007). Strain compatibility among the isolates was assessed using the streak and spot assay as described by Varada et al. (2022), with *Bacillus subtilis* NCDC 234 was used as the positive control, where NCDC refers to the National Collection of Dairy Cultures. The selected cultures were streaked perpendicularly and also spotted onto M17 agar plates, followed by incubation at 42°C for 24 h. After incubation, the plates were examined for inhibitory zones at the junctions of different streaks or spots. The absence of any inhibition zone was considered as an indication of compatibility between the strains.

**Optimization of culture combination for blend formulation**

Based on technological screening and compatibility assessment, three compatible strains of *S. thermophilus* were selected for blend formulation. These cultures were combined in four different ratios, i.e., 1:1:1, 1:0.5:0.5, 0.5:1:0.5, and 0.5:0.5:1 (v/v), to identify the most suitable combination for *dahi* preparation. Each blend was inoculated at 2% (v/v) into reconstituted skim milk (RSM) containing 11% (w/v) milk solids and incubated at 42°C until curdling of milk. The curd setting time, pH, titratable acidity and diacetyl production were determined as per standard methods. The time required for firm curd formation was recorded as the curd setting time. The blend showing desirable fermentation behavior and product characteristic was considered for further studies.

**Preparation of *dahi***

Toned milk procured from the local market was standardized to 14% total solids by adding 2.5% skim milk powder (SMP). The milk was heated to 90°C for 10 min, cooled to 42°C, and inoculated with 2% (v/v) of the selected *S. thermophilus* blend cultures. The milk inoculated with culture was 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* NCDC 325 from the National Collection of Dairy Cultures (NCDC), ICAR-NDRI, Karnal and a commercial Direct-Vat-Set (DVS) culture were used as positive controls, as described previously (Majumder et al. 2025).

**Analysis of *dahi***

The physico-chemical, microbiological, and sensory attributes of the *dahi* were evaluated to compare the performance of the developed blends with the standard cultures.

**Physico-chemical analysis**

The *dahi* samples prepared using different culture combinations were analysed for curd setting time, pH, titratable acidity, lactic count, and creatine test to compare their performance. For determination of syneresis, the refrigerated *dahi* samples were stirred 20 times alternately in clockwise and anticlockwise directions using a glass rod to ensure uniform mixing. About 30 g of the stirred sample was transferred into a 50 mL polypropylene centrifuge tube and kept for 2 h at 5°C for stabilization. The samples were then centrifuged at 3000 rpm for 15 min at 10°C to facilitate whey separation (Hussain et al. 2016). Syneresis (%) was calculated as the percentage of whey separated from the initial weight of the sample using the following formula:

$$\text{Syneresis (\%)} = \frac{\text{Volume of whey separated}}{\text{Initial yoghurt sample volume}} \times 100$$

**Microbiological analysis**

Microbiological evaluation of the *dahi* made using selected blends was carried out to assess the viability of LAB and the microbiological safety of the products. For each blend, viable *S. thermophilus* counts were determined by plating appropriate serial dilutions on M17 agar followed by incubation at 42°C for 48 h. The results were expressed as log10 CFU/mL. Microbial safety was assessed by screening for coliforms, yeasts, and molds. For coliform detection, violet red bile agar was used, while yeast and mold counts were determined using potato dextrose agar acidified to pH 3.5 with tartaric acid. Plates were incubated at 37°C for 24 h for coliforms and at 25°C for 5 days for yeasts and molds.

**Rheological analysis**

The flow behaviour of prepared *dahi* samples was determined using rheometer (MCR 52, Anton Paar, Ostifildern, Germany) sing cone and plate configuration (CP-75) having 75 mm diameter. Temperature was maintained at 20°C. The shear rate was increased stepwise from 0.01 to 100 $s^{-1}$, and the corresponding shear stress was recorded at each step (Steffe, 1996).

Texture profile of the prepared *dahi* samples were analyzed by using the method given by Hussain et al. (2016). Texture profiling was carried out by using a TA.HD + C Analyzer (Texture Technologies Corp., UK, Model TA.HD + C, version 05.26 along with 5 Kg load cell). A cylindrical probe (P25) having diameter of 25 mm was allowed to penetrate the *dahi* surfaces in cups of 50

mL volume at temperature of around 20-25°C. The textural properties measured included firmness, consistency, cohesiveness, and work of cohesion.

**Sensory analysis**

Sensory evaluation of *dahi* samples was carried out for flavour, body and texture, colour and appearance, acidity, wheying off, and overall acceptability using a 9-point hedonic scale. The assessment was performed by expert panel from the Divisions of Dairy Microbiology and Dairy Technology. Prior to evaluation, the samples were brought to room temperature to facilitate proper sensory analysis.

**Confirmation of presence of different strains in optimized blend**

To confirm presence of strains in the finally optimized blend, RAPD-PCR technique was employed. The diluted samples were then plated on M17 agar (HiMedia, India) using the pour plate method. After solidification, the plates were incubated at 42°C for 24h. Following incubation, 40 isolated colonies were randomly selected using a sterile inoculating needle and transferred into sterile M17 broth tubes. These broth cultures were further incubated at 42°C for 24h to obtain active cultures, from which template DNA was subsequently extracted for molecular analysis. The genomic DNA was subjected to RAPD-PCR using M13 primer and the amplified products were resolved on a 1.8% agarose gel and visualized under UV light after staining with ethidium bromide (EtBr). The RAPD fingerprints generated from the isolates recovered from *dahi* were compared with the standard RAPD profiles obtained earlier to confirm the presence of respective strains in the optimized blend.

**Statistical analysis**

Three independent trials (n = 3) were carried out for each experi-ment and the results are expressed as mean ± standard error of mean. One way ANOVA (Analysis of variance) followed by Tukey's test were carried out in GraphPad Prism (ver-sion 10) to determine significant differences among the mean values of a particular attribute ($p < 0.05$).

**Results and Discussion**

**Isolation and genotypic characterization of thermophilic lactic culture**

A total of 80 presumptive thermophilic lactic isolates were obtained from milk, *lassi*, and curd samples collected from different locations of Haryana. To ensure maximum recovery of thermophilic lactic cultures, the isolation was carried out at two incubation temperatures, 37°C and 42°C. The use of both incubation conditions facilitated effective isolation of thermophilic LAB from milk and fermented dairy products, as also reported in earlier studies (Goa et al. 2022; Khusboo et al. 2023). Among the isolates, 56 exhibited growth on BEA, which is a characteristic feature of *Enterococcus* spp. (Supp. Table 2). Twenty-four isolates, presumptively identified as *Streptococcus thermophilus,* were selected for further study based on specific criteria, such as absence of growth on BEA agar, characteristic colony morphology (cream to whitish, smooth, and convex colonies) and ability to grow at 42 °C. The colony characteristics observed were consistent with previous reports (Taye et al. 2021). Notably, milk samples predominantly yielded *Enterococcus* isolates, as revealed by their positive growth on BEA agar. In contrast, *dahi* and *lassi* samples provided isolates exhibiting characteristics typical of *S. thermophilus*, suggesting the predominance of this species in fermented dairy products. Minor morphological variations were observed under negative staining, indicating subtle phenotypic diversity among the isolates within the thermophilic LAB group.The nucleotide sequences obtained from 16S rRNA gene Sanger sequencing were trimmed and aligned using Chromas software (v2.6.6) and compared with reference sequences in the NCBI database through BLAST analysis. The isolates showed 92.25–99.75% similarity with reference *S. thermophilus* strains, confirming their genetic identity (Supp. Table 3). These findings clearly indicated that the isolated cultures matched closely with standard *S. thermophilus* reference strains available in public databases.

A maximum-likelihood phylogenetic analysis performed using MEGA12 provided a clear understanding of the genetic relationships among the *S. thermophilus* isolates. The phylogenetic tree, constructed using 1,483 non-coding sites under the Tamura–Nei model, produced several well-supported clusters with node supports ranging from 88–96%, and a total branch length of 0.621 (Supp. Fig. 1). The clade structure of the isolates closely aligned with previously reported *S. thermophilus* lineages described in population and comparative genomic studies (Alexandraki et al. 2019).

Based on the RAPD-PCR, most *S. thermophilus* isolates exhibited a conserved RAPD band of approximately 500 bp, along with distinct polymorphic bands differentiating the strains a pattern typical of M13-based RAPD (Fig. 1a). Under optimized amplification conditions, the RAPD profiles were reproducible with the core bands persisting across repeats and minor faint bands appearing occasionally due to thermocycler variations. These results confirm the genetic relatedness of the isolates with standard reference *S. thermophilus* strains, while highlighting intraspecies diversity among them (Olive and Bean, 1999).

Further, the presence of *Als* gene was evaluated among the isolates. Seventeen out of 24 isolates, isolated from dairy products, were positive for the *Als* gene, yielding a 397 bp amplicon (Fig. 1b). The positive control culture also showed amplification of the expected *Als* gene fragment. Majumder et al. (2025) mentioned screening of *Als* gene in fast-growing *S. thermophilus* strains at of 397 bp amplicon size. PCR screening

for the *epsA* gene revealed that 15 out of 24 isolates provided the expected 750 bp amplicon size (Fig. 1c). This indicated a conserved regulatory mechanism for EPS production among *S. thermophilus* isolates of dairy origin (Mozzi et al. 2006). The detection of the *Als* and *epsA* genes is significant because it indicates that most *S. thermophilus* isolates possess the genetic potential for flavour production and exopolysaccharide (EPS) synthesis. EPS production is particularly important, as it improves the texture, viscosity, and mouthfeel of fermented dairy products such as dahi, thereby enhancing the technological and functional quality of starter cultures used in the dairy industry. The identification of isolates carrying both genes provides a useful basis for selecting robust starter cultures with desirable fermentation performance and textural properties.

**Technological screening and compatibility assessment of *S. thermophilus* strains**

The technological performance of *S. thermophilus* strains was assessed in 11% (w/v) RSM based on CST, pH, and TA, which together indicate fermentation efficiency and curd quality. These interrelated parameters influence the development of desirable texture, flavour, and sensory characteristics in fermented milk products. The CST of the isolates varied from 3.43 ± 0.30 h (St16) to 7.15 ± 0.09 h (St15) **(**Suppl. Table 4). The pH values ranged from 4.35 ± 0.03 (St2) to 4.88 ± 0.04 (St12), while TA varied between 0.56 ± 0.02% (St12) and 0.76 ± 0.01% LA (St2 and St5). This variation indicated differences in acidification rates and metabolic activities among the *S. thermophilus* strains. Based on balanced acidification, desirable curd texture and flavour, three strains, St3, St7, and St17, were selected for blend formulation (Suppl. Table 4). They showed CST values of 4.05 ± 0.05, 4.87 ± 0.30, and 4.82 ± 0.26 h, with corresponding pH of 4.43 ± 0.07, 4.54 ± 0.01, and 4.53 ± 0.01, and TA of 0.70 ± 0.04, 0.69 ± 0.01, and 0.73 ± 0.01% LA, respectively. Faster and uniform acidification generally promotes better protein network formation, firm curds and reduced whey separation, whereas slow or uneven acidification results in weak curds and higher syneresis (Arab et al. 2023). The observed differences among strains are in line with these physicochemical properties and reflect strain-specific characteristics that influence product texture and sensory quality (Brodziak et al. 2020).

*S. thermophilus* St3 produced firm curd with pleasant flavour and clean cut along with positive diacetyl test. *S. thermophilus* St17 also showed diacetyl production, giving a mild buttery aroma, while *S. thermophilus* St7, though diacetyl-negative, formed firm curd with good texture and mild acidic flavour. Genotypic analysis revealed that St3 possessed both *Als* and *epsA* genes, St17 carried the *Als* gene, and St7 lacked both. The presence of the *Als* gene is associated with diacetyl production through á-acetolactate synthase activity (García-Quintáns et al. 2008), while *epsA* regulates exopolysaccharide synthesis that improves curd consistency and mouthfeel (Mozzi et al. 2006). The genetic profile of the isolates therefore correlated well with their technological performance.

Compatibility of three strains evaluated by spot and streak assay is presented in Supp. Fig. 2. The absence of inhibition zones indicated mutual compatibility and suitability of cultures for preparation of thermophilic starter culture blend. Similar results were reported by Majumder et al. (2025), who observed no inhibitory effects among *S. thermophilus* strains tested by co-streak assays. Likewise, Urcan et al. (2024) demonstrated compatible growth among *Lactiplantibacillus plantarum*, *Lactobacillus acidophilus* and *Apilactobacillus kunkeei* in co-culture studies, suggesting compatibility assay as prerequisite for starter blend evaluation. The three *S. thermophilus* strains, St3, St7, and St17, exhibited desirable technological traits and mutual compatibility, and were therefore considered suitable candidates for the development of a thermophilic starter culture blend for *dahi*.

**Optimization of culture combination for blend formulation**

Three *S. thermophilus* strains (St3, St7, and St17) were blended in different ratios such as D1 (St3:St7:St17, 1:1:1), D2 (St3:St7:St17, 1:0.5:0.5), D3 (St3:St7:St17, 0.5:1:0.5), and D4 (St3:St7:St17, 0.5:0.5:1) in RSM. For comparison, NCDC 325 and a commercial DVS culture were used. The performance of these strains was evaluated in terms of CST, pH, TA, and diacetyl production (Suppl. Table 5). All the blended cultures and the commercial DVS culture showed significantly ($p < 0.05$) reduced CST in RSM as compared to NCDC 325, with faster setting observed in blends D1 and D2. The pH and TA values after curd setting, though varied slightly among the blends and control cultures, remained within the acceptable range. Yadav et al. (2007) reported that prepared *dahi* had a pH of 4.93, titratable acidity of 0.89% lactic acid, and a total lactic count of approximately 8 log10 CFU/mL.

All four blends, NCDC 325 and commercial DVS culture were positive for the creatine test, confirming adequate diacetyl production, which contributed to the characteristic aroma of skim milk curd. The presence of *Als* positive isolates (St3 and St17) in the blends likely supported diacetyl production through alpha-acetolactate synthase activity (García-Quintáns et al. 2008). Majumder et al. (2025) also reported positive response in terms of presence of *Als* gene in four *S. thermophilus* strains.

The blended cultures exhibited suitable technological characteristics, like efficient acidification, lower syneresis, and adequate flavour production. Hence, *S. thermophilus* multi-strain blends were considered technologically suitable and were subsequently assessed for their performance in toned milk fermentation.

**Physico-chemical properties**

A comparative evaluation of the four *dahi* blends along with NCDC 325 and the commercial DVS culture was carried out, in which blend D1 showed the shortest curd setting time (3.20 ± 0.05 h) (Table 1). The TA, pH, and diacetyl production of *dahi* prepared from toned milk were nearly similar across all blends and control cultures. Syneresis analysis showed that *dah*i made with NCDC 325 and the commercial DVS culture had higher whey separation, while blend D1 recorded the lowest syneresis (32.45%), followed by D2, with D3 and D4 showing intermediate values (Table 1). This reduction in whey separation could be attributed to the combined activity of compatible strains and possible EPS production, which enhances water-holding capacity and curd firmness (Behare et al. 2013).

These variations could be attributed to the proportion of the EPS-producing strain (St3) present in each formulation. The blends D1 and D2, which contained a higher proportion of St3, exhibited comparatively lower acidity and reduced syneresis. The lower whey separation in these blends may be due to the formation of a protein–polysaccharide matrix, where the exopolysaccharides produced by St3 help retain water by entrapping moisture within the three-dimensional curd network (Nachtigall et al. 2023; Suthar et al. 2025).

**Microbiological properties**

In *dahi* the viable counts (log10 CFU/mL) were higher in D1, and Commercial DVS samples (~9.68–9.87), while D2, D3, and D4 had slightly lower counts (~9.1–9.3). Coliforms and yeast and mold were not detected in *dahi* samples. Majumder et al. (2025) reported the total lactic count of ~9 log10 CFU/mL which indicates our developed blend is equally effective for preparation of fermented milk.

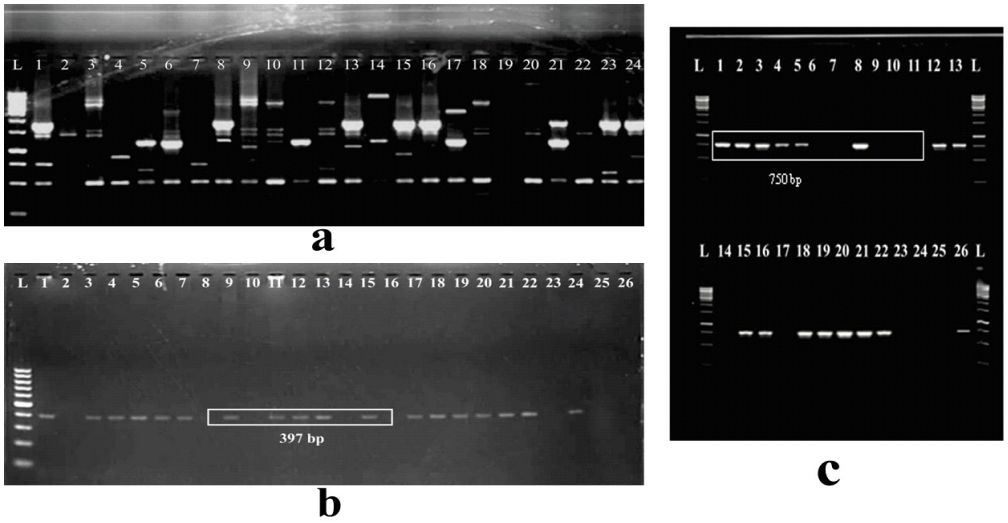



Fig. 1 Molecular characterization of *S. thermophilus* strains.(a) RAPD-PCR fingerprints; Lane L, 1 kb DNA ladder; Lanes 1–24, St1–St24. (b) PCR amplification of the *als* gene; Lane L, 100 bp DNA ladder; Lane 1, NCDC 325 (positive control); Lane 2, NCDC 75 (negative control); Lanes 3–26, St1–St24. (c) PCR amplification of the *eps*A gene; Lane L, 1 kb DNA ladder; Lanes 1–24, St1–St24; Lane 25, NCDC 75 (negative control); Lane 26, NCDC 757 (positive control).

Table 1. Physico-chemical, textural and microbiological properties of *dahi* prepared using different blends in toned milk

| Blend | Curd Setting Time (h) | pH | TA (%L.A.) | Syneresis (%) | Firmness (g) | Consistency (g.sec) | Cohesiveness (g) | Work of cohesion (g.sec) | Total viable count ($log_{10}$ CFU/ml) |
|---|---|---|---|---|---|---|---|---|---|
| NCDC 325 | 3.95 ± 0.23$^{a}$ | 4.33 ± 0.06$^{c}$ | 0.73 ± 0.01$^{ab}$ | 40.02 ± 0.50$^{ab}$ | 44.10 ± 1.43$^{a}$ | 285.14 ± 7.98$^{a}$ | -5.28 ± 0.61$^{a}$ | 172.77 ± 4.82$^{a}$ | 9.48 ± 0.14$^{a}$ |
| D1 | 3.20 ± 0.05$^{b}$ | 4.48 ± 0.01$^{ac}$ | 0.68 ± 0.02$^{b}$ | 32.45 ± 0.65$^{e}$ | 143.87 ± 2.26$^{b}$ | 1022.28 ± 45.35$^{b}$ | -15.54 ± 0.42$^{b}$ | 293.86 ± 3.81$^{b}$ | 9.87 ± 0.04$^{a}$ |
| D2 | 3.22 ± 0.04$^{b}$ | 4.58 ± 0.02$^{a}$ | 0.68 ± 0.02$^{b}$ | 37.05 ± 0.44$^{d}$ | 122.40 ± 3.92$^{c}$ | 753.80 ± 50.73$^{c}$ | -18.32 ± 1.89$^{bc}$ | 301.70 ± 7.26$^{b}$ | 8.97 ± 0.04$^{b}$ |
| D3 | 3.27 ± 0.09$^{b}$ | 4.37 ± 0.03$^{bc}$ | 0.74 ± 0.03$^{ab}$ | 38.89 ± 0.34$^{bc}$ | 106.04 ± 4.98$^{c}$ | 703.02 ± 26.83$^{c}$ | -13.86 ± 0.83$^{b}$ | 292.01 ± 8.60$^{b}$ | 9.02 ± 0.06$^{b}$ |
| D4 | 3.42 ± 0.06$^{b}$ | 4.48 ± 0.02$^{ab}$ | 0.69 ± 0.01$^{b}$ | 37.66 ± 0.45$^{cd}$ | 82.47 ± 2.56$^{d}$ | 540.41 ± 16.19$^{d}$ | -8.51 ± 0.59$^{a}$ | 212.73 ± 12.18$^{c}$ | 8.72 ± 0.12$^{b}$ |
| Commercial DVS | 3.87 ± 0.19$^{b}$ | 4.18 ± 0.02$^{d}$ | 0.74 ± 0.02$^{a}$ | 40.81 ± 0.34$^{a}$ | 176.63 ± 4.17$^{e}$ | 937.72 ± 14.19$^{b}$ | -21.04 ± 0.94$^{c}$ | 321.85 ± 3.40$^{b}$ | 9.68 ± 0.11$^{a}$ |

Data expressed as mean ± SEM (n=3); Different superscripts represent the significant difference ($p<0.05$) across the blends (*Analysed by one-way ANOVA following Tukey test).*

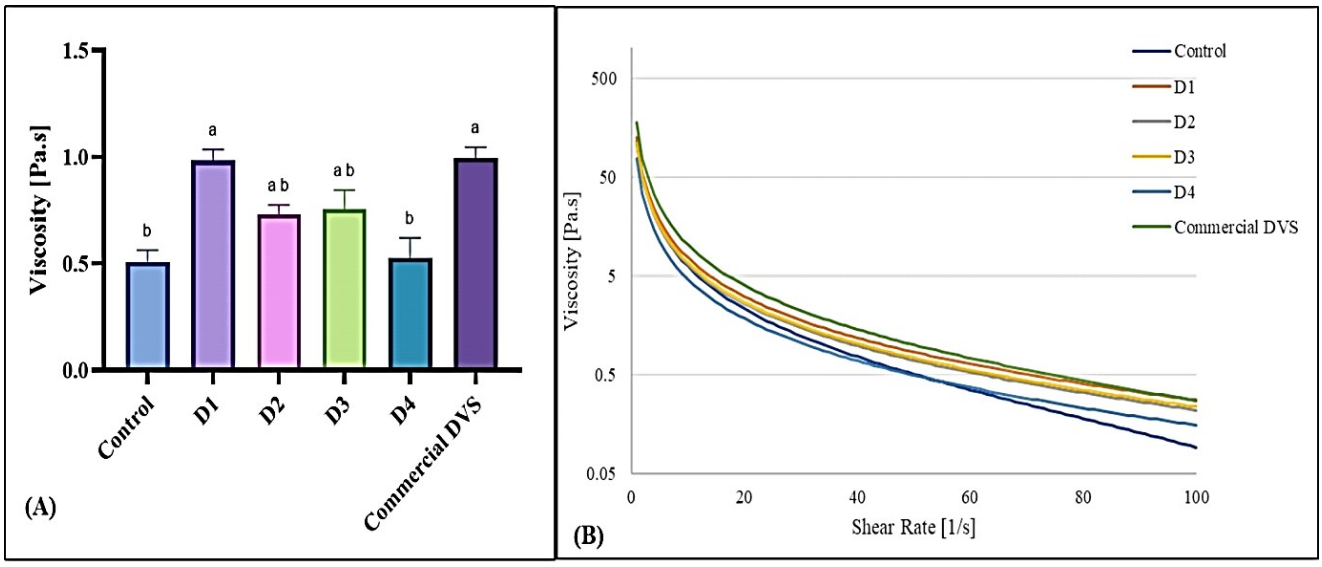



Fig. 2 Rheological analysis of *dahi* blends in toned milk. A. Viscosity of different blends at 50 $s^{-1}$ shear rate. B. Flow curve of different *dahi* blends. Data expressed as mean ± SEM (n=3); Different superscripts represent the significant difference ($p<0.05$) across the blends (*Analysed by one-way ANOVA following Tukey test)*

**Rheological properties**

The rheological analysis of *dahi* prepared using different blends and control cultures revealed notable variations in viscosity and textural characteristics. *Dahi* prepared using NCDC 325 exhibited a viscosity of 0.51 Pa.s, whereas blend D1 and the commercial DVS culture showed the highest viscosities (0.98 and 1.00 Pa.s, respectively), both being significantly ($p < 0.05$) higher than NCDC 325 at a shear rate of 50 $s^{-1}$ (Fig. 2A).

The flow curves (Fig. 2B) showed typical pseudoplastic behavior, with viscosity decreasing as shear rate increased. Blends D1 and the commercial DVS maintained higher viscosity throughout, indicating stronger gel networks, likely due to the EPS-producing strain (St3) enhancing protein–polysaccharide interactions. Intermediate viscosities in D2 and D3 suggest that balanced proportions of EPS-positive and EPS-negative strains moderately strengthen the gel, whereas D4 exhibited lower viscosity, reflecting a lesser EPS contribution. Shear-thinning behavior across all samples confirms the typical rheology of *dahi*, consistent with Zhang et al. (2016), who reported that EPS-producing cultures improve viscosity, mouthfeel, and stability. Texture profile analysis supported these observations, with D1 and D2 showing the highest firmness and consistency (Table 1), while other blends also demonstrated improvements compared to controls. The enhanced textural characteristics can be attributed to stronger protein–polysaccharide interactions, contributing to gel elasticity, cohesiveness, and reduced syneresis, in agreement with Madhubasani et al. (2020). Similar observations were made by Hassan et al. (2003) for fermented milk prepared with EPS-producing strains that exhibits greater consistency. Arab et al. (2023) also noted that stronger protein interaction and a higher protein-to-solids ratio leads to improved gel elasticity, reduced rupture, and better texture properties, which are clearly reflected in blends D1 and D2.

**Sensory properties**

Sensory evaluation of the prepared *dahi* samples was carried out using the 9-point hedonic scale and different parameters like flavour, body and texture, color and appearance, acidity, wheying-off, and overall acceptability was assessed whose results have been shown in Fig. 3. The parameters like body and texture and overall acceptability show significant differences in the blends against the NCDC 325 culture and commercial DVS, where D1 was highly preferred. Even the other blends were equally good. Majumder et al. (2025) performed a similar study where the prepared *dahi* blends had same scores against the *dahi* made using NCDC 325 and commercial DVS. The blend D1 showed better sensory scores as compared to the other followed by D2 and D3 which can be attributed to the balanced and better textural and rheological properties. A study conducted by Paul et al. (2016) affirmed that increasing the total solids of milk to 15% (from the typical 11.5% of toned milk) produced *dahi* having optimal firmness and consistency, resulted in highest overall acceptability scores among samples.

**Confirmation of presence of different strains in optimized blend**

The prepared *dahi* blends were confirmed for the presence of the individual strains in the prepared products by RAPD-PCR technique. In case of D1 the RAPD-PCR agarose gel electrophoresis image showed the presence of the three strains,

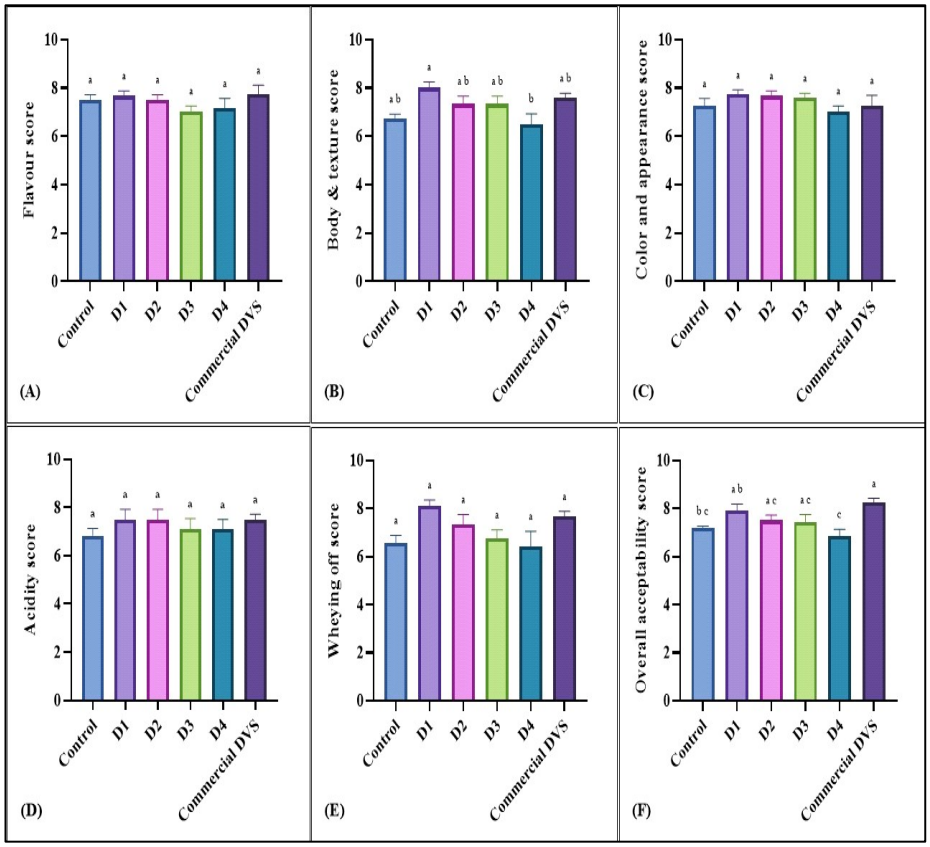



Fig. 3 Sensory evaluation of the *dahi* blends in toned milk. Data expressed as mean ± SEM (n=3); Different superscripts represent the significant difference ($p<0.05$) across the blends (*Analysed by one-way ANOVA following Tukey test).*

(St3, St17, St7) at approximately optimum ratio (out of 40 colonies, 12 shown similar pattern like St17, 14 shown pattern like St3 and rest shown similar pattern like St7) in the blend (Fig. 4). Majumder et al. (2025) reported different PCR amplicon pattern for different strains of *S. thermophilus*. Marasco et al. (2022) concluded significant difference in RAPD profiling of different isolates of *S. thermophilus*, *Lb. bulgaricus* and *L. lactis* ssp. *lactis*. Similarly, Collado and Hernа´ndez (2007) reported different DNA fingerprinting patterns at the strain level for different starter culture/ probiotic organisms, including *Bifidobacterium*, *Streptococcus* and *Lactobacillus*, indicating genetic differences even at the strain level.

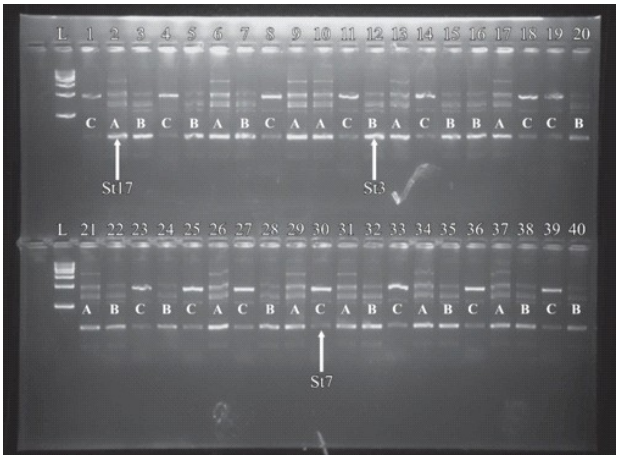



Fig. 4 RAPD profile of the *S. thermophilus* strains present in *dahi* blend D1. Lane L: 1 kb ladder, Lane 1-40: isolates; A: St17 strain, B: St3 strain, C: St7 strain

## Conclusion

This study successfully isolated, characterized, and optimized indigenous *Streptococcus thermophilus* strains for developing multi-strain starter culture blends for *dahi* production. Among the formulations, blend D1 (St3:St7:St17, 1:1:1) demonstrated the most desirable physico-chemical, rheological and sensory characteristics. The improved texture and water-holding capacity of D1 could be attributed to the synergistic activity of the EPS-producing strain (St3) and diacetyl-producing strains (St3 and St17), which together enhanced curd firmness, flavour, and overall product quality. The findings suggest that the D1 blend offers a promising indigenous alternative to commercial DVS cultures for *dahi* manufacture. Further pilot-scale validation and assessment of storage stability are recommended to establish its industrial applicability.

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