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Evaluation of Technological Attributes of *Lactococcus lactis* Cultures for Preparation of *Dahi* and *Misti Dahi* (Sweetened *Dahi*)

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ABSTRACT

Background: *Dahi* and *Misti dahi* are traditional Indian fermented milk products. The present study was aimed to evaluate the technological attributes of selected six *Lactococcus lactis* cultures namely NCDC 193, 125, 128, 94, 97 and 314 for preparation of *Dahi* and *Misti dahi*.

Methods: Six *Lactococcus lactis* were collected and tested for acidification profile as well as growth rate was also determined in milk with (15%) and without sucrose. Their flavor producing ability, proteolytic ability by OPA method and antimicrobial activity against four spoilage causing microorganisms (*Escherichia coli* NCDC 134, *Staphylococcus aureus* NCDC 109, *Micrococcus luteus* NCDC 131 and *Pseudomonas fluorescenes* NCDC 316) were evaluated.

Result: All the six cultures were able to grow and produce acidity in milk containing 15% sucrose and similar acidification profile as well as growth rate (K) were found in milk without sucrose. Cultures NCDC 193, 128 and 125 were found to produce flavoring compound (diacetyl). Proteolytic activity of the selected cultures ranged between 0.17 to 0.41 mg/mL of Leucine. Among six cultures three *Lactococcus lactis cultures* NCDC 314, 94 and 97 have shown zone of inhibition against all four test organisms.

Key words: Acidification activity, Antimicrobial activity, Growth rate, *Lactococcus lactis*, Sensory evaluation.

INTRODUCTION

Production of Dahi with defined single strain culture of *Lactococcus lactis* or a combination of cultures containing Lactobacillus and Lactococcus have reported in production of good quality product. Traditionally for preparation of *Dahi* is prepared by back slopping method known as small portion of product containing microbes used as a source of starter culture, is generally added to milk. Due to use of undefined cultures various flavor defects (fruity, alcoholic, acidic, flat taste) and textural defects such as gassiness, weak body, wheying off has been observed in fermented products. The sweetened variety of dahi is popularly known as *Misti dahi* or misthi doi (lal dahi, payodht) from the eastern part of India. Starter culture is added at ambient temperature (37°C) and filled into earthen pots followed by left undisturbed overnight for fermentation and served chilled (De 1980; Aneja *et al.* 2002; Singh 2007). A wide variation in total solids content (27-43%), non fat milk solids (11-16%), fat (1-12%) and sucrose (13-19%) in the market samples of Misti dahi have been reported by Ghosh and Rajorhia (1987). Production of *Dahi* and *Misti dahi* has taken a big leap from domestic scale to organized sector and is expected to grow further.

Lactococcus lactis (*L. lactis subsp. lactis, L. lactis* subsp. *cremoris* and *L. lactis* subsp. lactis biovar. diacetylactis) are widely used for the manufacture of fermented dairy products including cheese, butter, dahi *etc*. *Lactococcal* strains used in commercial starter cultures possess numerous characteristics such as lactose and citrate fermentation abilities, proteolytic activity, bacteriocin production, bacteriophage resistance, exopolysaccharides production *etc*. These properties can contribute to the desired ?avor and texture of the fermented dairy products and optimal growth on the milk components lactose and casein, as well as stability and survival. Due to mesophilic nature of *Lactococcus* cultures they perform optimally at ambient conditions and hence, good quality products can also be prepared at household level and unorganized sectors at room temperature. Thus, there is a need for defined strains of mesophilic cultures. Therefore, in the present study technological attributes of mesophilic cultures were evaluated for the preparation of good quality products at ambient temperature.

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MATERIALS AND METHODS

Six *Lactococcus lactis* cultures were procured from National Collection of Dairy Cultures (NCDC), ICAR-National Dairy Research Institute, Karnal, Haryana. All the procured starter cultures were inoculated in M-17 medium (HiMedia, Mumbai, India) followed by incubated at 30°C for 12 h for attaining high activity and stored at -20°C till further use. In addition, cultures were preserved in sterile reconstituted skimmed milk (RSM; 12 g skim milk powder + 100 mL distilled water). The study was performed during the interval of July 2016 to January 2017, in the Department of Dairy Microbiology and Department of Dairy Technology, ICAR-National Dairy Research Institute, Karnal, Haryana.

Growth rate determination

Growth performances of the each culture were evaluated in RSM containing 15% sucrose along with control (0% sucrose). Based on initial and final viable count growth rate (K) and generation time (G) was determined using equation:

$$n = Kt = \frac{N_t - N_0}{\log 2} \quad (1)$$

$$K = \frac{n}{t} \quad (2)$$

Where,

N_t= Log CFU/mL after 12 h.

N_0= Log CFU/mL after 0 h.

K= Grow rate constant.

G= Generation time (h).

t= Time (h).

Acidification profile

Acidification profile of the cultures was done by inoculation @ 1% in sterile 10% (w/v) RSM tubes as well as in heat treated RSM (25 mL) spiked with 15% sucrose followed by incubation at 30°C. Physico-chemical parameters such as pH and titratable acidity (TA expressed as % LA) were determined at 0, 4, 8 10 and 12h of incubation (AOAC, 1975).

Sugar tolerance test

A varied quantity of sucrose was added to 20 mL of fresh buffalo skim milk (9% SNF, 0.1% fat) test tubes to make the concentration of 5, 10, 15 and 20% sucrose (w/v). The contents were sterilized at 121°C for 15 min followed by inoculated @ 1% with the experimental cultures at ambient temperature. A positive control sample with 0% sucrose was also run simultaneously for accuracy.

Flavor production

Flavor production was estimated using O' Mearas Test with some modifications (Hammer, 1935). Fermented skim milk (2 mL) was mixed with an equal quantity of NaOH solution (40%) in clean sterilized test tube followed by small quantity (1-2 mg) of creatine powder was added and shaken. The tube was allowed to stand for 10 min in dark. Formation of pink ring on the surface of the content was indicated production of C-4 carbonyl compounds.

Proteolytic activity

Lactococcus cultures were assessed for proteolytic activity by measuring liberated amino acids and peptides by o-phthaldialdehyde (OPA) method (Church *et al.* 1983).

Antimicrobial activity

Antimicrobial activity of the strains were tested against selected known spoilage organisms (*Escherichia coli* NCDC 134, *Staphylococcus aureus* NCDC 109, *Micrococcus luteus* NCDC 131 and *Pseudomonas fluorescens* NCDC 316) of foods by using agar well diffusion assay as per method of Schillinger and Lucke (1989) with some modifications.

Preparation of *Dahi* and *Misti Dahi*

Dahi and *Misti dahi* was prepared using the method depicted by Ghosh and Rajorhia (1990) with slight modification (Fig 1).

Analysis of *Dahi* and *Misti dahi* samples

Total lactic count of the *dahi* and *misti dahi* samples were enumerated using serial dilution with sterile phosphate buffer and plated on M17 agar followed by incubated at 30°C for 24-48 h. The viable count was expressed in log10 colony forming units per gram (CFU/g). The pH of samples was determined with a pH meter (Orion Star A211, Thermo Scientific

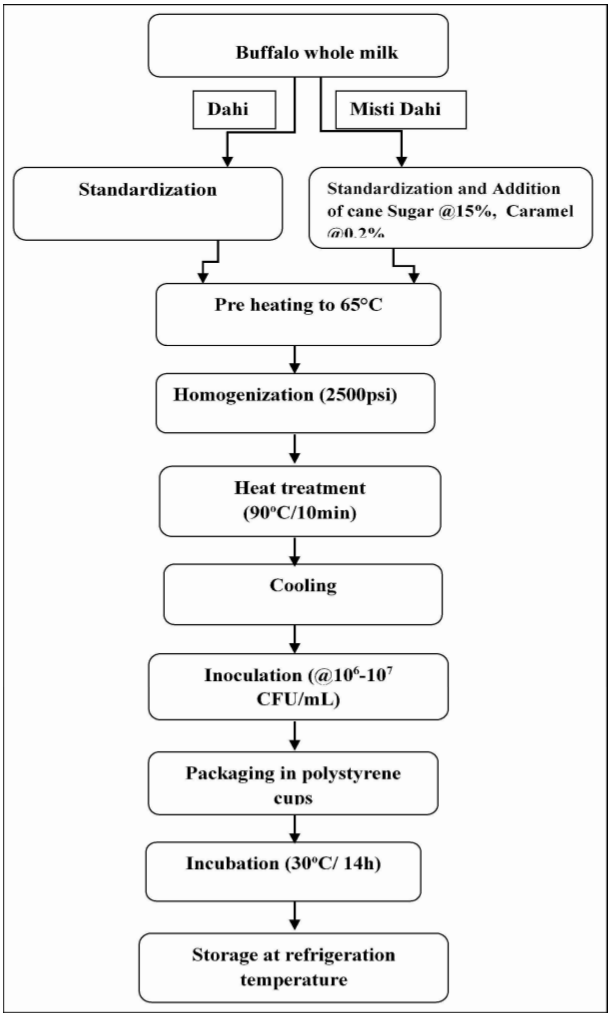

Fig 1: Flow diagram for preparation of *Dahi* and *Misti dahi.*

Orion, Waltham, MA USA). Titratable acidity was expressed in terms of % lactic acid (%LA) by adopting the method by AOAC (AOAC, 1975).

Sensory analysis

Dahi and *Misti dahi* samples was evaluated for sensory attributes such as flavor, body and texture, color and appearance, on 100 point composite score card with corresponding maximum scores being 45, 40 and 15, respectively. The panel consists of five discriminative judges from the Faculty of Dairy Technology, Dairy Chemistry and Dairy Microbiology Division of NDRI, Karnal. The sample was drawn from the refrigerator before serving for judging.

Statistical analysis

The results obtained in the present study were subjected to one-way analysis of variance (ANOVA) using Prism software (version 2005). Differences were compared by Tukey's test for all experiments and significant differences were determined at 95% level of confidence. The mean values and standard error were calculated from triplicate experimental data and results were expressed in Mean ± SEM for all experimental analysis.

RESULTS AND DISCUSSION

Effect of sucrose on growth and acidification profile

The effect of sucrose on the acidification profile of cultures was depicted in (Fig 2). Initially the pH and %TA of the milk (0% and 15% sucrose) was similar at the time of culture addition *i.e.* 6.6 and 0.12 respectively. After 12 h of incubation NCDC 193 and 125 were found to be the most sucrose tolerant cultures followed by NCDC 128 as the %TA was not affected by the presence of sucrose. The %TA of milk (15% sucrose) fermented by NCDC 125, 193 and 128 was 0.80, 0.81 and 0.72, respectively. Similarly, %TA of milk (0% sucrose) fermented by NCDC 125, 193 and 128 was 0.81, 0.85 and 0.81, respectively. The pH observed for NCDC 125, 193, and 128 cultured milk spiked with 15% sucrose was 4.44, 4.44 and 4.40 similar to fermented milk with 0% sucrose was 4.32, 4.40 and 4.32, respectively. However, growth of rest of the three cultures *i.e.* NCDC 314, 97 and 94 were less in milk with 15% sucrose as compared to milk with 0% sucrose. The %TA (with 15% sucrose) was 0.63, 0.63, and 0.65 as compared to %TA (milk without sucrose) of 0.85, 0.77 and 1.03, respectively. After 12h of fermentation the pH ranged from 4.27 to 4.60 which is more than 2 unit reduction in most of the cultures in both cases. It can be clearly observed form figures that the selected cultures (NCDC 125, 193, 128, 314, 97 and 94) were able to tolerate the 15% sucrose concentration as well as able to produce the desirable acidity and pH with consistent rate as in case of 0% sucrose. As per the results obtained, the reduction in pH and elevation in %TA, it can be interpreted that cultures are fast acidifying and sucrose tolerant. Our results were in concurrence with the observation made by Psoni *et al.*, (2007), Ozkalp *et al.*, (2007) and Dahou *et al.,* (2021). Moreover, no morphological changes were also observed during the experiment. Sucrose tolerance (14% and 16% sucrose concentration) of yoghurt culture and *Lactococcus lactis* has also been clearly demonstrated (Gosh and Rajorhia, 1990). Generation time of the selected cultures varied from 1.35 h (NCDC 193) to 2.27 h (NCDC 125). Similarly, slightly lower generation time was observed in case of milk containing 15% sucrose as compared to milk with 0% sucrose to lowest for NCDC 193 (1.45 h) and highest for NCDC 125 (3.14 h). The generation time (hours) was used as a tool to investigate the microbial growth dynamics of the pure cultures and demonstrate the variation in the growth of bacteria. The increase in generation time or prolonged lag phase was expected due to difficulty of the cultures to utilize the disaccharide sucrose (Desai *et al.*, 2004).

Flavor production (Diacetyl production)

Flavor production is mainly responsible for the consumer acceptance of the fermented products. The flavor forming capacity also varies between strains of same species (Cogan, 1982). Flavor production by *L. lactis* is found to be strain dependent, which may be due to specific transport and fermentation systems of citrate. The production of flavor compounds (Diacetyl) also varied according to the cultures used with highest color intensity produced by NCDC 193 and 128 followed by NCDC 125 after 12 h of incubation. NCDC 94 and 97 was produced minor quantity flavor whereas NCDC 314 was not able to produce any flavor compounds. The results obtained showed a clear relation was found between the rate of acid production and flavor compounds. As cultures NCDC 193 and 128 have shown higher acidification rate and flavor compounds production as compared to other selected *Lactococcus* cultures. Lindsay *et al.*, (1965) reported that different strains of *L. lactis* ssp. *lactis*, *L. lactis* ssp. cremoris and *L. lactis* ssp. *diacetylactis* produce diacetyl in the range of 0.1 to 3.24 ppm.

Protein hydrolysis of cultures in RSM medium

The proteolytic activity of skim milk fermentation by the cultures was illustrated in (Table 1). The activity varied among the different strains of *Lactococcus* cultures and have shown adequate proteolytic activity which ranged from 0.17 to 0.41 mg/mL of Leucine. Highest proteolytic activity was shown by NCDC 94 (0.41) recognized by producing the highest amount of free amino groups followed by NCDC 97 (0.36), NCDC 128, NCDC 314, NCDC 193 and NCDC 125. In addition, the results obtained from this study that the cultures were found fast acidifier with high proteolytic activity. The results obtained were in agreement with the observations by Nomura *et al.* (2006) in skim milk at 1% inoculation level. The final acidity (%LA), as well as the rate of acidification of yogurt cultures depend on the individual strains and their interaction (Béal *et al.*, 1999). Proteolysis results in production of essential growth factors as peptides and amino acids which provide protective effect and helps in improvement of survival of the starters (Ramchandran and Shah, 2010). These fast acidifiers and good proteolytic *Lactococcus* cultures could be used as starter cultures to start initial acidification. In OPA-method released a-amino groups

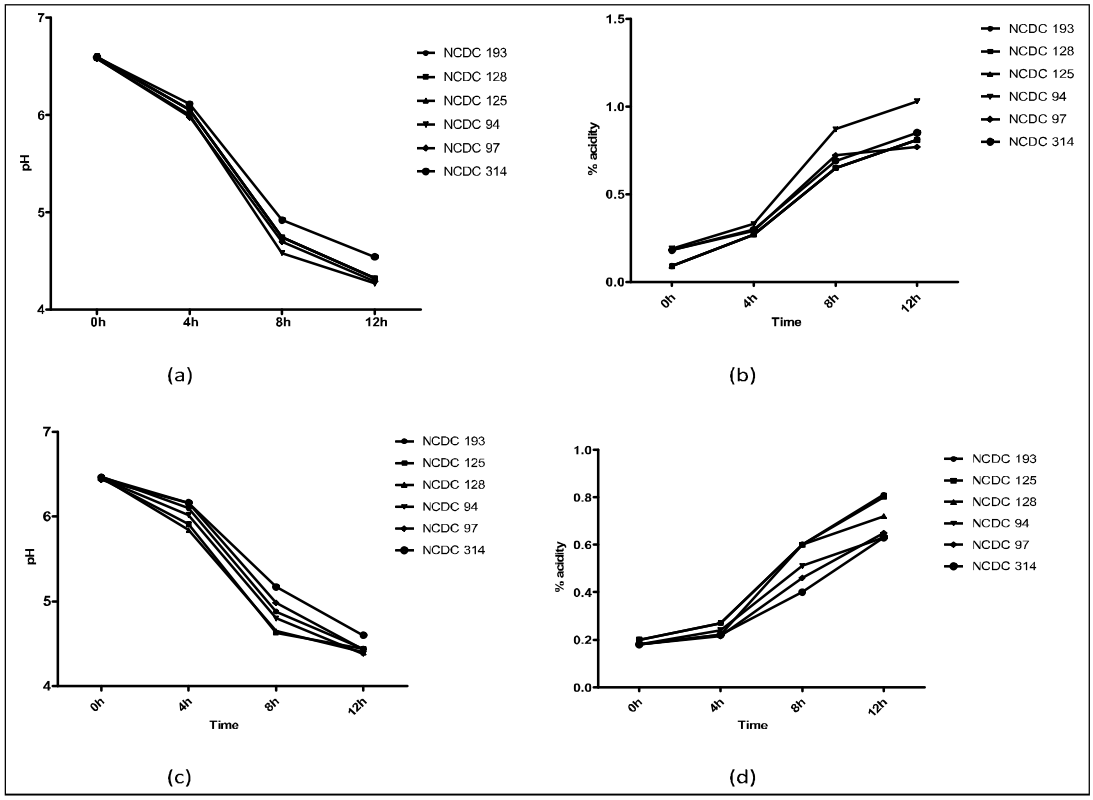

Fig 2: Acidification profile of cultures (a) pH at 0% sucrose (b) acidity at 0% sucrose (c) pH at 15% sucrose (d) acidity at 15% sucrose.

Table 1: Exhibition of different important technological attributes by six *L. lactis* strains after 12 h of growth.

Strain	Curd setting time (h)		Viable count (log CFU/mL)		Generation time (h)		Proteolytic activity (mg of leucine/mL)
	0% sucrose	15% sucrose	0% sucrose	15% sucrose	Without sucrose	15% sucrose	
NCDC 193	10	12	9.42±0.04	9.34±0.03	1.35	1.45	0.17±0.24
NCDC 97	12	11.5	9.59±0.05	9.30±0.07	1.43	1.58	0.36±0.21
NCDC 314	10	11.5	9.47±0.02	9.39±0.04	1.41	1.70	0.23±0.34
NCDC 125	12	14	9.31±0.04	8.91±0.06	2.27	3.14	0.10±0.15
NCDC 94	11	13	9.23±0.06	9.21±0.05	1.82	2.15	0.41±0.28
NCDC 128	11	13.5	9.41±0.03	9.32±0.06	1.40	1.47	0.22±0.33

Values are means ± standard error, n=3.

produced after the proteolysis of milk protein is detected spectrophotometrically, thus giving a direct measurement of proteolytic activity. The degree of protein hydrolysis is expressed as content of mg/mL of Lucine after 24 h of fermentation at 30°C in reconstituted skim milk. *L. lactis* possesses a number of genes whose products are involved in the utilization of proteins present in milk, such as an extracellular protease, peptide transport systems and intracellular peptidases (Kok and Buist, 2003). LAB proteinases (*Lactococcus* sp. and *Lactobacillus* sp.) are able to hydrolyse more than 40% of the peptide bonds of aS1-CN and β-CN, which results in the formation of more than100 different oligopeptides that are further degraded by the peptidase system (Choi *et al*., 2012).

Antagonistic activity against spoilage and pathogenic bacteria

The zone of inhibition by different *Lactococcus* strains against spoilage and pathogenic bacteria are shown in Fig 3. Among all the cultures NCDC 97 have shown good zone of inhibition against the four test organism with maximum against spoilage bacteria *i.e*. *P. fluorescenes* NCDC 316 (16 mm). NCDC 94 (13 -23 mm) and 314 (14-22 mm) were also found to have inhibitory activity against all the test organisms with varying zone of inhibition. As NCDC 97, 314 and 94 had wider range of inhibitory effect as they are active against all the test organisms whereas NCDC 193 was found to be least active culture as zone of inhibition was shown

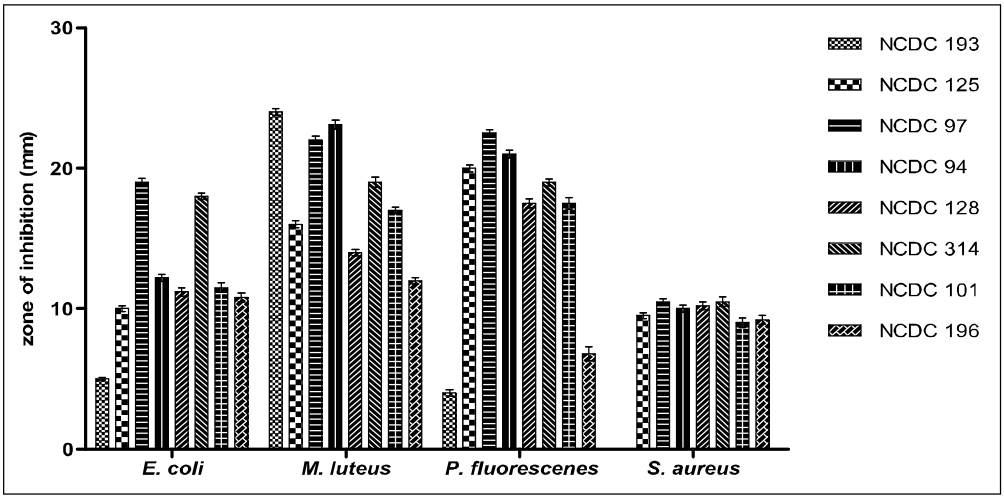

Fig 3: Antimicrobial activity of six *L. lactis* cultures against four test organisms Data are expressed as means ± SEM.

against *M. luteus* only. Similarly, cultures NCDC 128 and 125 have shown zone of inhibition against *M. luteus* and *P. fluorescenes.* The main activity of *Lactococcus* cultures was found against *M. luteus* NCDC 131 which is a pathogenic organism in dairy products. In a study conducted by Bachanti *et al*. (2017) out of 12 selected *Lactobacilli* spp all the cultures have shown antagonistic activity against *S. aureus* and weak to moderate antibacterial activity was observed against *E. coli* by most of the cultures. The effect of inhibition is probably due to proteinaceous nature of the cultures, which is yet to be proved. This inhibitory effect is also contributed by the lowering of pH below 6.0 and production of compounds such as lactate, acetate, organic acid and bacteriocins and this fact may contribute to their colonizing and competitive ability (Balciunas *et al.,* 2013). The results obtained were supported by previous studies (Atanasova *et al*., 2014 and Alomar *et al*., 2008) that the variable antimicrobial activity had been showed by *Lactococcus lactis*.

Microbiological and physio-chemical properties of *Dahi* and *Misti dahi*

The microbiological and physio-chemical properties of prepared *Dahi* and *Misti dahi* after 12 h of incubation at 30°C is shown in (Table 2). The pH value for dahi ranged from 4.53 to 4.62 and 4.64 to 4.73 for *Misti dahi.* The lowest value was reported in *Dahi* prepared using NCDC 94 (4.53) followed by NCDC 125 (4.57), in case of *Misti Dahi* NCDC 314 (4.64) exhibited minimum pH value followed by NCDC 94 (4.65). The range of pH values observed here are in accordance with the values recommended by Frye (2013) for yoghurt. Lowering the pH by Lactic acid bacteria is due to production of lactic acid during fermentation of milk- lactose (Eke *et al,* 2013). These pH values are satisfactory in order to prevent any pathogenic growth on the products, thus increasing its shelf life (Donkor *et al.*, 2006).

The titratable acidity also ranged from 0.71 to 0.81% LA after 12h of incubation at 30°C for *Dahi* and 0.74 to 0.81% LA for *Misti dahi*. Maximum acid production was observed by NCDC 94 (0.81%) for *Dahi*, whereas in case of *Misti dahi* NCDC 193 and 128 (0.81%) produced highest amount of lactic acid during fermentation. It is suggested that acidity in fermented products should be within definite limits for consumer acceptance. The TA of all yoghurt formulations in our results was more than the minimum recommended limit of 0.6% by Codex (2011). The TA obtained for *Misti Dahi* was found less than the results obtained by Chatterjee *et al.*, (2015).

The total lactic count present in *Dahi* and *Misti dahi* samples ranged from 9.41 to 9.58 log CFU/mL and 8.94 to 9.28 log CFU/mL respectively. Maximum growth was observed by NCDC 193 in both the products, which shows that the culture was able to tolerate high amount of sucrose (15%) as well as total solids. All the cultures performed well in *Misti dahi* samples also which has higher total solid content and less difference was found in viability of cells as compared to *Dahi* samples. Our results were in agreement with the findings of Mahdian and Tehrani (2007) who showed that growth and activity of starter bacteria improved in samples with higher amounts of total solids. All the *Dahi* and *Misti dahi* samples had a total lactic count of >9 log10 CFU/g which is the minimum limit recommended by National Yoghurt Association of the USA. A higher bacterial cell produces metabolites during fermentation which has beneficial effects on human health.

Sensory evaluation

Acceptable scores for Flavor, body and texture and color and appearance in case of *Misti dahi*, were ranged between 40.2-44.8 ot of 45, 36.0-36.8 out of 40 and 8.6-9.2 out of 15, respectively. The sensory scores for *Dahi* and *Misti dahi* are presented in Table 3. *Dahi* highest score for flavor, body and texture was obtained by NCDC 97 (46.75 and 38.0), and for color and appearance NCDC 125 obtained highest

Table 2: Physico-chemical and microbiological characteristics of *Dahi* prepared with *L. lactis* ssp. *lactis* cultures.

Strain	Viable count (log_{10} CFU/g)		pH		Titratable acidity (%LA)	
	Dahi	*Misti dahi*	*Dahi*	*Misti dahi*	*Dahi*	*Misti dahi*
NCDC 193	9.58±0.28	9.28±0.28	4.58±0.05	4.70±0.05	0.71±0.05	0.81±0.05
NCDC 97	9.46±0.22	9.18±0.22	4.59±0.03	4.72±0.04	0.72±0.04	0.74±0.08
NCDC 314	9.52±0.21	9.31±0.21	4.58±0.04	4.64±0.06	0.74±0.08	0.76±0.05
NCDC 125	9.41±0.16	8.94±0.16	4.57±0.02	4.73±0.08	0.80±0.09	0.78±0.06
NCDC 94	9.43±0.19	9.03±0.19	4.53±0.06	4.65±0.08	0.81±0.06	0.80±0.04
NCDC 128	9.27±0.18	9.07±0.18	4.62±0.03	4.68±0.06	0.72±0.04	0.81±0.08

Table 3: Sensory score of *Dahi* and *Misti dahi* prepared by selected *L. lactis* ssp. *lactis* cultures.

Strains	Parameters							
	Flavor		Body and texture		Color and appearance		Overall acceptability	
	Dahi	*Misti dahi*	*Dahi*	*Misti dahi*	*Dahi*	*Misti dahi*	*Dahi*	*Misti dahi*
NCDC 314	43.5±1.5^{a}	44.2±0.68^{a}	37.0±0.7^{a}	36.2±0.35^{a}	8.5±0.28^{a}	8.6±0.80^{a}	89.0±1.20^{a}	89.0±0.90^{a}
NCDC 97	44.7±0.47^{a}	44.8±0.36^{a}	36.75±0.25^{a}	36.6±1.02^{a}	8.25±0.12^{a}	8.8±0.31^{a}	89.75±0.85^{a}	90.0±0.65^{a}
NCDC 128	41.75±0.36^{b}	40.2±0.65^{b}	37.63±1.02^{a}	36.6±0.68^{a}	9.12±0.42^{a}	8.9±0.12^{a}	88.5±0.29^{a}	85.1±0.85^{b}
NCDC 94	46.75±0.75^{a}	45.0±0.90^{a}	38.0±0.15^{a}	36.6±0.30^{a}	8.5±0.20^{a}	8.6±0.40^{a}	93.25±0.60^{b}	90.0±0.28^{a}
NCDC 193	41.75±0.31^{b}	40.5±0.75^{b}	36.13±0.65^{a}	36.8±0.40^{a}	8.65±0.68^{a}	8.8±0.28^{a}	88.5±0.35^{a}	85.0±0.6^{b}
NCDC 125	44.25±0.8^{a}	40.75±0.3^{b}	35.38±0.12^{b}	36.0±1.2^{a}	9.25±0.3^{a}	9.2±0.47^{a}	90.13±0.2^{a}	84.0±0.25^{b}

^{a,b,c,d} Mean (±SE) values with different superscript with in a column differ significantly ($p<0.05$).

score (9.25). Overall acceptable sensory scores of *Dahi* for flavor, body and texture and color and appearance were ranged between 41.75-46.75, 35.38-38.00 and 8.50-9.25, respectively. NCDC 97 scored highest for flavor and body and texture attributes but for color and appearance NCDC 125 found to obtain maximum score. Overall, *Dahi* prepared with NCDC 97 (93.25) obtained significantly highest total score ($P<0.05\%$) due to its flavor producing ability whereas, NCDC 314 (89.0), NCDC 94 (90.0) and NCDC 97 (90.0) obtained significantly higher total score scores for *Misti dahi* preparation, as the texture obtained with NCDC 314 is most acceptable. The sensory scores obtained clearly prove that both the products prepared using the six starter cultures were acceptable.

CONCLUSION

All the six cultures were able to grow and ferment in presence of 15% sucrose as well as without sucrose after 12h of incubation at 30°C. As the cultures have antagonistic property against common pathogenic and spoilage microorganisms, the shelf life of products will be enhanced. Based on the sensory analysis results obtained, it was concluded that acceptable quality of *Dahi* and *Misti dahi* could be prepared using these six cultures with good amount of viable bacteria.

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