www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(10): 701-705

www.thepharmajournal.com
Received: 02-07-2022
Accepted: 06-08-2022

Shivaraj Shivashimpar
M-Tech (Dairy Microbiology), Department of Dairy Microbiology, Dairy Science College, KVAFSU, Hebbal, Karnataka, India

Poornima
M-Tech (Dairy Technology), Department of Dairy Technology, Dairy Science College, KVAFSU, Hebbal, Karnataka, India

Ramachandra B
Associate Professor and Head, Department of Dairy Microbiology, Dairy Science College, KVAFSU, Hebbal, Karnataka, India

Corresponding Author:
Shivaraj Shivashimpar
M-Tech (Dairy Microbiology), Department of Dairy Microbiology, Dairy Science College, KVAFSU, Hebbal, Karnataka, India

Characterization of lactic cultures from domestic dahi samples: A research study

Shivaraj Shivashimpar, Poornima and Ramachandra B

Abstract
Curd or dahi is one of the most consumed fermented milk products. Ample of microorganisms are responsible for the curdling of milk including bacteria and yeasts. The curd samples collected were analyzed for their microflora and the samples consists of lactococci viable count ranged with an average of 7.90 $\log_{10}$ cfu/g whereas Leuconostocs with an average of 6.4 $\log_{10}$ cfu/g streptococci with an average of 5.94 $\log_{10}$ cfu/g. In respect of lactobacilli with an average of 7.90 $\log_{10}$ cfu/g. In the case of LFY with an average of 4.55 $\log_{10}$ cfu/g. It is observed that Lactococci and Lactobacilli were highest and nearly the same in all the samples, and these were followed by Leuconostoc, streptococci and LFY. In characterization, the obtained lactic isolates were obtained as Lactococci isolates were identified as *Lactococcus lactis* ssp. *lactis*, Leuconostoc isolates were identified as *Leuconostoc mesenteroides* ssp. *mesenteroides.* The *Streptococcus thermophilus* isolates were identified as *Streptococcus thermophilus.* The lactobacilli isolates were identified as *Lactobacillus delbrueckii* ssp. *bulgaricus.* Finally, lactose fermenting yeast isolates were identified as *Kluyveromyces lactis.*

Keywords: Curd, dahi, lactic acid bacteria, lactose fermenting yeast

Introduction
Milk is an almost complete food as it contains both energy-supplying nutrients in the form of lactose, and milk fat and body-building nutrients such as milk protein and minerals like calcium, and phosphorus are part and parcel of milk. In order to increase the keeping quality, among the various preservation methods, heat treatment is one the most popular method of preservation followed by fermentation.

Archaeological evidence has indicated that the process of fermentation in foods was discovered accidentally thousands of years ago. However, over time it has soon become apparent that many fermented foods had longer storage lives and improved nutritional values compared to their unfermented equivalents, making this form of food processing a popular technique. The role of fermented milk in human nutrition is well documented and the virtues of these products were known to man even during the ancient days of civilization. These products have long been an important component of a nutritional diet. The medicinal and nutritional properties of various fermented foods have been experienced for several generations. Recently, importance has been given to producing fermented milk with improved health attributes particularly the therapeutic properties of these products. A fermented milk product has been defined by the International Dairy Federation as a milk product prepared from skimmed milk or not with specific cultures. The microflora is kept alive until the sale to the consumers and may not contain any pathogenic germs (Ray and Sonali, 2014) [13]. The consumer's interest in fermented milk products is gaining momentum due to the development of new food processing techniques, changing social attitudes; scientific evidence of the health benefits of certain ingredients. Some cultured dairy foods such as Bioghurt, Yakult, Actimel etc. are already marketed as therapeutic and dietetic products.

Fermentation is the conversion of carbohydrates to organic acids or alcohol and carbon dioxide, using lactic bacteria and yeasts under anaerobic conditions to produce fermented milk products. The most common microorganisms observed in fermented milk products belong to the strains of LAB, *Lactobacillus, Leuconostoc, Lactococcus*, etc. (Hao *et al.*, 2010) [4]. Recently, there is a growing interest to develop a variety of fermented milk products for other beneficial purposes, particularly for health purposes and preventing toxins produced by foodborne pathogens and spoilage bacteria that enter the human body. The beneficial effects of fermented milk products are produced by a variety of bioactive compounds of LAB. LAB represent the most extensively studied bacteria for milk fermentation.

The presence of LAB in milk fermentation can be either spontaneous or inoculated starter cultures. Milk itself is known as one of the natural habitats of LAB. Although under spontaneous fermentations the growth of LAB cannot be predicted or controlled, this procedure has been practised and carried out traditionally for years. A procedure called back slopping is often used (Widyastuti and Febrisiantosa, 2014) [14]

LAB play a major role in determining the positive health effects of fermented milk. The health benefits of fermented milk include prevention of gastrointestinal infections, reduction of serum cholesterol levels reducing the per cent of atherosclerosis and possessing anti-mutagenic activity etc. The fermented milk is recommended for consumption by lactose intolerant individuals as most of the lactose will be metabolized by the starter cultures. LAB are the gram-positive, facultative anaerobic, non-spore former, catalase negative, litmus milk reducers. They utilize lactose as the sole source of carbon and in turn, release lactic acid as the principal acid.

Enumeration and isolation of LAB and LFY from dahi have led to the identification of major LAB species that are commonly present in domestic dahi samples. There is a predominance of lactobacilli with a viable log count of 7.845 to 8.477 on acetate agar followed by streptococci of 0-8.17 and a total lactic count of 8.041 to 8.579 on Eliker agar in 15 domestic dahi samples of Bangalore (Mohanan *et al.*, 1984) [7]. Pradeep (2007) [9] found a predomination of lactococci (7.82 $\log_{10}$ cfu/g) followed by lactobacilli (5.45 $\log_{10}$ cfu/g) and leuconostocs (3.20 $\log_{10}$ cfu/ml) by selective plating technique using NRCLA, Rogosa Agar and Sucrose Agar respectively among the microflora of four domestic curd samples collected from Bangalore city, Karnataka.

Rajasekhar *et al*. (2013) [10] enumerated LAB from Dahi samples (50 samples) collected from 10 different zones of Karnataka. Among dahi samples collected, lactococci show an average viable log count per gram of 4.16, streptococci show 3.68, leuconostoc of 3.79 and lactobacilli of 4.43. They isolated 5 isolates of lactococci belongs to *Lactococcus lactis ssp. lactis* (1), *Lactococcus lactis ssp. cremories* (1), *Lactococcus lactis ssp. lactis bv. diacetylactis* (3), among 9 isolates of streptococci, *Streptococcus thermophilus* (2), *Enterococcus faecium* (6), *Enterococcus gallinarum* (1), among 5 isolates of leuconostocs, it included *Leuconostoc mesenteroides ssp. lactis* (1), *Leuconostoc mesenteroides ssp. mesenteroides* (4), and 21 isolates of lactobacilli traced to *Lactobacillus rhamnosus* (1), *Lactobacillus fermentum* (5), *Lactobacillus hilgardii* (1), *Lactobacillus plantarum* (10), *Lactobacillus delbrueckii ssp. bulgaricus* (1), *Lactobacillus equi* (1), *Lactobacillus agilis* (1) and *Lactobacillus viridescens* (1). Further, Bhattarai *et al*. (2013) [2] also isolated LAB from 39 dahi (indigenous dairy product) samples collected from different districts of eastern Nepal. The isolates consist predominately *Lactobacillus fermentum*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Streptococcus thermophilus*, *Lactococcus* ssp. *cremoris*, *Lactococcus lactis* ssp. *lactis biovar diacetylactis* and *Leuconostoc mesenteroids* ssp. *mesenteroides* was found consistently in most of the samples examined. Another study stated that around ~59.38% of lactobacilli, ~21% of streptococci ~8.2% of lactococci and ~11.42% leuconostocs were isolated from indigenous dahi. In the case of lactose fermenting yeast, Ramachandra *et al*. (2009) [12] revealed that among 85 isolates obtained from three whey sources (channa, paneer, cheese), all the 85 isolates of yeasts belonged to the species *Kluyveromyces lactis*.

Materials and Methods

Materials: Curd samples from three different sources, sterilized glass wares, microbiological plating media (M17, MRS and MEA), sterile skim milk and phosphate buffer

Methods: Domestic Curd samples of 10 numbers were collected in sterile sample bottles (100 ml) from KVAFSU campus, Bengaluru and brought to the laboratory and subjected to the enumeration of lactic cultures.

For enumeration, serially diluted 4^{th}, 5^{th}, and 6^{th} dilutions were transferred to the labelled sterile Petri plates for the enumeration of lactococci, streptococci, leuconostocs, lactobacilli and yeast using sterile pipettes. Respective sterile molten agar media such as M17, and MRS maintained between 45°-50 °C of 10-15 ml were poured to labelled lactococci, streptococci and leuconostocs plates as well as for lactobacilli plates respectively. Malt Extract Agar (MEA) was poured into the yeast plates with pH adjusted to 3.5 using 10% Lactic Acid (LA). Later the poured agar plates were allowed to solidify (Harrigan, 1976) [5].

All lactococci and leuconostoc plates were incubated at 30^0 C/24-48 h and lactobacilli and streptococci at 37 °C/24-48 h in an anaerobic candle jar and aerobically at 30 °C/3-5 days for yeast and enumerated after the incubation period. The colonies of LAB and yeast obtained by plating curd samples on agar media were selected, purified, and characterized up to species level based on preliminary and biochemical tests. The typically selected colonies from M17 agar plates as lactococci and streptococci colonies were transferred to sterile M17 broths while leuconostoc & lactobacilli colonies from the MRS agar plates to sterile MRS broth tubes and incubated at respective time-temperature combination, till turbidity was observed. Typical yeast colonies were selected from the Malt extract agar plates transferred to malt extract broth and incubated at 30 °C for 2-3 days. All the isolates were purified thrice by streaking onto sterile poured respective agar plates. The isolates were propagated in respective sterile broths as well as in sterile skim milk and sub-cultured once a week. The yeast isolates were simple stained for confirmation. Species identification of yeast isolates were performed by lactose fermentation, ascospore formation and utilization of ethanol.

Characterization of LAB isolates

The LAB isolates were subjected to preliminary identification tests like Gram's staining, catalase test, litmus milk reaction and carbon dioxide from glucose as per the procedure given by Harrigan (1976) [5]. The final species level identification was successful only after conducting specific biochemical tests for each of the LAB isolates such as lactococci, streptococci, leuconostoc, lactobacilli, LFY.

Preliminary tests were conducted for the isolated lactic cultures like Gram's staining, catalase test, litmus milk reaction, gas from glucose and for lactococci specific biochemical tests were conducted like growth at 40 °C, diacetyl in milk, growth at 45 °C, growth in 6.5% NaCl, growth in 4% NaCl and growth at pH 9.2.

For leuconostocs, dextran production from sucrose, acid from sucrose/trehalose/arabinose, survival at 55 °C/30 min, growth in 3% and 6.5% salt whereas for lactobacilli, growth at 15 °C, 45 °C and fermentation of sucrose were conducted as specific tests to confirm genus lactobacillus up to species level (De Vos *et al.*, 2009) [3].

To identify the lactose fermenting yeasts, tests like simple staining, lactose fermentation, ascospore formation and utilization of ethanol (Ainsworth, 2008) [1].

Result and Discussion

Enumeration of lactic cultures in curd samples from different sources

Curd samples were collected from different locations and their microflora was determined in this research study. Results showed that lactococci viable count ranged from 7.85 to 7.9 with an average of 7.90 log_{10} cfu/g whereas leuconostocs count accounted for around 6.4 log_{10} cfu/g streptococci count were in the range of 5.62 to 6.23 with an average of 5.94 log_{10} cfu/g. In respect of lactobacilli the count ranged from 7.80 to 7.96 with an average of 7.90 log_{10} cfu/g. In the case of LFY counts were between 4.50 and 4.60 with an average of 4.55 log_{10} cfu/g (Table 1). It is observed that lactococci and lactobacilli were highest in the count and nearly the same in all the samples and then these were followed by leuconostocs, streptococci and LFY.

Table 1: Enumeration of LAB and LFY in Curd samples

Sl. No.	Code No.	Curd Samples	Lactococci	Leuconostocs	Streptococci	Lactobacilli	Lactose Fermenting Yeast (LFY)
1	H1	Hostel	7.90	6.40	6.23	7.80	4.60
2	R1	Resident	7.85	6.40	5.62	7.96	4.50
Average			7.90	6.40	5.94	7.90	4.55

Note: H1 and R1 results are an average of 5 samples each

The preliminary and specific tests conducted for the lactic cultures

Identification of lactococci isolates

The lactococci isolates (LC1, LC2) were subjected to the preliminary and specific biochemical tests. The results of these tests and the identity of these isolates were given in the Table 2. Based on these results the lactic isolates viz. LC1 and LC2 were identified as *Lactococcus lactis* ssp. *lactis*.

Table 2: Identification of lactococci isolates from curd sample

Isolates		Growth at 40 °C	Growth at 45 °C	Growth in 4% NaCl	Growth in 6.5% NaCl	Growth at pH 9.2	Diacetyl in milk	Identified isolate
Lactococci	LC1	+	-	+	-	+	-	*Streptococcus thermophilus*
	LC2	+	-	+	-	+	-	*Streptococcus thermophilus*

Note: All these isolates were Gram positive, cocci, catalase negative, ARC in litmus milk, No CO_2 produced

Identification of leuconostocs isolates

The leuconostocs isolates (LU1, LU2) were screened for preliminary tests and specific biochemical tests. Based on the results of these tests, the identity of these isolates was given in the Table 3. By these results, both the LU1 and LU2 were identified as *Leuconostoc mesenteroides* ssp. *mesenteroides*.

Table 3: Identification of leuconostocs isolates from curd sample

Isolates		Dextran production from sucrose	Acid from sucrose	Acid from arabinose	Survival at 50 °C/30 min	Growth in 3% NaCl	Growth in 6.5% NaCl	Identified isolate
Leuconostocs	LU1	+	+	+	-	+	–	*Leuconostoc mesenteroides* ssp. *mesenteroides*
	LU2	+	+	+	-	+	–	*Leuconostoc mesenteroides* ssp. *mesenteroides*

Note: All these isolates were Gram positive, cocci, catalase negative, ARC in litmus milk, CO_2 produced from glucose

Identification of Streptococcus thermophilus isolates

The Streptococcus thermophilus isolates (ST1, ST2) were tested for preliminary and specific biochemical tests. The results of these tests and the identity of these isolates were given in the Table 4. Based on these results both the ST1 and ST2 were identified as *Streptococcus thermophilus*.

Table 4: Identification of *Streptococcus thermophilus* isolates from curd sample

Isolates		Growth at 15 °C	Growth at 45 °C	Growth in 4% NaCl	Growth in 6.5% NaCl	Growth at pH 9.2	Diacetyl in milk	Identified isolate
Streptococci	ST1	+	+	+	-	+	_	*Streptococcus thermophilus*
	ST2	+	+	+	-	+	_	*Streptococcus thermophilus*

Note: All these isolates were Gram positive, cocci, catalase negative, ARC in litmus milk, No CO_2 produced

Identification of lactobacilli isolates

The lactobacilli isolates (LB1, LB2) were tested for preliminary tests and the results of these tests and the identity of these isolates are given in the Table 5. Based on these results both the LB1 and LB2 were identified as *Lactobacillus delbrueckii* ssp. *bulgaricus*.

Table 5: Identification of lactobacilli isolates from curd sample

Isolates		Growth at 15 °C	Growth at 45 °C	Fermentation of sucrose	Identified isolate
Lactobacilli	LB1	-	+	+	*Lactobacillus delbrueckii* ssp. *bulgaricus*
	LB2	-	+	+	*Lactobacillus delbrueckii* ssp. *bulgaricus*

Note: All these isolates were Gram positive, rods, catalase negative, ARC in litmus milk, no CO_2 produced

Identification of LFY isolates

The isolates (LFY1, LFY2) were tested for preliminary tests and the results of these tests and the identity of these isolates are given in the Table 6. Based on these results both the LFY1 and LFY2 were identified as *Kluyveromyces lactis*.

Table 6: Identification of LFY isolates from curd sample

Isolates		Simple staining	Lactose fermentation	Ascospore formation	Identified isolate
Lactose Fermenting Yeast (LFY)	LFY1	+	+	+	*Kluyveromyces lactis*
	LFY2	+	+	+	*Kluyveromyces lactis*

Note: All these isolates were Oval, CO2 produced from lactose, Ethanol utilization positive

Hence, the preliminary and specific tests revealed that the lactococci isolates were identified as *Lactococcus lactis* ssp. *lactis.*, leuconostoc isolates that were identified include *Leuconostoc mesenteroides* ssp. *Mesenteroides*, the streptococcus isolate was identified as *Streptococcus thermophilus, and* the lactobacilli isolate was identified as *Lactobacillus delbrueckii* ssp. *bulgaricus.* Finally, lactose fermenting yeast isolates were identified as *Kluyveromyces lactis.*

The lactic culture lactococci showed the log count with an average of 7.90 $\log_{10}$ cfu/g, leuconostoc counts of 6.4 $\log_{10}$ cfu/g, streptococci count of 5.94 $\log_{10}$ cfu/g and lactobacilli counts of 7.90 $\log_{10}$ cfu/g. In the case of LFY, the average counts were 4.55 $\log_{10}$ cfu/g. By the obtained results, it is observed that lactococci and lactobacilli were highest and nearly the same in all Curd samples and these were followed by leuconostocs, streptococci and LFY. A similar study was reported by Pradeep (2007) [9] where, in domestic dahi samples collected from Bengaluru, the predominance was of lactococci (7.82 $\log_{10}$ cfu/g) followed by lactobacilli (5.45 $\log_{10}$ cfu/g) and leuconostocs (3.20 $\log_{10}$ cfu/g) by selective plating technique using NRCLA, Rogosa Agar and Sucrose Agar respectively. Further, a similar observation was supported by Patel and Patel (2012) [8] where it was observed that the predominance of lactobacilli from dahi prepared from household buffalo milk of Gujarat and have opined that the abundance of lactobacilli could be due to their use as starter culture in the manufacture of buffalo milk-based fermented milk products.

Further Rajashekar *et al*. (2013) [10] have reported a predominance of leuconostoc having a viable $\log_{10}$ count per gram of 4.54 followed by lactobacilli of 4.20, lactococci of 3.7 and streptococci of 3.22 from 10 domestic dahi samples of different zones of Karnataka and Mahesh *et al*. (2017) [6] has also reported that the occurrence of lactococci, leuconostoc, streptococci and lactobacilli was with a viable count ranging from 4.80 to 6.11 $\log_{10}$ cfu/g from 4 dahi samples obtained from different sources on Bengaluru.

While Ramachandra, (2016) [11] collected dahi samples (30 nos.) from 10 locations of Bengaluru and he also reported a similar pattern of the lactic predominance i.e., lactococci having viable $\log_{10}$ count of 4.44 followed by lactobacilli of 4.21, leuconostocs of 3.50 and streptococci 3.56. In the study conducted by Ramachandra *et al.* (2009) [11], on lactose agar (pH- 6.8) viable log count of yeast cells after an incubation period of 72 h at 25 °C was 5.58.

Conclusion

Domestic dahi samples of 10 numbers were collected from every two locations in the sterile sample bottles (100 ml) and subjected to the enumeration of lactic cultures. The Dahi samples were serially diluted and plated using the respective medium for lactococci, leuconostoc, *Streptococcus thermophilus*, lactobacilli and LFY. The plates were incubated at respective time-temperature combinations. It was observed that the viable log count of dahi for lactococci, leuconostoc, streptococci, lactobacilli and lactose fermenting yeasts were 7.90, 6.4, 5.94, 7.90, and 4.55 respectively. It was observed that lactococci and lactobacilli were highest and nearly the same in all the samples and these were followed by leuconostocs, streptococci and lactose fermenting yeast. It can be concluded that in domestic dahi or curd among ample lactic cultures, lactobacillus species dominate.

References

1. Ainsworth GC. Ainsworth & Bisby's dictionary of the fungi. Cabi; c2008.
2. Bhattarai RR, Das SK. Scientific study on indigenous technology of Dahi making of eastern Nepal. Journal of Food Processing and Technology; 2013, 4(8).
3. De Vos P, Garrity GM. Bergey's manual of systematic bacteriology. Springer; c2009.
4. Hao Y, Zhao L, Zhang H, Zhai Z, Huang Y, Liu X, *et al*. Identification of the bacterial biodiversity in koumiss by denaturing gradient gel electrophoresis and species-specific polymerase chain reaction. Journal of dairy science. 2010 May 1;93(5):1926-33.
5. Harrigan WF, McCance ME. Laboratory methods in food and dairy microbiology. Academic Press Inc. (London) Ltd.; c1976.
6. Mahesh Patil, Manjunatha H, Ramachandra B. Shelf life studies of carrot juice enriched probiotic shrikhand. Int. J App. Natu. Sci. 2017;5:95-102
7. Mohanan KR, Shankar PA, Laxminnarayana H. Growth and acid production of dahi starter cultures at sub-optimum temperatures. Indian journal of dairy science; c1984.
8. Patel N, Patel V. Isolation, identification, and antimicrobial activity determination of lactic acid bacteria from buffalo milk in Gujarat state, India. International Journal Pharma World Research; 2012, 3(2).
9. Pradeep JA. Characteristics of exopolysaccharides produced by selective lactic acid bacteria. *MSc. Thesis*. KVAFSU, Bidar, India, 2007.
10. Rajasekhar P, Prabha R, Ramachandra B, Suchitra N, Manjunatha H. Characterization of Lactic Acid Bacteria Obtained from Dahi Samples of Agricultural Zones of Karnataka. Frontier J Vet. Anim. Sci. 2013;2(2):1-10.
11. Ramachandra B. Performance of *dahi* samples in the development of honey enriched probiotic lassi. Ph.D. Thesis. KVAFSU, Bidar, 2016.
12. Ramachandra B, Manjunath H, Prabha R, Krishna R. Standardization of bread dough rise capacity using *Kluyveromyces lactis* and *Saccharomyces cerevisiae* yeast cultures. Journal of Dairying, Foods and Home Sciences. 2009;28(2):124-6.
13. Ray RR, Sonali R. Tradition, trend and prospect of fermented food products: a brief overview. World Journal

of Pharmacy and Pharmaceutical Sciences (WJPPS). 2014;3(10):272-86.
14. Widyastuti Y, Febrisiantosa A. The role of lactic acid bacteria in milk fermentation. Food and Nutrition Sciences. 2014 Feb 7;2014.