

# A Study On Isolation And Screening Of Lactic Acid Bacterial Isolates For Dairy Product

Madhusudan,N.M, Manjunath.H, Dhavalagi.Pallavi

**ABSTRACT:** Dahi is an indigenous milk product, obtained by the fermentation of lactic acid bacteria (LAB). Different brands of dahi samples (24) were collected from various BBMP Zones of Bengaluru and analysed for the LAB. The results convey that, the enumeration of LAB present in dahi were lactococci, streptococci, leuconostoc, lactobacilli, the study found that incubation condition with respect to temperature and time was different for different parameters based study. The findings related to log count for LAB. The results revealed that lactobacilli was found to have highest log count compared to other isolates. The findings for screening of LAB isolates using sterile skim milk. The results conveyed that Titratable acidity (TA) and Direct microscopic count (DMC) carried out for each isolate and the selection of isolates is based on the early setting time and more DMC counts. Further more than 50% of streptococci isolates set the milk early with curdling followed by lactococci and least was found in case of leuconostoc isolates.

## INTRODUCTION

Food is one of the basic requirements for human beings which provide nutritional support. Milk is considered as nature's most nearly perfect food and also considered as culture medium for the growth and proliferation of microorganism. Milk is considered as perishable which needs special care to preserve the nutrients. Fermentation has been used as a means of improving the keeping quality of food for more than 6000 years (Holzapfel, 1997). Fermentation of milk preserves it for longer time than normal milk and fermentation is made possible by inoculating the milk with lactic acid bacteria. Milk products obtained by fermentation of lactic acid bacteria have their own importance in the human diet. These products extend potential therapeutic benefits to the consumers. The recent classification of fermented milk products was given by (Tamime and Robinson, 1998). It includes fermented milk products such as mesophilic, thermophilic and therapeutic; yeast and mold lactic milk products. LAB are a group of microaerophilic, Gram-positive, non-spore forming organisms that ferment lactose to produce primarily lactic acid (Emiliano *et al.*, 2014). Dahi, an Indian fermented milk product has mixed strains of lactic cultures, such as lactococci, streptococci, leuconostoc and lactobacilli. LAB includes a variety of industrially important genera such as *Lactococcus*, *Pediococcus*, *Streptococcus*, *Leuconostoc* and *Lactobacillus*. Thus, there is a need to enumeration and isolation of LAB from dahi samples collected from different BBMP zones of Bengaluru.

## MATERIALS AND METHODS

**Dahi samples:** Different market brands of dahi samples were collected from eight different Bruhath Bengaluru Mahanagara Palike (BBMP) zones approximately 100 g were collected aseptically in sterile sample bottles.

**Plating of samples:** The dahi samples collected were serially diluted in sterile phosphate buffer. Dilution of first was prepared by transferring 11 g of dahi to 99 ml of sterile phosphate buffer and mixed thoroughly. Using the first dilution, required dilutions were prepared for lactococci, leuconostoc and lactobacilli. For streptococci, the first dilution was subjected to laboratory pasteurization of 63°C for 30 min., cooled to room temperature immediately and separately diluted for required dilutions. The required dilutions of 1 ml were transferred to labeled sterile petri plates poured with, 10-15 ml of molten agar medium maintained at 50°C water bath, mixed gently allowed to solidify and anaerobically incubated invertedly in a candle jar as given in Table 1. The colonies were counted by selecting the countable plates and average count was expressed as cfu per g. After enumeration, colonies were selected based on the morphology and transferred as well as maintained in respective broth media. After purification, lactic isolates were individually inoculated into sterile skim milk and incubated, time taken for setting was noted and also tested for Titratable acidity (TA) and Direct Microscopic Count (DMC).

## RESULTS AND DISCUSSION

### Enumeration of lactic acid bacteria (LAB) present in dahi collected from different BBMP Zones

Dahi samples (24 No's) were collected from 8 different locations in the BBMP zones of Bengaluru and were plated using M17 agar and MRS agar. Table.1 reveals the incubation conditions for the enumeration of LAB the findings vary over time and incubation temperature and also with type of media used for incubation. Two medias were used for the study, viz M17 agar and MRS (De man rogosa sharpe) agar to estimate the parameters such as lactococci, streptococci and leuconostoc lactobacilli respectively. However, these parameters were having different incubation temperature and time such as streptococci and lactobacilli 37°C for 24-48 hours was needed where as for lactococci and leuconostoc 30°C temperature for 24-48 hours time was needed to ascertain required parameter.

- Madhusudan,N.M, Manjunath.H, Dhavalagi.Pallavi
- Dairy Science College, Mahagaon Cross, Kalburgi-585316, Karnataka Email: [madhu.nm30@gmail.com](mailto:madhu.nm30@gmail.com)

**Table 1: Incubation conditions for the enumeration of lactic acid bacteria (Harrigan, 1998)**

Media	Parameter	Incubation temperature and Time
M17 agar	<i>Lactococci Streptococci</i>	30°C for 24-48 h
		37°C for 24-48 h
MRS agar	<i>Leuconostoc Lactobacilli</i>	30°C for 24-48 h
		37°C for 24-48 h

The findings related to log count related to Lactic Acid bacteria (LAB) are presented in the Table 2, the finds show that lactococci count ranged from 4.00 to 5.91 log<sub>10</sub>cfu/g with an average of 4.89 log<sub>10</sub>cfu/g of dahi. In case of streptococci the counts were in the range of 4.07 to 5.61 log<sub>10</sub>cfu/g with an average of 4.48 log<sub>10</sub>cfu/g. Leuconostoc counts ranged from 3.07 to 4.66 log<sub>10</sub>cfu/g with an average of 3.82 log<sub>10</sub>cfu/g. In respect of lactobacilli the counts ranged from 4.36 to 5.34 log<sub>10</sub>cfu/g with an average of 4.98 log<sub>10</sub>cfu/g. These results are in agreement

with Pradeep (2007) found predomination of lactococci (7.82 log<sub>10</sub>cfu/g) followed by lactobacilli (5.45 log<sub>10</sub>cfu/g) and leuconostoc (3.20 log<sub>10</sub>cfu/g) by selective plating technique using Neutral Red Chalk Lactose Agar, Rogosa Agar and Sucrose Agar respectively among microflora of four domestic dahi samples collected from Bengaluru. A similar study by Rajasekhar *et al* (2013) recorded the predominance of leuconostocs of 4.54 (log<sub>10</sub>cfu/g) followed by lactobacilli of 4.20, lactococci of 3.71 and streptococci of 3.22 in domestic dahi samples from Bengaluru market.

**Table 2: Enumeration of log counts of lactic acid bacteria of market dahi samples collected from BBMP zone**

Sl. No.	Zone	Code	Location	Mean viable count log <sub>10</sub> cfu/g			
				Lactococci	Streptococci	Leuconostoc	Lactobacilli
1	Bengaluru East	B1	Hebbal, Shivajinagar	5.41	5.04	3.69	4.54
2	Bengaluru South	B2	Basavanagud, Jayanagar	5.91	5.61	3.66	5.34
3	Bengaluru West	B3	Yeshavanthaur, Sanjayanagar	5.59	4.69	3.91	5.17
4	Bommanahalli	B4	Bommanahalli, HSR Layout	4.91	4.38	3.30	5.27
5	Dasarahalli	B5	Dasarahalli, Mallasandra	4.54	4.49	3.77	4.77
6	Mahadevapura	B6	K R Puram, HAL Airport	4.04	4.43	3.07	4.36
7	Rajarajeshwari Nagar	B7	Kengeri, Kottigepalya	4.00	4.17	4.54	5.34
8	Yelahanka	B8	Atturu, Yelahanka satellite town	4.27	4.07	4.66	5.07
<b>Average</b>				<b>4.89</b>	<b>4.48</b>	<b>3.82</b>	<b>4.98</b>

### Isolation of lactic acid bacteria from dahi samples

Based on colony morphology after the enumeration of lactic acid bacteria on agar medium, selections of colonies were made. *Lactococcal* and *streptococcal* colonies were individually transferred to M17 broth incubated anaerobically at 30°C and 37°C respectively. MRS broth was used to grow the colonies of leuconostoc and lactobacilli with anaerobic incubation at 30°C and 37°C respectively. Table 3 reveal that total of 82 lactic acid

bacterial isolates were picked from the respective agar plates and out of which 24 were lactococci; while 15 numbers were streptococci; 10 numbers were leuconostoc and 33 numbers were lactobacilli respectively. The results obtained were on par with Mahesh *et al.* (2016) reported that 8 lactococci and 6 streptococci followed by 2 leuconostoc and 6 lactobacilli out of 22 lactic isolates obtained from 8 domestic dahi samples from Bengaluru.

**Table 3: Distribution pattern of lactic acid bacteria isolates in dahi sample**

Sl.No	Group	Isolate code	No. of isolates
1	Lactococci	LL 1 to LL 24	24
2	Streptococci	ST 1 to ST 15	15
3	Leuconostoc	LE 1 to LE 10	10
4	Lactobacilli	LB 1 to LB 33	33
<b>Grand Total</b>			<b>82</b>

### Screening of the lactic acid bacterial isolates from dahi samples in sterile skim milk

All the 82 purified lactic isolates were individually inoculated into sterile skim milk and incubated at 30/37°C until milk got set. The time taken for setting was noted and also tested for Titratable acidity (TA) and Direct Microscopic Count (DMC). Table 4 showed that among 24 lactococcal isolates only 8 isolates set the milk early with a curdling time of 9h, TA of 0.67% LA and DMC ranging from 7.28 to 7.34 log<sub>10</sub> cells/g. In respect of streptococci only 8 isolates showed early curdling time of 6h, %TA of 0.67%LA and DMC ranging from 7.52 to 7.68 log<sub>10</sub>cells/g out of 15 isolates. Among 10

leuconostoc isolates only 3 isolates set the milk in 14h. These isolates at the time of curdling showed an acidity of 0.66 % LA and DMC of 7.59-7.71 log<sub>10</sub>cells/g. Out of the 33 isolates of lactobacilli tested, 8 isolates curdled the milk in 8h, acidity of 0.68% LA and cell count ranged from 7.57 to 7.79 log<sub>10</sub> cells/g. Remaining lactic isolates took longer hours to set a milk with low number of cells. Mahesh *et al.* (2016) found that out of the 22 lactic isolates obtained from the dahi samples in Bengaluru 15 isolates set the milk at 18 h with the acidity 0.67%LA and DMC ranged from 7.5 - 8.25log<sub>10</sub> cells/g.

**Table 4:** Screening of lactic acid bacteria isolates for acid production and growth in milk

Sl. No.	Group	No. of isolates positive	Curdling time (h)	Titrateable Acidity (% LA)	DMC (log <sub>10</sub> cells/g)
1	Lactococci	LL4,LL7,LL9,LL10,LL15,LL17,LL18,LL21	9	0.67	7.28-7.34
		LL1,LL6,LL8,LL11,LL12,LL19	10	0.73	6.23-6.58
2	Streptococci	ST1,ST2,ST3,ST5,ST7,ST11,ST14,ST15	6	0.67	7.52-7.68
		ST4,ST6,ST8,ST9,ST12	8	0.72	5.76-6.87
3	Leuconostoc	LE1,LE3,LE4	14	0.66	7.59-7.71
		LE2,LE5	15	0.69	5.87-6.43
4	Lactobacilli	LB1,LB2,LB4,LB5,LB8,LB10,LB21,LB24	8	0.68	7.57-7.79
		LB11,LB26,LB28	9	0.82	6.76-7.12

**Note:**

- *Lactococci and Leuconostoc isolates incubated at 30°C*
- *Streptococci and Lactobacilli isolates incubated at 37°C*

**Conclusion**

The findings of isolation and screening of LAB for dairy products especially Dahi samples which have exhibited wide differences based on the time and duration of incubation and its culture. The findings helped us to conclude the below mentioned points. The study concludes that, on an average, almost all the LAB distributed nearly to 4 log numbers in dahi samples and also percent of lactococci, streptococci, leuconostoc and lactobacilli are around 25, 24, 20 and 31 respectively. Predominance of certain group of LAB in dahi may be due to variations in ambient temperature and type of inoculum used. If the culture held at room temperature, the culture which grows rapidly will dominate and the culture with stands low acid for long time will dominate, if the culture used is from commercial dahi the cultures will dominate and also the culture which accelerates milk growth faster and dominates the other microflora.

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