© 2017 IJSRST | Volume 3 | Issue 8 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
Themed Section: Science and Technology

Growth of Lactic Acid Bacteria in Milk for the Preparation of Functional Frozen Misti Dahi (Sweet Curd)

Gayatri Saikia*, B. K. Mishra
Department of Rural Development and Agricultural Production, North-Eastern Hill University, Tura, Meghalaya, India

ABSTRACT

This study was conducted to evaluate the growth of lactic acid bacteria in the milk for preparation of functional frozen Misti Dahi (Sweet Curd). The identity of the culture was carried out by macroscopy (morphology) and microscopy (Gram straining) method. Cultures were cultivated in 10% (w/v) reconstituted skim milk to check their pH, titratable acidity and lactic acid bacteria count during 0, 3, 6, 9 and 12 hours fermentation at 37^0C. On the basis of all of the identification tests one strain was identified as *Lactobacillus helveticus*, and the other strain was identified as *Streptococcus thermophilus*. The pH of fermented milk decreased from pH 6.42 to 3.11and titratable acidity increased from 0.10% to 0.98% by *Lactobacillus helveticus*, *Streptococcus thermophilus* and combination of *Lactobacillus helveticus* and *Streptococcus thermophilus*, when incubated for 12 h at 37°C. The number of lactic acid bacteria counts in fermented milk was maximized at this incubation condition. *Lactobacillus helveticus*, *Streptococcus thermophilus* and combination of *Lactobacillus helveticus* and *Streptococcus thermophilus* strains produced 7.80 to 8.85log cfu/ml of lactic acid in fermented milk at 37^0C. The present findings suggests that the both the *Lactobacillus helveticus* and *Streptococcus thermophilus* cultures alone or in combinations are potential to be used for the preparation of functional frozen Misti Dahi (Sweet curd).

Keyword: Identification, pH determination, Acidity determination and Lactic acid bacteria count.

I. INTRODUCTION

Milk has played a major contribution in the human diet in many different countries across the world. In a predominantly vegetarian population like India, people consume milk as part of their daily diet. It is not surprising therefore, that over many years considerable attention has been paid to improve the quality of milk. Milk and milk products is a suitable food, support vigorous multiplication of a number of microorganisms. Milk contains many health promoting constituents including immunoglobulin, bioactive fatty acids and peptides amongst others. The healthy image of milk has resulted in dramatic growth in the diversification of dairy products in recent years and in huge increase in the varieties of products such as dairy desserts, flavoured milk drinks, cheeses, yoghurt, dahi, Misti Dahi etc. Apart from the milk components, the health attributes are associated with fermented and probiotic milks and dairy products. Milk has been preserved by fermentation through the action of lactic acid bacteria (LAB), which convert lactose to lactic acid and other organic acids, thereby lowering the pH and subsequently inhibiting the growth of pathogenic and spoilage bacteria.

Lactic acid bacteria (LAB) are a group of Gram positive, non-spore forming, cocci or rods which produce lactic acid as major end product from fermentation of carbohydrates. Majority of microorganisms used as probiotics belong to the LAB and bifidobacteria.

In recent years, there has been a worldwide increase in interest in the addition of intestinal bacterial species (*Lactobacillus helveticus* and *Streptococcus thermophilus*) to fermented milks. The addition of probiotic bacteria to fermented milk products is made not only as a consequence of certain claimed health-promoting effects of their presence in the intestinal tract of consumers, but also due to the

expanding variety of products that can be formulated with probiotics bacteria (Liu *et al*., 2002).

Since product quality and consumer satisfaction are fundamental factors to the successful and repeated sale of dairy products, the evaluation of Misti Dahi quality becomes very important laboratory analysis, a very important part of quality evaluation includes microscopic examination, titratable acidity (TA), pH and chemical composition.Microbiological examination usually includes a check on the survival of the starter organism, as well as a check for the presence of undesirable spoilage or pathogenic organisms. Therefore, the survival of the starter organism is very important for evaluation of milk qua1ity. Thus considering its importance in human health and also due to the growing research interest in lactic acid bacteria (LAB), the present study was undertaken to evaluatethe growth of lactic acid bacteria in milk.

II. METHODS AND MATERIAL

Microorganisms and Media

The strains used in this study, are collected from Anand Agricultural University, India. Pure strains of *Lactobacillus helveticus* (V_3) and *Streptococcus thermophillus* (MD_2) were activated from their frozen forms by transferring once in MRS broth (*Lactobacillus helveticus*) and in M17 broth (*Streptococcus thermophillus*) at 37°C.

Macroscopic assessment

This experiment was performed by pour plating technique. In preparation for macroscopic assessment, two cultures i.e. *Lactobacillus helveticus* (V3) and *Streptococcus thermophillus* (MD2) were grown in MRS (De Man Rogosa and Sharp) and M17 broth respectively at 37^0C for 12 hours @ 1%.

Microscopic assessment : Microscopic assessment was carried out by Gram staining method.

Determination of pH

After activation of the cultures, fermentation of milk was carried out by inoculating 1% overnight grown LAB in MRS and M17 medium. pH was carried out with the help of digital pH meter (Electronic India, Model112) after fermenting skim milk at 0, 3, 6, 9 and 12 h of incubation at 37^0C. The readings were taken according to the time intervals.

Determination of Titratable Acidity

Acidity was determined as method suggested by (Hati *et al*., 2015). For this, 10 gm sample was taken in a porcelain dish and distilled water was added to make up the volume 20 ml. Few drops of phenolphthalein indicator was added and the material was titrated against 0.1 [N] NaOH. End point was considered when light pink color was developed from white. The percent acidity was expressed in terms of % lactic acid. The titratable acidity was calculated as percent lactic acid as follows: % of acidity = ml of 0.1 N NaOH used in titration x 0.009x100/ grams of sample used.

Determination of Lactic acid bacteria counts

Lactic counts were performed through serial dilution and pour plating technique. To carry out the experiment, 1ml of culture was inoculated into 9ml each sterile skim milk containing tubes which was labeled as 0, 3, 6, 9 and 12 respectively. The same were kept for fermentation at 37^0C. Serial dilutions were made by diluting 1ml of the fermented skim milk in 0.1% peptone water upto 10^{-6}dilutions. Then 0.1ml is taken from the respective dilutions and spreading on MRS and M17 agar plates. The plate count was performed by using the following formula that is given below:

CFU/ml= No. of colonies x Dilution factor / volume of inoculum

Statistical Analysis

All the tests were carried out in triplicate for each bacterial strain, with all analyses performed at least in duplicate. Significant differences between treatments were tested by analysis of variance (ANOVA) with a level of significance at0.05%.

III. RESULTS AND DISCUSSION

Macroscopic assessment: Macroscopic characterization was done on the basis of shape, colour, opacity, texture, margin and elevation of the colony on the plates. Colony characteristics of *Lactobacillus helveticus* and *Streptococcus thermophilus* were studied by picking up the typical, well isolated and representative colony that appeared on the plate. Results presented in Table 1demonstrate that the macroscopic observation of *Lactobacillus helveticus* and *Streptococcus thermophillus* which were comparable with the study of Hoque *et al.* (2010).

Table 1. Macroscopic observation of Lactobacillus helveticus and Streptococcus thermophillus on Agar plates

Cultures	Shape	Colour	Opacity	Margin texture	Elevation	Medium Used
***Lactobacillus helveticus* (V_3)**	Rounded	Off white	Opalescent	Smooth	Raised	MRS Agar
***Streptococcus thermophillus*(MD$_2$)**	Rounded	White	Opalescent	Smooth	Convex	M17 Agar

Microscopic Assessment

In present study both the bacterial cultures match the characteristics of genus *Lactobacillus* and *Streptococcus* as they are Gram positive, rod shaped and cocci in chains. Their characteristics have shown in Table 2. Similar findings have also been reported by Masud *et al*. (1991) and Amoroso *et al.* (1992).

Table 2. Morphological examination of Lactobacillus helveticus and Streptococcus thermophillus

Cultures	**Type**	**Gram staining**
Lactobacillus helveticus	Rods in shape	Gram positive
Streptococcus thermophillus	Cocci in chain	Gram positive

Determination of pH : The culture performance in the present study was assessed by the production of lactic acid as the primary metabolite and it was measured by decline in pH. The average pH values were 4.32, 5.62 and 4.48 for *Lactobacillus helveticus*, *Streptococcus thermophilus* and combination of *Lactobacillus helveticus* and *Streptococcus thermophilus* in 1:1 ratio respectively (Table 3). The strain *Streptococcus thermophilus* produced maximum pH value (6.42) and it was significantly higher than *Lactobacillus helveticus* (5.47) and combination of *Lactobacillus helveticus* and *Streptococcus thermophilus* (5.81). It was also observed thatthe 12 hours incubation period lowered value of pH to 3.87 and differs significantly from the periods of 3, 6 and 9 hours. However zero hour periods showed maximum value of pH i.e. 5.9. pH decreased with time due to sugar fermentation and conversion of lactose to lactic acid. Similar findings were reported by Hati *et al.* (2014).

Table 3. Mean Summary table for pH of Lactic Acid Bacterial strains at different Incubation Periods

pH						
Strains	**Incubation Periods (h)**					
	0	**3**	**6**	**9**	**12**	**Mean**
Lactobacillus helveticus	5.47	4.73	4.10	3.73	3.59	**4.32**
Streptococcus thermophilus	6.42	6.05	5.61	5.08	4.92	**5.62**
Mixed	5.81	5.06	4.35	4.08	3.11	**4.48**
Mean	**5.9**	**5.28**	**4.65**	**4.31**	**3.87**	**4.80**
C.D $_{0.05}$ Strain : 0.40 : S, Period : 0.54 : S and Strain x Period : 0.98 : NS						

Determination of Total Titratable Acidity

The average results presented in Table 4 demonstrated the Toatal Titrable Acidity produced by Lactobacillus helveticus (0.47%), Streptococcus thermophilus (0.26%) and combination of Lactobacillus helveticus and Streptococcus thermophilus (0.61%). Similar observation were made by Younus et al. (2002) where they found that the mean acidity per cent in dahi ranged

from 0.87 to 1.13 in three brands. The maximum acidity was showed by mixed strain i.e. the combination of Lactobacillus helveticus and Streptococcus thermophilus with the value of 0.98% and followed by Lactobacillus helveticus with value of 0.71% and Streptococcus thermophilus with the value of 0.41% at 12 hours. Whereas minimum acidity was given by streptococcus thermophilus with the value of 0.1% followed by combination of Lactobacillus helveticus and Streptococcus thermophilus (0.18%) and Lactobacillus helveticus (0.26%) at 0 hours. Ordonez et al. (1999) also found that the titrable acidity was increased with incubation periods.

Table 4. Mean Summary table for Total titrable acidity of Lactic Acid Bacterial strains at different Incubation Periods:

Titratable Acidity						
Strains	**Incubation Periods (h)**					
	0	**3**	**6**	**9**	**12**	**Mean**
Lactobacillus helveticus	0.26	0.35	0.45	0.6	0.71	**0.47**
Streptococcus thermophilus	0.10	0.16	0.23	0.38	0.41	**0.26**
Mixed	0.18	0.41	0.68	0.81	0.98	**0.61**
Mean	**0.18**	**0.31**	**0.45**	**0.60**	**0.7**	**0.45**
C.D $_{0.05}$ Strain : 0.04 : S, Period : 0.04 : S and Strain x Period : 0.06 : S						

Lactic Acid Bacteria Count:

From the study; it was found that the average counts of 8.69, 8.57 and 8.46 log cfu/ml were obtained by the *Lactobacillus helveticus, Streptococcus thermophilus* and the combination of *Lactobacillus helveticus* and *Streptococcus thermophilus* (Table 5). The counts of lactic acid bacteria were raised with increased incubation periods i.e. 8.24, 8.54, 8.63, 8.68 and 8.77 log cfu/ml for 0, 3, 6, 9 and 12 hour periods. Hoque *et al.*(2010) also reported that the viable cell counts of *Lactobacillus* spp. on MRS agar media were increased from (1.5×10^4 CFU/ml) at 0 h to 1.59×10^4 CFU/ml at 24 h.The maximum Lactic Acid Bacterial Count was showed by mixed strain i.e. the combination of *Lactobacillus helveticus* and *Streptococcus thermophilus* with the value of 8.85log cfu/ml and followed by *Lactobacillus helveticus* with value of 8.81logcfu/ml and *Streptococcus thermophilus* with the value of 8.66log cfu/ml. Whereas minimum counts were observed in case of combination of *Lactobacillus helveticus* and *Streptococcus thermophilus* (7.80log cfu/ml) then *Streptococcus thermophilus* (8.40log cfu/ml) and *Lactobacillus helveticus*(8.52logcfu/ml).

Table 5. Mean Summary table for Lactic Acid Bacterial counts at different Incubation periods

Lactic Acid Bacteria						
Strains	**Incubation Periods (h)**					
	0	**3**	**6**	**9**	**12**	**Mean**
Lactobacillus helveticus	8.52	8.64	8.73	8.75	8.81	**8.69**
Streptococcus thermophilus	8.40	8.57	8.61	8.63	8.66	**8.57**
Mixed	7.80	8.42	8.54	8.67	8.85	**8.46**
Mean	**8.24**	**8.54**	**8.63**	**8.68**	**8.77**	**8.57**
C.D $_{0.05}$ Strain : 0.02: S, Period : 0.03 : S and Strain x Period : 0.04 : S						

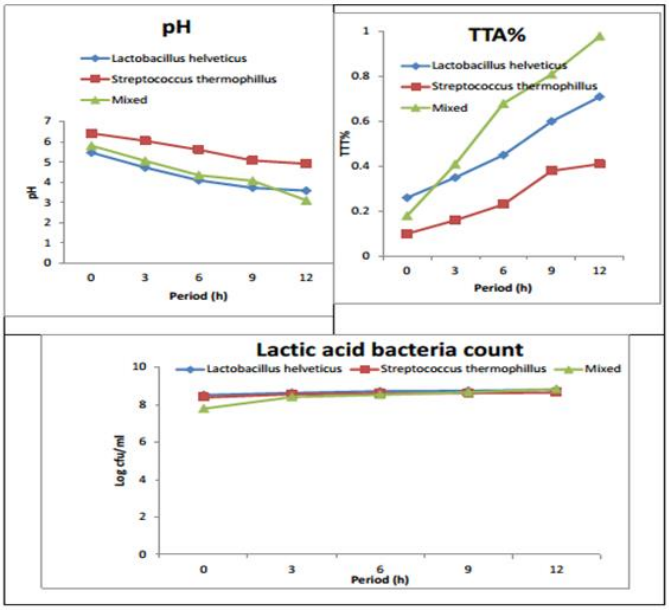

Figure 1 : The change of pH, titratable acidity and lactic acid bacteria in milk during incubation periods.

IV. CONCLUSION

Lactic acid bacteria are widely applied to several milk products due to their specific properties. The growth of lactic acid bacteria in milk was evaluated.From the results, it is found that the both the culture was identified

as *Lactobacillus helveticus* and *Streptococcus thermophilus*. pH values of the cultures decreased with increasing periods significantly. There is a close relationship between the acidity and lactic acid bacteria counts increasing patterns during fermentation. It could be concluded that the functional frozen Misti Dahi can be prepared by using probiotic cultures viz., *Lactobacillus helveticus* and *Streptococcus thermophilus* alone or in combination of this two bacteria.

V. REFERENCES

[1]. M. J . Amoroso and M. C. Manca de Nadra. 1992. World Journal of Microbiology and Biotechnology 8:50-54

[2]. S. Hati , V. Sreeja, J. Solanki and J . B. Prajapati. 2015. Indian J. Dairy Sci. 68 (6):584-591

[3]. M. Z. Hoque, F. Akter, K. M. Hossain, M. S. M. Rahman, M. M. Billah and K. M. D. Islam. 2010 World Journal of Dairy & Food Sciences. 5 (1): 39-46

[4]. F. V. Kosikowski. 1981. Journal of Food Protection. 44 (11):853-856

[5]. Y. Liu, M. Chen and C. Lin. 2002. Asian-Aust. J. Anim. Sci. 15 (4):602-609

[6]. T. Musud, K. Sultana and M. A. Shah. 1991. AJAS. 4 (4):329-331

[7]. G. A. Ordonez, I. J. Jeon and H. A. Roberts. 2000. Journal of Food Processing Preservation. 24:163-176

[8]. S. Younus, T. Musud and T. Aziz. 2002. Pakistan Journal of Nutrition. 1 (5):226-230

[9]. T. Zahoor, S. U. Rahmant and U. Farooq. 2003. Internationl Journal of Agriculture & Biology. 5(1):46-48