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ENUMERATION *OF LACTOBACILLUS ACIDOPHILLUS* IN CURD AT DIFFERENT STORAGE CONDITIONS

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ABSTRACT

The Human gut microbiota plays a significant role in Gut health. The concept of Probiotics has led to wide spread consumption of food preparation containing pro biotic microbes such as curd and yogurt. Most of the peoples in Southern India consume home made curd which is prepared from cow milk, buffalo milk or commercial milk. The main aim of the study was to determine the best storage conditions for the curd. In this study we foundthat the samples having adequate pH (6.8) in buffalo milk. In this study *Lactobacillus acidophillus* in different curd sampleswas enumerated at different storage condition by using Pour plate method and found that the curd sample was cow milk, buffalo milk, commercial milk was maintaining adequate count of *Lactobacillus acidophillus* and organoleptically acceptable. Hence the home made curd can be stored at 4⁻⁻5°C in refrigerator after fermentation for 2 days with good probiotic value.

Keywords: Gut microbiota, Probiotics, Cow milk, Buffalo milk, *Lactobacillus acidophillus*

INTRODUCTION

Lactobacillus:

Lactobacillus is a genus of gram-positive, aerotolerant anaerobes or microaerophilic, rod-shaped, non-spore-forming bacteria. Until 2020, the genus *Lactobacillus* comprised over 260 phylogenetically, ecologically, and metabolically diverse species; a taxonomic revision of the genus assigned *lactobacilli* to 25 genera.

Lactobacillus is a type of "friendly" bacteria. It lives in your body but doesn't cause

disease. You can also get it in food and food supplements **[1]**.

Lactobacillus may help your body: Break down food, Absorb nutrients, Resist infections in the gastrointestinal track **[2]**.

***Lactobacillus* role in human health**

People take lactobacillus for many reasons.

- Digestive system. People take *lactobacillus* to try to treat or prevent diarrhea. Some research shows that one type of *lactobacillus* may help children get over diarrhea caused by rotavirus more quickly.
- *Lactobacillus* may also help prevent diarrhea for:
  - Hospitalized adults
  - People taking antibiotics
  - Patients getting cancer treatment.

Probiotics

Probiotics are living organisms that are used as food additives with beneficial effects on the healthy body by setting microbial balance in gastrointestinal tract. *Lactic acid bacteria (LAB)* as protective cultures are common pro biotic organisms that are considered safe due to having specific characteristics **[1]**.

Probiotics such as *lactobacillus* prevent the development of a wide range of human and animal's pathogens

Curd

Curd is made by bacterial fermentation of milk. In this process, lactose in milk is converted into lactic acid by several Probiotics microorganisms. The species involved in the fermentation depends on the temperature and humidity of the environment **[3]**.

The quantity of curd one should consume daily to gain health benefits can vary Special depending on individual factors such as age, sex, overall diet, and any specific health condition However, general dietary guidelines suggest consuming about one serving of dairy or dairy alternatives daily, which typically equates to:

For adults: 1 to 1.5 cups (approximately) (225 to 350 grams) of yogurt per day.

For children: Half to one cup (approximately 115 to 225 grams) of yogurt per day **[13]**.

Types of *lactobacillus* in different types of curds:

Curd prepare from cow milk

- *Lactobacillus acidophilus* **[5]**
- *Lactobacillus casei*
- *Lactobacillus rhamnosus*
- *Bifidobacterium bifidum*
- *Bifidobacterium lactis*
- *Lactococcus lactis*

Curd prepare from buffalo milk:

- *Lactobacillus acidophilus*
- *Lactobacillus casei*
- *Bifidobacterium bifidum*
- *Lactococcus lactis*
- *Leuconostoc spp.* **[5]**

- **In commercial curd:**
  - *Lactobacillus casei*
  - *Lactobacillus rhamnosus*
  - *Bifidobacterium bifidum*

AIM AND OBJECTIVES:

Aim: To enumerate *L.acidophilus* in different curs at different storage conditions.

Objects: To prepare curd from different types of milk

- To evaluate the physical chemical properties of fermented cow milk, buffalomilk, commercial milk
- To enumerate *L.acidophillus* of fermented cow milk, buffalo milk, commercial milk.

MATERIALS AND METHODS

Materials

- **Samples**
  - Cow milk (Sample -1)
  - Buffalo milk (Sample-2)
  - Commercial milk (Sample-3)
- **Chemicals**
  - Nutrition Agar
  - Distilled water
  - Hydrogen peroxide (H_2O_2)

Methods

The process of making curd from milk is called "curdling" or "fermentation". During this process, milk is heated and mixed with a small amount of starter culture, which contains lactic acid bacteria such as Lactobacillus. The bacteria consume the lactose in the milk and convert it into lactic acid, which causes the milk proteins to coagulate and form curd Different Types Of curd samples like Cow Milk curd, Buffalo Milk curd and Commercial milk curd.

Preparation method of sample-1, sample-2 and Sample-3:

Buffalo milk is traditionally considered better for making yogurt than cow milk due to its higher fat content making a thicker yogurt mass. Buffalo, Cow and commercial curd is obtained by bacterial fermentation of milk. In this process, lactose in buffalo and Cow milk is converted into lactic acid using several micro- organisms.

Cow, Buffalo and commercial milk to make at home typically heated to around 85-90^{0}c and sterilized and cooled down around 35-40°C the stater culture (1-1.5%) is than added to the warm milk the mixture is undisturbed several hours to allow the bacteria to grow and ferment the milk, once the curd has formed.

- **Identification test of bacteria**

Catalyse test:

To perform this test, a single isolated colony was streaked on a glass slide and one drop of 3 % hydrogen peroxide (Merck, Germany) was added on to it. The effervescence of oxygen indicated the positive response of the bacteria to catalyse test **[8]**.

Agar plate preparation and incubation

Method name: Pour plate method

→ Dissolve nutritional agar in hot

double distilled water until agar solution was prepared. Stir the solution for 2hrs. Pour the solution into polystyrene petridish (of diameter 9cm). Keep it aside till dry. At T=60^0C for 48hrs the film was formed, dry it and stored **(Figure 2)**.

Dilution standards of different samples

Take 1ml of curd in test tube-1 Add 9ml of distilled water until diluted to 10ml. Pipette out 1ml of solution from test tube-1 to test tube-2, repeat the procedure for 5 serial dilutions **(Figure 3)**.

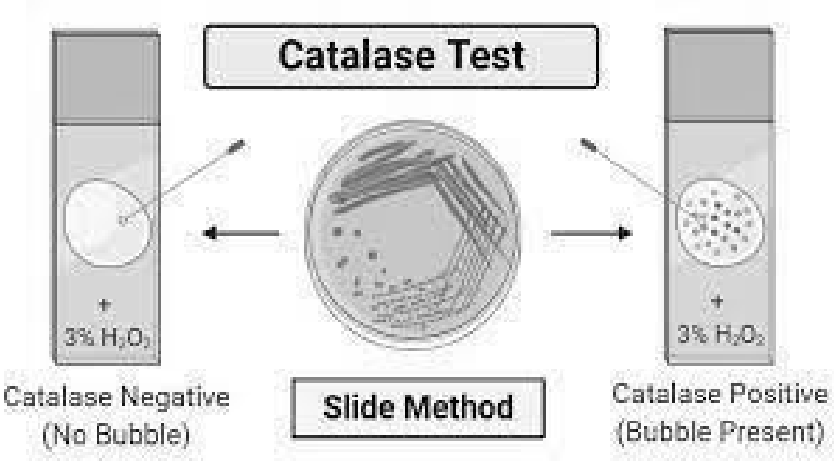

Figure 1: Catalyse test

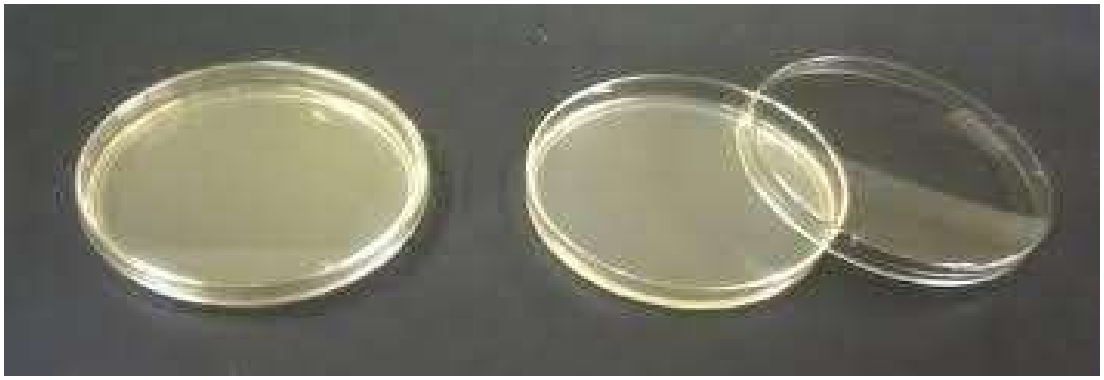

Figure 2: Agar Plate Preparation and Incubation

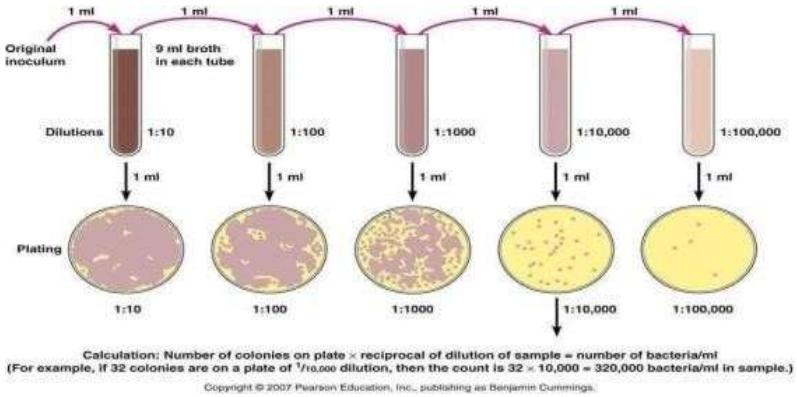

Figure 3: Dilutions standards of different samples [12]

Inoculation of lactobacillus bacteria by pour plate method

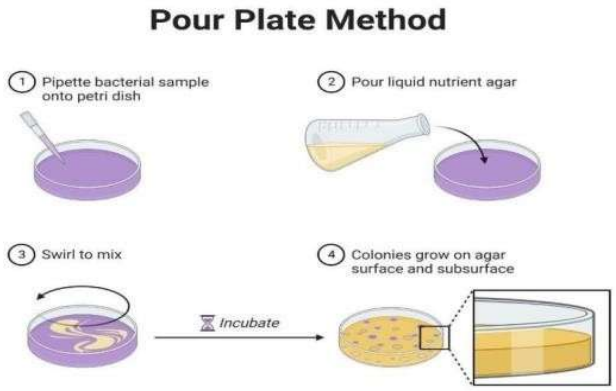

Figure 4: Pour Plate method

Enumeration of *Lactobacillus acidophilus* at different samples:

Method: Microscopic Counting

This method involves direct counting of bacterial cells under a microscope using a counting chamber or a hemocytometer. It requires staining the bacteria to improve visibility and accuracy.

Method of collection

RESULTS

This cross-sectional study was carried out after obtaining the permission of institutional review board Sri Padamavathi School of Pharmacy, in febraury 2024, Tiruchanoor, Tirupati, AP, India.

Physicochemical properties:

Table 1:CS-1

Physicochemical properties	12hrs (CS-1-A)	24hrs (CS-1-B)	48hrs (CS-1-C)
pH	4.9	4.8	4.7
Colour	Pale yellowish	Pale yellowish	Pale yellowish
Order	Slightly acidic	Acidic	Acidic
Taste	Slightly sour	Sour	Sour
Temperature	25^0C	25^0C	25^0C

Table 2: CS-2

Physicochemical properties	12hrs (CS-2-A)	24hrs (CS-2-B)	48hrs (CS-2-C)
pH	4.5	4.5	4.5
Colour	Pale yellowish	Pale yellowish	Pale yellowish
Order	Slightly acidic	Slightly Acidic	Slightly Acidic
Taste	Slightly sour	Slightly Sour	Slig
Temperature	25^0C	4^0C	4^0C

Table 3: CS-3

Physicochemical properties	12hrs (CS-3-A)	24hrs (CS-3-B)	48hrs (CS-1-C)
pH	5.01	4.85	4.25
Colour	Creamy white	Creamy white	Creamy white
Order	Slightly acidic	Acidic	Acidic
Taste	Slightly sour	Sour	Sour
Temperature	25^0C	25^0C	25^0C

Table 4: CS-4

Physicochemical properties	12hrs (CS-4-A)	24hrs (CS-4-B)	48hrs (CS-4-C)
pH	6.87	6.80	6.75
Colour	White	White	White
Order	Slightly acidic	Slightly Acidic	Slightly Acidic
Taste	Slightly sour	Slightly Sour	Slightly Sour
Temperature	25^0C	4^0C	4^0C

Enumeration of *Lactobacillus.acidophils*

Table 5: *CS-1*

Enumeration	12hrs (CS-1-A)	24hrs (CS-1-B)	48hrs (CS-1-C)
No.of colonies	128	136	148
No.of Bacteria	$128 \times 10^3 = 128000$	$136 \times 10^3 = 136000$	$148 \times 10^3 = 148000$

Figure 5: Colony count growth of CS-1-A,CS-1-B,CS-1-C

Table 6: CS-2

Enumeration	12hrs	24hrs	48hrs
No. of colonies	128	131	131
No. of Bacteria	$128 \times 10^3 = 128000$	$131 \times 10^3 = 131000$	$131 \times 10^3 = 131000$

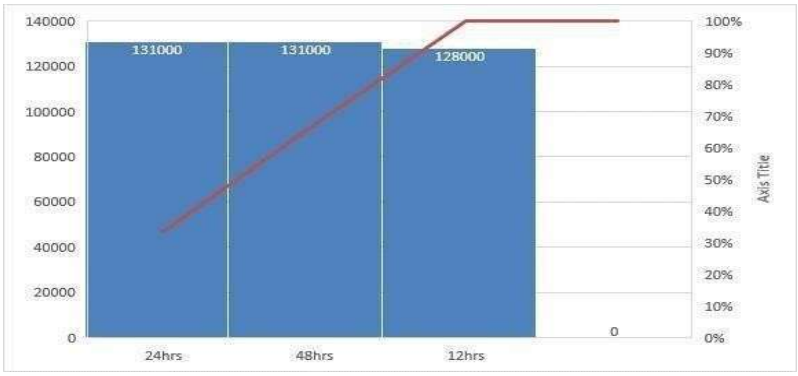

Figure 6: Colony count growth of CS-2-A, CS-2-B, CS-2-C

Table 7: CS-3

Enumeration	12hrs (CS-3-A)	24hrs (CS-3-B)	48hrs (CS-3-C)
No. of colonies	275	425	525
No. of Bacteria	275 ×10^3 = 275000	425×10^3 = 425000	525×10^3 = 525000

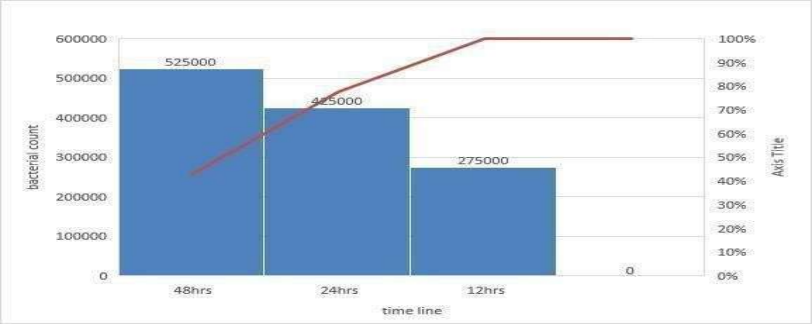

Figure 7: Colony count growth of CS-3-A, CS-3-B, CS-3-C

Table 8: CS-4

Enumeration	12hrs (CS-4-A)	24hrs (CS-4-A)	48hrs (CS-4-A)
No. of colonies	275	280	280
No. of Bacteria	275 ×103 = 275000	280×10^3 =280000	280×10^3 =280000

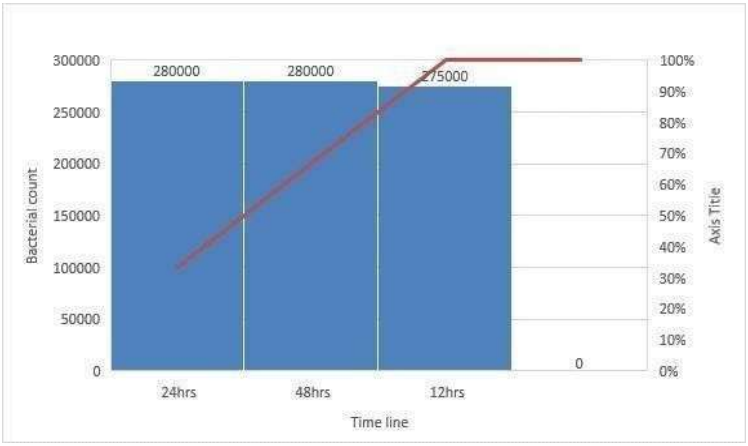

Figure 8: Colony count growth of CS-4-A, CS-4-B, CS-4-C

Table 9: CS-5

Enumeration	12hrs (CS-5-A)	24hrs (CS-5-B)	48hrs (CS-5-C)
No. of Colonies	92	92	92
No .of Bacteria	92×10^3=92000	92×10^3=92000	92×10^3=92000

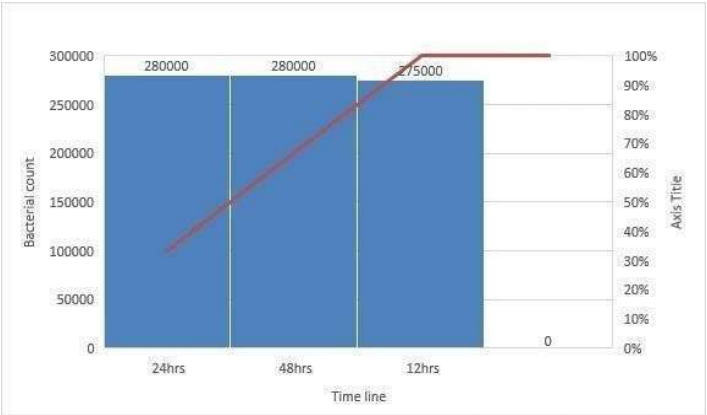

Figure 9: Colony count growth of CS-5-A, CS-5-B, CS-5-C

DISCUSSION

In this study we have enumerated *L.acidophilus* in different curd samples at different storage conditions. We fermented cow milk, buffalo milk and commercial milk samples and stored at different storage conditions. After 12 hrs of fermentation the samples were stored in two different temperature i.e $4^{\circ}C$ and at room temperature for 24 hrs and 48 hrs .we found the presence of *L.acidophilus* in all samples at different storage conditions and temperatures . We identified *L.acidophilus* in all samples through catalyst test and we enumerated *L.acidophilus*. We observed no change of colour in all the samples at different storage conditions. But change of pH and taste was observed in samples CS1 & CS3.

We enumerate *L.acidophilus* in all samples and found that sample CS-1-A,CS-1-B,CS-1-C,CS-2-A,CS- B,CS-2-C,CS-3-A,CS-3-B,CS-3-C,CS-4-A,CS-4-B,CS-4-C,CS-5-A,CS-5-B,CS-5-C have no.of colonies 128,136,148,128,131,131,275,425,525,275, 280,280,92,92,29 colonies per / unit.

CS3 sample has more amount of colonies compared to CS1, CS2, CS4, CS5. According the study we realized that amount of *L.acidophilus* was increased in samples CS1 ,CS3 and in the samples there was no significant increase According to **[2]**, at $4^{\circ}C$ *L.acidophilus* can survive but no growth was observed. As per our study also we obsereved viability of *L.acidophilus* but no *L.acidophilus* growth in sample CS1, CS3. Hence the home made curd could be stored at $4^{\circ}C$ without decreasing the probiotic potential for 2 days (48hrs).

CONCLUSION

The fermented milk (curd) is having good probiotic potential **[1]** at 12 hours after fermentation. But the organoleptic properties like taste and odour have been changed due to long storage conditions of24 hours and 48 hours at room temperatures. So, that curd might not be accepted by most of the consumers.

Hence for long storage the fermented milk (curd) could be stored at 4^0-5^0C for two days at 48 hours without loosing it's good taste and smell and also the amount of *lactobacillus acidophilus.*

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