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Formation of Yogurt by Lactic acid bacteria with Addition of Vitamin B12

Het Joshi[1], Dhwani Upadhyay[2] , Indrani Bhattacharya[2], Anjali Thakur[2] , Pooja Bhowmick [1], Prasad Andhare[3]*

[1-2]Parul Institute of Applied Science, Parul University, Post Limda, Waghodiya , Gujarat
[3] Assistant Professor, Biological Sciences, PDPIAS, Charotar University of Science and Technology, Changa, Anand, Gujarat.
*Corresponding Author: Dr. Prasad Andhare;
E-Mail: prasadandhare.as@charusat.ac.in

ABSTRACT

The lactic acid bacteria arose from an early curiosity and a desire to transmit and teach a broad range of literary knowledge on their activities as starter cultures, in the production of fermentation products such as dairy and alcoholic drinks, and their contribution to improved health. The lactic acid bacteria (LAB) that alter the scent and flavour of yogurt have amassed a substantial amount of information. Lactic acid bacteria (LAB) are well-known for their ability to produce antibacterial compounds as well as other high-value products. Yogurt used to be manufactured entirely of milk, with no extra additives. Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus, among other lactic acid bacteria, generate yogurt, a popular fermented dairy product. These bacteria create lactic acid during yogurt manufacture, which lowers pH and causes milk protein to coagulate. Camel, cow, goat, sheep, buffalo, reindeer, mare, and yak milk are among the nine species utilised commercially. The content of these milks varies, which has a significant impact on the quality of yogurt. Carbonyl substances, nonvolatile or volatile acids, and exopolysaccharides are among their metabolites that have a significant impact on yogurt quality. To enhance yogurt texture, nonfat dry milk (NDM), whey protein concentrate (WPC), and other dairy or plant-based substances were added to milk. Vitamin B12 can only be synthesised by bacteria, and this needs a sufficient amount of Co. Animal products, particularly those from ruminants, provide a natural supply of vitamin B12 in human diets.

KEY WORDS : Yogurt, *Lactic acid bacteria (LAB)*, Milk, Fermentation, *Streptococcus thermophilus, Lactobacillus bulgaricus, Vitamin B12.*

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INTRODUCTION

Yogurt is a popular dairy product that is prized for its health and nutritional value, as well as its sensory qualities. *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* work together to produce yogurt from milk through lactic acid fermentation. Lactic acid is created by bacterial cultures from lactose (milk's major carbohydrate), which lowers pH and causes milk protein to coagulate, giving it a thick gel-like shape. The components of milk are transformed into carbonyl compounds, volatile and nonvolatile acids such as acetone, acetate, diacetyle, acetaldehyde, and acetone, which are responsible for yogurt's distinct flavour. Furthermore, certain strains of yogurt starter culture generate an excess of exopolysaccharides (EPS), which diminish syneresis and improve the texture and viscosity of the finished product [1]. Depending on the lactic acid bacteria starters and their metabolites, the key quality attributes of yogurt, such as texture, taste, and flavour, might change. Set type yogurt and strained yogurt are the most prevalent commercially accessible forms of yogurt, while frozen yogurt and sipping yogurt have recently gained popularity [2]. Firmness and the capacity to hold water are the two most essential textural properties of yogurt. Compared to yogurt prepared with encapsulated strains or by direct acidification, yogurt manufactured with encapsulated cultures had a more open structure with wider holes [3].

MATERIAL AND METHODS

BACTERIAL SPECIMEN AND CULTURE

Milk (dairy product) bought from a local market in Vadodara, Gujarat, without any preservatives. The 1ml milk was first serially diluted up to 10^{-5} times in the test tubes. After that, MRS (De Man, Rogosa and Sharpe) agar composition in Table 1, which is used to culture *Streptococcus thermophilus* or *Lactobacillus delbrueckii subsp. bulgaricus* , was produced on 5 petri plates of standard size (100mm x 15mm). Then, using the spread plate approach, 1ml of 10^{-4} serially diluted samples were put on the MRS agar plate. The MRS agar plates should then be incubated for 24 hours at 37°C. Check the MRS agar plates that have been incubated for 24 hours for contamination. After inspecting the plates, place them in an incubator set at 37°C for later use.

TABLE 1: MRS AGAR'S INGREDIENTS [4].

COMPOSITION	CONCENTRATION (G/L)
Agar	15 g
Dextrose	20 g
Beef Extract	10 g
Enzymatic Digest of Animal Tissue	10 g
Yeast Extract	5 g
Sodium Acetate	5 g
Ammonium Citrate	2 g
Potassium Phosphate	2 g
Polysorbate 80	1 g
Magnesium Sulfate	0.1 g
Manganese Sulfate	0.05 g

Final pH: 6.5 ± 0.2 at 25°C.

CONFORMATION TEST FOR *STREPTOCOCCUS THERMOPHILUS* STRAIN

The isolated colony produced on MRS agar plates was identified using Gram stain, biochemical tests, and an automated method for rapid identification of bacteria. The identification was made using Bergey's definitive bacteriology manual. The culture was cultured on an MRS agar slant, which was stored at 4°C. One loop of bacteria was combined in a sterile vial containing porous beads maintained in glycerol for cryo-preservation and functioning as microbe transporters, and kept at -20°C for long-term storage [5].

GRAM STAINING TEST

Using the gram staining approach described by Collins and colleagues, the isolated bacteria were viewed using a compound microscope at a magnification of 100x with oil immersion [6].

MOTILITY TEST

Carigie's approach and the hanging-drop moist method were used. To evaluate the bacteria's movement, the slide was examined using a light microscope at 40x magnification. [7].

CATALASE TEST

A single isolated colony was streaked on a glass slide and one drop of 3 % hydrogen peroxide was poured to it to perform this test. The bacteria's positive reaction to the catalase test was demonstrated by effervescence of oxygen [8].

CARBOHYDRATE FERMENTATION TEST

The media for this experiment was phenol red broth base medium. Lactose, sucrose, glucose, arabinose, sorbitol and maltose were among the sugar substrates utilised. In 100 mL of medium, 0.1 g (0.1 % w/v) of each sugar substrate was added. Each tube received 5 mL of each combination. Durham tube was placed into a glucose test tube for gas detection. At 121°C for 15 minutes, all tubes were sterilized. A single colony of the bacterium under investigation was added to each tube. Changes in the medium's colour showed that the bacteria were reacting positively [9].

YOGURT PREPARATION

Fresh milk was obtained from local shopes in Vadodara. Total fat was 6.0 % and SNF (solid-non-fat) was 9.0 % in the early stages of Amul Gold milk. With the use of a funnel, the 500 mL milk was first prefiltered in a 1000 mL sterile conical flask. After pouring the milk into a sterile conical flask, a loop full of *Streptococcus thermophilus* or *Lactobacillus delbrueckii subsp. bulgaricus* was introduced and thoroughly stirred [10,11]

FERMENTATION OF YOGURT WITH VITAMIN B12

The cultured milk was placed in a conical flask and fermented in an incubator for 24 hours under observation. Then, using a mortar and pestle, ground the vitamin B12 pill. Before fermentation, a vitamin B12 pill (MBSON-SL) with 1500 mcg of Mecobalamin was introduced to the flask. Following fermentation, a sample was obtained to assess attributes such as acidity, antibacterial activity, pH, antioxidant activity, vitamins, and viable LAB count that would allow them to be deemed functional drinks. Furthermore, the level of vitamin B12 was checked to confirm that it did not interfere with the growth of microbes [12].

RESULT AND DISCUSSION

The bacteria that had been isolated were examined under a light microscope. The bacteria were rod-shaped, gram-positive *coccobacilli* that may be found alone or in groups. Based on the gram staining results, the isolated bacterium was identified as *Streptococcus thermophilus* or *Lactobacillus delbrueckii subsp. bulgaricus*.

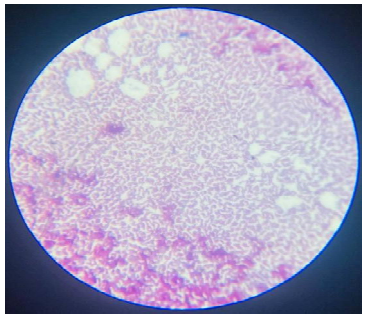

FIGURE 2: ISOLATED *LACTOBACILLUS* BACTERIA UNDER MICROSCOPE

Using the hanging-drop wet technique, the isolated bacteria were determined to be nonmotile. In contrast, Carigie's approach indicated that the bacteria exclusively grew along the stab line in the medium. As a consequence, these methods proved that the bacterium in issue was nonmotile. *Streptococcus thermophilus* or *Lactobacillus delbrueckii subsp. bulgaricus* is notable for its non-motility.

The catalase test is one of the most helpful diagnostic tests for detecting bacteria due to its simplicity. During the catalase test, no bubble was observed, indicating that the isolated bacteria lacks catalase and is unable to mediate the breakdown of H_2O_2 to produce O_2. *Streptococcus thermophilus* or *Lactobacillus delbrueckii subsp. bulgaricus* is a catalase-negative bacteria [13].

The carbohydrate fermentation test's main purpose is to check if bacteria can ferment different kinds of carbohydrates. The phenol red broth base medium was used as a marker to differentiate the bacteria based on their carbohydrate use patterns. Result shows that the isolated bacteria could ferment lactose, maltose, glucose, sucrose but not sorbitol or arabinose. No bubbles were formed when glucose was fed through the Durham tube, showing that no gas was created as a result of the growth. As a consequence, the obtained data matched the features of *Streptococcus thermophilus* or *Lactobacillus delbrueckii subsp. bulgaricus* strains [14].

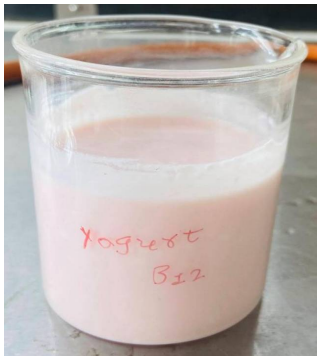

FIGURE 2: FERMENTED B12 YOGURT

This study looked at the survivability and fermentation performance of Lactobacillus bacteria in yogurt that had been supplemented with vitamin B12. Only a few studies on yogurt fermentation have been done thus far. Exopolysaccharide production utilising milk, as well as *L. bulgaricus* and *Streptococcus thermophilus* growth and survival on a yogurt-based media, are just a few instances. Generally, L. delbrueckii ssp. Bulgaricus S. thermophilus and are used to make yogurt, and the relationship between the two species, known as protocooperation, has been explored in terms of metabolic interactions[15]. All

of these tests detected microbial growth in yogurt. We utilised yogurt as the only fermentation medium, without any additions, in comparison to those used in previous research, to guarantee that the yogurt was the only raw material that dictated the development and metabolism of the bacteria in our study [16].

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CONFLICT OF INTREST
The authors declare that they have no conflict of interest.

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