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Short Research Communication

Production of Yogurt by locally isolated starters: *Streptococcus thermophilus* and *Lactobacillus bulgaricus*

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Abestract: The aim of this study was to produce yogurt using isolated native starters *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus salivarius subsp. thermophilus*. The native starters were isolated, purified and identified according to Bergey's manual of determinative bacteriology**.** Yogurt was made by two methods, the first method was made by the combination of *L . bulgaricus* and *S. thermophilus* in equal ratios and which were inoculated directly into a pasteurized milk. The second method was done using the isolated bacteria that was prepared from the culture media MRS and M17 respectively, through two transfers in 10% skimmed milk medium overnight, and then 3% of these cultures were inoculated into milk separately. The mixture was incubated at 42ºC for 6-8 hours and coagulation was observed. Yogurt PH and volume were measured of and our results indicate that the first method was better than the second one. It is well known that yogurt production using native starter cultures instead of commercial ones is beneficial in respect of both economic and organoleptic aspects. Our results indicate that the isolated native yogurt starters can be used in yogurt manufacturing in an industrial large scale.

Keywords: Yogurt, Dairy products, Starter cultures, *Lactobacillus bulgaricus*, *Streptococcus thermophilus*

INTRODUCTION: The consumption of fermented milk products increased during last year's. This event is attributed to the expanding variety to sensory aspects and to nutritional and therapeutic properties of these products **(1)**. One aspect of biotechnology is the directed use of organisms for making yogurt. It is a dairy product produced by bacterial fermentation of milk sugar into lactic acid that gives yogurt its gel-like texture and characteristic tang **(1)**.

Yogurt contains thermophilic starter culture such as *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus salivarius subsp. thermophilus* **(2-5)**. *Lactobacillus delbrueckii* are rod with rounded ends shape, but *Streptococcus thermophilus* has a spherical to ovoid shape with an irregular segments **(6)**. Both are Gram-positive, facultative anaerobic, non-motile and non-spore-forming bacteria **(7)**.

Successful preparation of yogurt depends upon the proper symbiotic relationship between the two organisms at equal proportion **(8)**. Strains belonging to the same species may show more or less marked activities, and give clearly different products **(9)**. The aim of this study is to produce yogurt from isolated native starters *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus salivarius subsp. thermophilus.*

Materials and Methods

Bacterial strains and growth conditions

The native starters *L . bulgaricus* and *S. thermophilus* were isolated from locally prepared yogurt on Elliker agar and M17 (HiMedia, India) respectively **(8)**. These isolates were purified and identified on the basis of their morphological and biochemical characteristics. Bergey's manual of determinative bacteriology criteria were applied in the identification of the isolated microorganisms **(10)**.

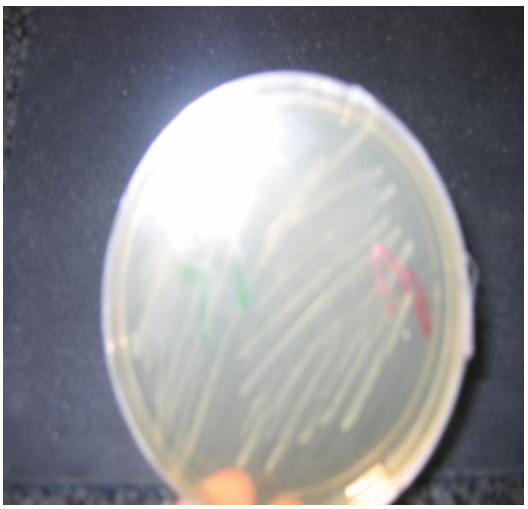
The isolated bacteria were maintained as frozen stock culture at -70ºC in MRS (HiMedia, India) and M17 broth respectively, containing 20 % glycerol (Merck, Germany).

Preparation of Yogurt

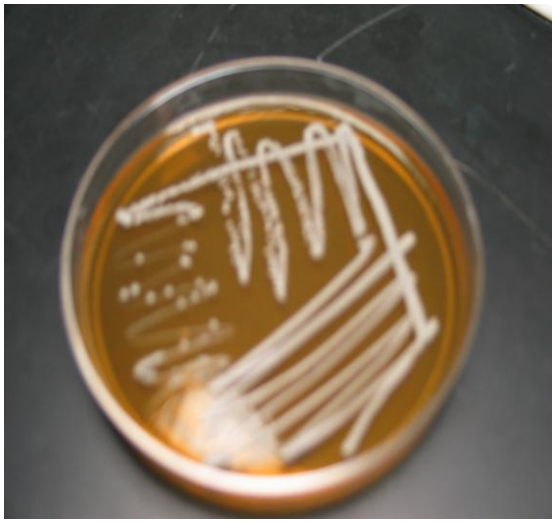
Yogurt was made by two methods, the first method (A): A combination of *L. bulgaricus* & S. *thermophilus* in equal ratio 1:1 (18 hours old) were directly inoculated to pasteurized milk and incubated at 42 ±1ºC for 8 hours in the incubator (NB-201, N-BIOTEK, Korea) **(8)**. In the second method (B), the isolated bacteria were prepared from MRS and M 17 respectively, through two transfers in 10 percent skimmed milk medium (HiMedia, India) overnight and after that 3 % of these cultures was separately inoculated into the milk. Finally mixtures were incubated at 42ºC for 6-8 hours and after that coagulation was observed and the flasks were cooled immediately **(11)**. In each case curdling was observed and the product was tested for acidity by pH meter (MARTINI instrument, Romania).

RESULTS

Our results matched with the expected results, based on characteristics listed in the Bergey's manual. For instance, *S. thermophilus and L. bulgaricus* are positive on Gram stain **(Figure 1)**, negative for the capsule stain and endospore stain tests. Our results also showed that they were catalase and oxidase negative, and non-motile **(10)**.



Lactobacillus. bulgaricus



Streptococcus thermophilus

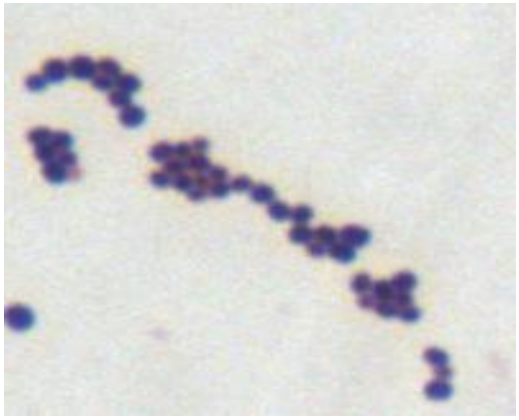


Figure (1) Gram stain and shape of the isolates.

Table (1) Methods of making yogurt

Yogurt	Method A	Method B
Volume (ml)	80	50
PH directly	4.9	4.5
PH after 3days	4.84	4.42

Table 1 show that the amount of yogurt produced by method (A) is higher in volume than in method (B). The results show that the initial pH is considerably higher in method (A) than in method (B). In both cases pH was decreases after three days.

DISCUSSION: Our first attempt was to plate the locally prepared yogurt straight on the Elliker agar, but only *Lactobacillus bulgaricus* grew when we tried it by this way. So we decided to inoculate the M17 media with yogurt, and this proved successful because growth was occurred and Gram-staining showed that the organism is gram positive cocci **(Figure 1)**. Thus we re-streaked the Elliker agar plates with this culture and we were able to enrich *S. thermophilus* **(12)**.

The isolated bacteria were then purified and identified according to Bergey's manual and were inoculated in the same ratio 1:1 into low fat milk (2%) or skimmed milk. The coccus colonies were grown faster than the rod colonies and it is primarily responsible for acid production **(12)**. It renders milk anaerobic respiration and weak acid production, and then the rod colonies acidifies the milk even more. The associative growth of the two organisms ferments almost all lactose to lactic acid at a rate

greater than that produced by either when growing alone. The increased acidity, in turn causes proteins in milk to tangle into a solid mass (crude, denature). Our results show that the pH was acidic and decreased considerably with time in different methods which confirm the continuous conversion of lactose to lactic acid by the inoculated starter culture. These results correlates with the other studies **(1, 4 & 11).** The increased acidity (PH 4-5) also prevents the proliferation of other potentially pathogenic bacteria **(7)**.

A temperature of 43°C was maintained for 4-6 hours and this temperature was optimized for the two microorganisms (ST 39°C; LB 45°C). Then product cooling and storing was done at (5°C) to slow down the physical, chemical and microbiological degradation and stop fermentation **(13)**.

Recommendation: In order to have a good yogurt we recommend these points:

1. Shorten incubation time and refrigerate yogurt as soon as it becomes firm.
2. Sufficient heat treatment of milk.
3. Active culture, commercial, unflavored yogurt used for starter must be fresh and contain live culture.
4. Incubate milk at 42 ºC, higher temperature may cause the separation or curdling and can destroy the active yogurt culture, while lower temperature may stop the growth.
5. Wash and thoroughly rinse all yogurt-making equipment and container(s) before making new yogurt.
6. Use fresh milk with a good flavor and fresh dry milk powder.

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