



ISOLATION AND IDENTIFICATION OF LACTIC ACID BACTERIA

Dr. Sanjay Kumar Yadav

Asst. Prof. Dept. Of chemistry, Major S.D. Singh P.G. College, Mohammdabad Farrukhabad (UP)

Abstract

*Microorganisms vary greatly in their nutritional requirements and consequently no single medium and set of growth conditions will permit the growth of all microorganisms present in a natural population. In their present investigation the isolation and identification of Lactic acid bacteria from curd (which contains *L. bulgaricus*, *S. thermophilus*, *S. faecalis*, *S. lactis*, *L. casei* and *L. plantarum*) used the agar medium containing 500 mg glucose, 500 mg lactose, 600 mg sodium acetate, 600 mg, peptone, 500 mg yeast-extract, 50 mg each of KH_2SO_4 and K_2HPO_4 20 mg $MgSO_4 \cdot 7H_2O$, 1.0 mg each of $NaCl$, $MnSO_4 \cdot 5H_2O$ and $FeSO_4 \cdot 7H_2O$ and 1.5 g agar-agar in distilled water and total volume was made up-to 100 ml.*

Keyword: *Microorganisms, Lactic acid bacteria, Curd, agar medium, Yeast extract.*

Introduction

The yield of lactic acid produced by fermentation varies with strains of lactic acid bacteria. Therefore, before any detailed study of lactic acid fermentation is done, it becomes necessary to isolate a good lactic acid producing strain of lactic acid bacteria.

Microorganisms are ubiquitous, so the preparation of a pure culture involves not only the isolation of a given microorganism from a mixed natural microbial population which contains two or more species of the organisms growing together, but also the maintenance of the isolated individual and its progeny in an artificial environment to which the access of other microorganisms is prevented. A pure culture consists of only one species of the microorganism growing in or on the nutrient media. It is needed for the morphological and physiological studies of the microorganisms.

A study of 305 strains of lactic acid bacteria to determine the effect of species origin of the bacteria and the source of their isolation on the bacterial property to accumulate fatty acids was done by TerKazar'yan. Pereverzevadn Zodyana isolated 350 cultures of lactic acid bacteria (rod like forms) from corn silage at various stages of ripening and studied their properties. Callieri and Malacalza isolated *Lactobacillus plantarum*, *L. brevis*, *L. delbrueckii*, *L. buchneri*, *Pediococcus cerevisial*, *Leuconostoc mesenteroides* and *Streptococcus lactis* from sugarcane tops silage and studied their characteristics. Paul isolated 480 cultures of lactic acid bacteria from milk.

The microbiology of natural milk cultures was studied by Offogalli and Antonietta", Misuoka examined 27 samples of 11 brands of yogurt and 67 samples of 34 brands of acid milk drinks bacteriologically with special emphasis on the taxonomic problems of lactobacilli which were identified as *L. bulgaricus*, *L. jurti*, *L. Casei* and *L. Acidophilus*. Any nutrient or combination of the nutrients so prepared as to be directly usable for the growth and cultivation of the microorganisms of any kind is termed as culture media. Many media 34-39 have been found useful not only to keep the organism alive and growing but also to assist in differentiating them. Upon or in the media of various kinds, the organisms are able to exhibit physiological activities as well as their morphology and growth characteristics.

Preparation of medium for isolation of Bacteria

Microorganisms vary greatly in their nutritional requirements and consequently no single medium and set of growth conditions will permit the growth of all microorganisms present in a natural population. In their present investigation the author has used the agar medium containing 500 mg glucose, 500 mg lactose, 600 mg sodium acetate, 600 mg, peptone, 500 mg yeast-extract, 50 mg each of KH_2SO_4 and K_2HPO_4 20 mg $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.0 mg each of NaCl , $\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$ and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and 1.5 g agar-agar in distilled water and total volume was made upto 100 ml.

Streak plate method for isolation of bacteria

Growth medium was prepared as above. It was sterilized, 50°C and poured into sterile petri-dishes. When the agar became firm, a loop full of mixed culture of lactic acid bacteria from curd (which contains *L. bulgaricus*, *S. thermophilus*, *S. faecalis*, *S. lactis*, *L. casei* and *L. plantarum*) was removed and was spread at the upper and petri-dish to thin in out. Then streaks were made back and forth the surface of agar. With the same loop, streaks were made on the second and the third plate in the same manner. The plates were then inverted and incubated at 37°C for 48 hrs.

After incubation, colonies were examined and it was seen that on the first plate, there was a very thick growth and colonies were not separated. On proceeding to succeeding plates, the growth became thinner and colonies separated. Ultimately, single isolated colonies, resulting from the growth of a single cell, were found. A little growth from one isolated colony was transferred on a slide and stained. The stained preparation was examined under microscope to ensure that all bacteria were similar morphologically. A loopful growth was transferred from the colony examined to an agar slant. It was incubated at 37°C for 48 hrs, to get a pure culture.

Identification of isolated culture

The identification of isolated culture was done according to "Bergeys' a Manual of Determinative Bacteriology The strain was identified as *Lactobacillus bulgaricus*. It was designated as *L. bulgaricus* MG.

Morphological characteristics of *L. bulgaricus* MG.

Vegetative cells

Form-Red shaped	-	Ends-rounded
Arrangement-chains of 2 – 3 cells	-	Motility-non-motile
Flagella-Absent	-	Capsule absent
Spores absent	-	Staining character - gram positive

Cultural characteristic of *L. bulgaricus* MG Surface colonies

Macroscopic appearance Form – irregular	:	Size - 2-3 in diameter
Margin- circulat to irregular	:	Optical characters- dull
Consistency viscid	:	Elevation flat
Miscroscopic appearance Margin erose	:	Internal structure granular
Agar streak growth Form-filliform:	:	Amount - upto depth of inoculation

Biochemical characteristics of *L. bulgaricus* MG

1. Fermentation reactions in substrate media :

Table 2

<u>Chemical nature of the substrate</u>	<u>Compound</u>	<u>Reaction</u>
Monosaccharide	Ribose	Fermentable
	Arbinose	Fermentable
	Glucose	Readily fermentable
Disaccharide	Lactose	Readily fermentable
	Sucrose	Very mildly fermentable
Poysaccharide	Starch	Unfermentable
Alcohol	Mannitol	Unfermentable
Glycoside	Salicin	Unfermentable

2. Production of organic acids

<u>Produced</u>	<u>Not-produced</u>
Butyric acid	Oxalic acid
Lactic acid	Formic acid
	Acetic acid
	Benzoic acid
	Succinic acid
	Salicylic acid

Gallic acid

Tannic acid

3. Neutral volatile substance**Produced**

Alcohol

(Ethanol)

Not-produced

Acetaldehyde

Acetone

4. Gas productionNo production of H₂S and NH₃**5. Aromatic products (indole)**

Not produced

6. Temperature

Optimum 47°C

Minimum 20°C

Maximum 65°C

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