



Lactobacillus Bulgaricus Agar Base

M927

Lactobacillus Bulgaricus Agar with acetate buffer is used for isolation and identification of *Lactobacillus bulgaricus*.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	10.000
Yeast extract	5.000
Beef extract	10.000
Dextrose	20.000
Dipotassium phosphate	2.000
Tomato juice	2.000
Polysorbate 80	1.000
Agar	20.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 70 grams in 920 ml distilled water and heat to boiling to dissolve the medium completely. Add 80 ml Acetate Buffer (11.355% Sodium acetate and 0.99% Acetic acid). Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. DO NOT OVERHEAT THE MEDIUM. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Lactobacillus bulgaricus (*Lactobacillus delbrueckii* subsp. *bulgaricus*) is one of several bacteria used for the production of Kisselo mlyako (Bulgarian) - “Sour milk” yoghurt (yogurt). The bacterium was first identified in 1905 by the Bulgarian doctor Stamen Grigorov. It is named after Bulgaria, the country where it was first used (it thrives freely on the Balkan Peninsula). The bacterium feeds on milk and produces lactic acid which also helps to preserve the milk. Lactobacillus Bulgaricus Agar was originally formulated by Kulp and White (1) for the recovery of Lactobacilli. Further modification is recommended by APHA (2) for isolation and identification of *L. bulgaricus* from foods. *Streptococcus thermophilus* and *L. bulgaricus* are the essential microbial species and are active in symbiotic relationship in yoghurt. Because of the emphasis on maintaining a balance between cocci and rods, techniques are needed to determine the relative proportions of *S. thermophilus* and *L. bulgaricus* when grown together in milk cultures.

Casein enzymic hydrolysate, yeast extract and beef extract in the medium provide nitrogenous compounds, minerals, vitamins and trace ingredients. Polysorbate 80 supplies fatty acids required for the metabolism of Lactobacilli. Dextrose is the fermentable carbohydrate. Tomato juice along with acetate maintains the low pH of the medium and thus inhibits microorganisms other than Lactobacilli. Acetate also restricts the swarming of *L. bulgaricus* and along with dipotassium phosphate forms the buffering system.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% agar gel.

Colour and Clarity of prepared medium

Medium amber coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of the medium (7% w/v aqueous solution of base containing 8% v/v acetate buffer) at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

M927: Cultural characteristics observed with added acetate buffer, after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery
<i>Lactobacillus bulgaricus</i> ATCC 11842	50-100	good-luxuriant	>=50%

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. Kulp W. L. and White V., 1932, Science, 76:17.
2. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., APHA, Washington, D.C.

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