



## Rogosa Agar, Modified

M1899

Recommended for the selective cultivation of Lactobacilli from food

### Composition\*\*

Ingredients	Gms / Litre
Tryptone	10.000
Yeast extract	5.000
Glucose	20.000
Potassium dihydrogen orthophosphate	6.000
Tween 80	1.000
Triammonium citrate	2.000
Sodium acetate	15.000
Magnesium sulphate, 7H <sub>2</sub> O	0.575
Manganese (II) sulphate, H <sub>2</sub> O	0.110
Iron (II) sulphate, 7H <sub>2</sub> O	0.034
Agar	15.000
Final pH ( at 25°C)	6.2±0.1

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Suspend 74.40 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Add 1.32 ml glacial acetic acid and mix thoroughly. DO NOT AUTOCLAVE. Mix well and pour into sterile Petri plates.

### Principle And Interpretation

Rogosa Agar is primarily a selective medium for the cultivation of *Lactobacillus* (1). High acetate concentration and low pH effectively suppress other bacteria, but also many strains of other lactic acid bacteria. The modification of the pH to 6.2 instead of 5.5 alters the selectivity of the medium for the whole group of lactic acid bacteria (2,3).

Casein enzymic hydrolysate, yeast extract provide nitrogenous compounds, sulphur, trace elements and vitamin B complex, essential for growth of Lactobacilli. Glucose acts as fermentable carbohydrate. Polysorbate 80 is the source of fatty acids. Ammonium citrate and sodium acetate inhibit moulds, Streptococci and many other organisms. Monopotassium phosphate provides buffering capability. Magnesium sulphate, manganese sulphate and ferrous sulphate are sources of inorganic ions. Low pH of the medium and addition of acetic acid makes the medium selective for Lactobacilli, inhibiting other bacterial flora (4). It is recommended that the plates should be incubated at 30°C for 5 days or at 37°C for 3 days in an atmosphere of 95% hydrogen and 5% carbon dioxide (5). High acetate concentration and acidic pH suppress many strains of other lactic acid bacteria.

### Quality Control

#### Appearance

Cream to yellow homogeneous soft lumps which can be easily broken down to powder form.

#### Gelling

Firm, comparable with 1.5% Agar gel

#### Colour and Clarity of prepared medium

Light yellow coloured opalescent gel forms in Petri plates

#### Reaction

Reaction of 7.44% w/v aqueous solution with 0.132% v/v acetic acid at 25°C. pH : 6.2±0.1

#### pH

6.10-6.30

#### Cultural Response

Cultural characteristics observed in presence of 5% Carbon dioxide (CO<sub>2</sub>) and 95% H<sub>2</sub> after an incubation at 35-37°C for 40-48 hours.

### Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery
<b>Cultural Response</b>			
<i>Lactobacillus casei</i> ATCC 9595	50-100	good - luxuriant	≥50%
<i>Lactobacillus fermentum</i> ATCC 9338	50-100	good to luxuriant	≥50%
<i>Lactobacillus leichmanni</i> ATCC 4797	50-100	good to luxuriant	≥50%
<i>Lactobacillus plantarum</i> ATCC 8014	50-100	good-luxuriant	≥50%
<i>Staphylococcus aureus</i> ATCC 25923	≥10 <sup>3</sup>	inhibited	0%

### 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. Rogosa, J., Mitchell J.A. and Wiseman, R.F. (1951) A selective medium for the isolation and enumeration of oral and fecal lactobacilli. *J. Bacteriol.* 62, 132-133.
2. ISO (1984) Drafts reports. Enumeration of Lactobacteriaceae in meat and meat products. ISO/TC 34/SC 6/WG 15, no. 3 and no. 5. International Organization for Standardization, Geneva.
3. Reuter, G. (1985) Elective and selective media for lactic acid bacteria. *Int. J. Food Microbiol.* 2, 55-68.
4. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification- Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore. Md.
5. Sharpe M. L. (Ed.), 1960, Lab-Practice, 9(4): 223.

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