



MRS Agar, Modified (Lactobacilli Heteroferm Screen Agar)

M1163

Modified MRS Agar is recommended for the isolation and cultivation of *Lactobacillus* species from salad dressings.

Composition**

| Ingredients | Gms / Litre |
|-----------------------|-------------|
| Dextrose | 20.000 |
| Proteose peptone | 10.000 |
| Yeast extract | 5.000 |
| Sodium acetate | 5.000 |
| 2-Phenylethyl alcohol | 3.000 |
| Ammonium citrate | 2.000 |
| Dipotassium phosphate | 2.000 |
| Magnesium sulphate | 0.100 |
| Manganese sulphate | 0.050 |
| Bromocresol green | 0.040 |
| Cycloheximide | 0.004 |
| Agar | 15.000 |
| Final pH (at 25°C) | 5.5±0.2 |

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 62.2 grams in 1000 ml distilled water containing 1 ml polysorbate 80. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. If necessary, adjust the pH with glacial acetic acid after sterilization. Mix well and pour into sterile Petri plates.

Warning : Cycloheximide is very toxic. Avoid skin contact or aerosol formation and inhalation.

Principle And Interpretation

Mayonnise, cooked starch-based dressings resembling mayonnise and pourable dressings are the types of salad dressings available. Microorganisms in salad dressings come from the ingredients from manufacturing equipments and from air. The microflora causing salad dressing to spoil seems quite restricted and consists of few species of *Lactobacillus*, *Saccharomyces* and *Zygosaccharomyces*. MRS Agar, Modified (Lactobacillus Heteroferm Screen Agar) recommended by APHA (1), is used for the isolation and cultivation of *Lactobacillus* species from salad dressings (2).

MRS Agar, Modified is the modification of MRS medium of deMan et al (3). Proteose peptone and dextrose supply nitrogen, carbon and other elements essential for the growth of Lactobacilli. Polysorbate 80 a mixture of oleic esters, supplies fatty acids required by Lactobacilli. Ammonium citrate, sodium acetate, 2-phenylethyl alcohol and cycloheximide inhibit gram-negative organisms, moulds and certain gram-positive bacteria. Certain yeasts are also suppressed because of presence of cycloheximide. Bromocresol green is the pH indicator, which under acidic conditions, changes colour from green to yellow.

Inoculate 1 ml of 1:10 dilutions of the dressing sample into three MRS Broth, Modified (M1164) tubes. Incubate at 32°C for 72 hours ± 2 hours. Positive tubes have trapped CO₂ in the Durhams tubes or bubbles of CO₂ clinging to the inside of the tube and a colour change from green to yellow indicating acid production. These presumptive cultures can be confirmed by streaking on MRS Agar, Modified plates.

Quality Control

Appearance

Light yellow to bluish grey homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Green coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 6.22% w/v aqueous solution at 25°C. pH : 5.5±0.2

pH

5.30-5.70

Cultural Response

M1163: Cultural characteristics observed in presence of 5-10% Carbon dioxide(CO₂) after an incubation at 35-37°C for upto 3 days.

| Organism | Inoculum (CFU) | Growth | Recovery |
|--|----------------|-----------|----------|
| Cultural Response | | | |
| <i>Lactobacillus casei</i> ATCC 9595 | 50-100 | luxuriant | >=50% |
| <i>Lactobacillus acidophilus</i> ATCC 4356 | 50-100 | luxuriant | >=50% |
| <i>Lactobacillus fermentum</i> ATCC 9338 | 50-100 | luxuriant | >=50% |
| <i>Lactobacillus plantarum</i> ATCC 8014 | 50-100 | luxuriant | >=50% |

Storage and Shelf Life

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

Reference

1. Vanderzant C. and Splittstoesser D. F., (Eds.), 1992, Compendium of Methods for the Microbiological Examination of Foods, 3rd Ed., APHA, Washington, D.C.
2. Smittle R. B. and Flowers R. M., 1982, J. Food Protection, 45:977.
3. DeMan J. D., Rogosa M. and Sharpe M. E., 1960, J. Appl. Bacteriol., 23:130.

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