Modified Rogosa Agar (M16 Agar)

M600

M16 Agar is recommended for cultivation and enumeration of lactic streptococci used in manufacture of cheddar cheese.

Composition**

Ingredients	Gms / Litre
Papaic digest of soyabean meal	5.000
Tryptose	5.000
Beef extract	5.000
Yeast extract	2.500
Dextrose	5.000
Ascorbic acid	0.500
Sodium acetate	3.000
Agar	10.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 36 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

Principle And Interpretation

A variety of acid-producing bacteria are found in nature, in the soil, on raw agricultural products and in certain processed foods. One of the most important groups of acid producing bacteria in food industry is the lactic acid bacteria. Streptococci belong to the lactic acid bacteria group.

Streptococcus is a genus of spherical, gram-positive bacteria, and a member of the phylum Firmicutes (1). M16 Agar is a modification of Rogosa Sodium Lactate Agar recommended by APHA (1, 3). This medium was developed to support growth of lactic streptococci used in cheddar cheese manufacturing in New Zealand (2). This medium can also be used as selective medium for the cultivation of oral and faecal lactobacilli. Since some lactobacilli do not grow on this medium if incubated aerobically, incubation in a CO2-enriched atmosphere is recommended.

The large number of media proposed for lactic acid bacteria, particularly for streptococci and /or lactobacilli, is an indicative of the variability in growth features of different species, thereby the difficulties encountered in growing some strains of this group of organisms. While the lactic acid bacteria in general are tolerant to low pH, they can be very sensitive to other adverse conditions. Freezing and thawing prior to analysis may be detrimental to cell growth. Dilution process may also damage lactic acid bacteria in samples, thus it is best to use sterile 0.1% Peptone Water (M028) as the diluent.

Papaic digest of soyabean meal, tryptose and beef extract provide the essential nutrients like amino acids, minerals etc. Yeast extract supplies vitamin B complex to the lactic streptococci. Dextrose is the fermentable carbohydrate and energy source. Sodium acetate inhibits other contaminating bacteria and suppresses swarming growth. Ascorbic acid provides vitamin C to the organisms.

The samples to be tested are processed to enumerate bacteria by pour plate technique.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.0% Agar gel.

Colour and Clarity of prepared medium
Light amber coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 3.6% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH
7.00-7.40

Cultural Response
M600: Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours in CO2 enriched atmosphere.

Organism	Inoculum (CFU)	Growth	Recovery
Cultural Response			
Lactobacillus lactis ATCC 8000	50-100	good-luxuriant	>=50%
Streptococcus cremoris ATCC 19257	50-100	good-luxuriant	>=50%

Storage and Shelf Life
Store below 8°C and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference
1. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., APHA, Washington, D.C.
2. Lowrie R. J. and Pearce L. E., 1971, New Zealand, J. Dairy Sci. Technol., 6: 166.
3. Rogosa M., Mitchell J. A. and Wiseman R. F., 1951, J. Bacteriol., 62 : 132-133

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