Nutrient Agar w/ Manganese

M931

Nutrient Agar with Manganese is used for promoting sporulation in *Bacillus* species.

Composition**

Ingredients	Gms / Litre
Beef extract	3.000
Pancreatic digest of gelatin	5.000
Manganese sulphate	0.030
Agar	15.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 23.03 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

Nutrient Agar w/ Manganese, conventionally abbreviated as NAMn favours culture and sporulation of aerobic *Bacillus* species especially from canned foods.

Beef extract and pancreatic digest of gelatin provide necessary nutrients for growth of *Bacillus* species. Manganese is known to influence and enhance sporulation in *Bacillus* species (1- 4). It has been reported that organisms recovered from spoilage of foods such as fruit drinks, tomatoes, acidified onions and other canned foods sporulate well aerobically on Nutrient Agar with added manganese (5).

Thermophilic bacteria such as *B. stearothermophillus* are capable of growth at 55-65°C while an incubation temperature of 30 to 35°C is favorable for culture and sporulation of mesophilic spore formers (5). This property is exploited to grow and therefore differentiate mesophilic and thermophilic spoilage bacteria. As recommended by APHA, in routine diagnosis for spoilage in canned foods, microbiological cultural procedures involve the use of primary recovery media and subculture media to identify spoilage bacteria and study its growth characteristics. Recovery media for aerobes generally include DTA (Dextrose Tryptone Agar) (M092) or DTB (Dextrose Tryptone Bromocresol Broth) (M122). Use of Cooked Meat Medium (M149) is recommended for recovery of anaerobic organisms. NAMn is widely used as subculture media for aerobes.

Nutrient Agar with Manganese supports growth and enhances sporulation by aerobic spore-formers and can be used primarily to differentiate mesophilic from thermophilic *Bacillus* species. When rod shaped aerobes in pure culture are isolated on DTA (or DTB) media (M092/ /M122) and sporulation is not evident, the isolates should be subcultured on Nutrient Agar with Manganese, at the temperature of initial isolation. After incubation for upto 10 days, if spore production has taken place, the spores are heat shocked to destroy all vegetative cells and cultured on Nutrient Agar w/ Manganese at both 30-35°C and 55°C. The temperature at which outgrowth occurs from the spore state indicates whether the isolate is an obligate mesophile (growth at 30 to 35°C), an obligate thermophile (growth at 55°C) or a facultative thermophile (growth at 30 to 35°C and at 55°C).

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

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

Reaction
Reaction of 2.3% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH
6.60-7.00

Cultural response
Cultural characteristics observed after an incubation at 35-37°C for upto 5 days .

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery
Cultural response			
Bacillus stearothermophilus ATCC 7953	50-100	luxuriant (incubated at 55°C for upto 5 days)	>=50%
Bacillus coagulans ATCC 8038	50-100	luxuriant (with sporulation)	>=50%
Bacillus licheniformis ATCC 9945a	50-100	luxuriant (with sporulation)	>=50%
Bacillus megaterium ATCC 9855	50-100	luxuriant (with sporulation)	>=50%
Bacillus polymyxa ATCC 8526	50-100	luxuriant(with sporulation)	>=50%
Bacillus subtilis ATCC 6633	50-100	luxuriant (with sporulation)	>=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. Charney, J., Fisher, W. P. and Hegarty C. P., 1951, J. Bacteriol., 62:145.
2. Curran, H. R. and Evans F. R., 1954, J. Bacteriol., 67: 489.
3. Maunder D. T., 1970, “Examination of canned foods for microbial spoilage.” Microbiology, Metal Div. R. and D, Continental Can Co., Inc., Oak Brook, III.
4. Penna T. C., Machoshvili I. A., Taqueda, M. E and Ferraz, C. A. 1998, PDA J. Pharm. Sci. Technol., 52 (5):198.
5. 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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