Nutrient Agar w/ Manganese

M931

Intended Use:

Recommended for promoting sporulation in *Bacillus* species.

Composition**

Ingredients	Gms / Litre
HM peptone B #	3.000
Gelatin peptone	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

- Equivalent to Beef extract

Directions

Suspend 23.03 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. 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.

HM peptone B and gelatin peptone provide necessary nutrients required for growth of *Bacillus* species. Manganese is known to influence and enhance sporulation in *Bacillus* species (1,2,5,6). 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 (7).

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 (7). 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 M Medium (M149) is recommended for recovery of anaerobic organisms. NAMn is widely used as subculture media for aerobes. 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). After use, contaminated materials must be sterilized by autoclaving before discarding. 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.

Type of specimen

Food samples

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (7).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Further biochemical and serological tests must be carried out for complete identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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 .

Organism	Inoculum (CFU)	Growth	Recovery
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 subsp. *spizizenii* ATCC 6633 (00003*)	50-100	luxuriant (with sporulation)	>=50%

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

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