Ashby's Mannitol Agar

M706

Intended Use:

Recommended for isolation of *Azotobacter* species from soil that can use mannitol and atmospheric nitrogen as source of carbon and nitrogen respectively.

Composition**

Ingredients	Gms / Litre
Mannitol	20.000
Dipotassium hydrogen phosphate	0.200
Magnesium sulphate	0.200
Sodium chloride	0.200
Potassium sulphate	0.100
Calcium carbonate	5.000
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40.7 grams in 1000 ml purified / distilled water. Heat just to boiling. 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.
Note: Due to presence of calcium carbonate, the prepared medium forms opalescent solution with white precipitate.

Principle And Interpretation

Azotobacter is a genus of free-living diazotrophic bacteria which have the highest metabolic rate compared to any other microorganisms. *Azotobacters* are chemoorganotrophic, using sugars, alcohols and salts of organic acids for growth. *Azotobacters* can non-symbiotically fix atmospheric nitrogen aerobically due to their unique mode of metabolism. Besides the ability to fix atmospheric nitrogen, *Azotobacter* also synthesize biologically active substances, which attributes to improving seed germination, plant growth etc.
Ashby's Mannitol Agar are formulated as described by Subba Rao (3). It is used for isolation of *Azotobacter*, a non-symbiotic nitrogen fixing bacteria which uses mannitol as a carbon source and atmospheric nitrogen as nitrogen source. Dipotassium phosphate provides buffering to the medium. Various essential ions required for promoting growth of *Azotobacter* are also available in this medium

Type of specimen

Soil samples

Specimen Collection and Handling

For soil samples, follow appropriate techniques for sample collection and processing as per guidelines (3).
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. Due to variable nutritional requirements, some strains show poor growth on this medium.

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
White to cream homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Whitish opalescent gel forms in Petri plates

Reaction
Reaction of 4.07% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

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

Organism	Growth
Azotobacter nigricans ATCC 35009	good-luxuriant
Azotobacter vinelandii ATCC 478	good-luxuriant

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 (1,2).

Reference

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
3. Subba Rao, 1977, Soil Microorganisms and Plant Growth, Oxford and IBH Publishing Co., India.

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