Asparagine Nitrate Medium

M724

Intended Use

Recommended for the isolation and cultivation of denitrifying bacteria from soil samples.

Composition**

Ingredients	Gms / Litre
Potassium nitrate	1.000
L-Asparagine	1.000
Sodium citrate	8.500
Potassium dihydrogen phosphate	1.000
Magnesium sulphate	1.000
Calcium chloride	0.200
Ferric chloride	0.0001
Agar	15.000

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 27.7 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

Asparagine Nitrate Medium is formulated as per Subba Rao (3). Nitrogen transformation in soil results in the loss of molecular nitrogen. The conversion of nitrate and nitrite into molecular nitrogen or nitrous oxide through microbial processes is known as denitrification. Denitrification of bound nitrogen to gaseous nitrogen is mediated by numerous species of bacteria, which normally use oxygen as hydrogen acceptor (aerobic). These bacteria also posses the ability to use nitrate and nitrite in the place of oxygen as the hydrogen acceptor (anaerobically).

Asparagine is source of organic nitrogen and is readily available for microbial energy and growth while the salts in the medium help for growth of microorganisms.

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
Light amber coloured clear to slightly opalescent gel forms in Petri plates

Cultural Response
Cultural characteristics observed after an incubation at 25-30°C for upto 7 days.

Organism	Growth
Achromobacter denitrificans ATCC 14648	luxuriant
Bacillus subtilis subsp. *spizizenii ATCC 6633* (00003*)	luxuriant
Micrococcus luteus ATCC 10240	luxuriant
Pseudomonas aeruginosa ATCC 27853 (00025*)	luxuriant
Thiobacillus denitrificans ATCC 29685	good

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 (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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