Ashby's Sucrose broth

M2024

Intended Use:

Recommended for growth and maintenance of *Azotobacter chroococcum.*

Composition**

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

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 25.7 grams in 1000 ml purified / distilled water. Heat just to boiling. Dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

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 microorganism.

Azotobacters are chemoorganotrophic, using sugars, alcohols and salts of organic acids for growth.

Ashby's Medias are formulated as described by Subba Rao (3). It is used for isolation of *Azotobacter*, a non-symbiotic nitrogen fixing bacteria which uses sucrose as a carbon source and atmospheric nitrogen as nitrogen source. Besides the ability to fix atmospheric nitrogen, *Azotobacter* also synthesize biologically active substances which attributes to improving seed germination, plant growth etc. Dipotassium hydrogen phosphate provides buffering to the system. Various essential ions required for promoting growth of *Azotobacter* are also available in this medium

Type of specimen

Soil Sample

Specimen Collection and Handling

For soil samples follow appropriate techniques for handling specimens as per established 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. Other Nitrogen fixing bacteria capable to fix atmospheric nitrogen, grows when cultured on this nitrogen-free 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

Colour and Clarity of prepared medium

Colourless, opalescent solution in tubes with precipitate

Reaction

Reaction of 2.57% 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 25-30°C for upto 5 days.

Organism	Growth
Azotobacter chroococcum MTCC 7724	good-luxuriant

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°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 clinical 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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