



Isolation Medium For Iron Bacteria

M622

Isolation Medium For Iron Bacteria is used for the isolation of iron bacteria, especially those belonging to *Sphaerotilus* Leptothrix group.

Composition**

Ingredients	Gms / Litre
Glucose	0.150
Ammonium sulphate	0.500
Calcium nitrate	0.010
Dipotassium phosphate	0.050
Magnesium sulphate	0.050
Potassium chloride	0.050
Calcium carbonate	0.100
Cyanocobalamin(Vitamin B12)	0.00001
Thiamine	0.0004
Agar	10.000

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 10.88 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml 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 dispense into sterile test tubes.

Note: Due to the presence of calcium carbonate, the prepared medium forms opalescent solution with white precipitate.

Principle And Interpretation

Sphaerotilus-Leptothrix group are filamentous bacteria that form sheath. The sheathed bacteria have the ability to deposit ferric hydroxide and sometimes manganese dioxide on their sheaths (1). The specific deposition of ferric ions on the sheath of *S.discophorus* (also *Leptothrix* species) was demonstrated by Rogers and Anderson (2, 3). Iron bacteria obtain energy by the oxidation of iron from the ferrous to ferric state. Some bacteria that do not oxidize ferrous ions may dissolve or deposit it indirectly. During their growth, they either liberate iron by utilizing organic radicals to which the iron is attached, or alter environmental conditions to permit the deposition of iron. Isolation Medium for Iron Bacteria is recommended by APHA (4) for the isolation of iron bacteria, especially those belonging to the *Sphaerotilus-Leptothrix* group. The medium has been proven helpful for identifying various groups of filamentous organisms including iron bacteria (5).

Magnesium sulphate, ammonium sulphate, potassium chloride and calcium nitrate are sources of ions that stimulate metabolism. Glucose acts as the carbon source. Dipotassium phosphate buffers the medium. The bacteria of both genera, *Sphaerotilus* and *Leptothrix* require vitamin B12 as an essential growth factor. A number of *Leptothrix* strains have been found to require additionally thiamine as growth factor.

Prepare agar slants of these media and aseptically pipette 3 ml test water sample on to surface of slants. Inoculate the tubes and incubate at room temperature until turbid growth develops in the liquid layer (4).

Quality Control

Appearance

Cream to beige homogeneous free flowing powder

Gelling

Firm, comparable with 1.0% Agar gel.

Colour and Clarity of prepared medium

Medium yellow coloured, clear to slightly opalescent gel forms in tubes as slants

Cultural Response

M622: Cultural characteristics observed after an incubation at 22-25°C upto 5 days.

Organism	Growth
<i>Leptothrix discophora</i> ATCC 43182	good-luxuriant
<i>Sphaerotilus natans</i> ATCC 13338	good-luxuriant
<i>Ferrobacillus ferrooxidans</i>	good-luxuriant

Storage and Shelf Life

Store below 30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

Reference

1. Balows A., Truper H. G., Dworkin M., Harder W., Schleifer K. H., (Eds.), The Prokaryotes, 2nd Edition, Vol. III, Springer-Verlag.
2. Rogers S. R., Anderson J. J., 1976, J. Bacteriol., 126: 257-263.
3. Rogers S. R., Anderson J. J., 1976, J. Bacteriol., 126: 264-271.
4. Eaton A. D., Clesceri L. S., Rice E. W., and Greenberg A. W., (Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st Ed., APHA, Washington, D.C.
5. VanVeen W. L., 1973, Antonie Van Leeuwenhoek (Holland), 39:189.

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