Rhizobium Medium

M408

Rhizobium Medium is used for cultivation and isolation of *Rhizobium* species.

Composition**

Ingredients	Gms / Litre
Mannitol	10.000
Dipotassium phosphate	0.500
Magnesium sulphate	0.200
Yeast extract	1.000
Sodium chloride	0.100
Agar	20.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 31.8 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Rhizobium Medium is used in the large scale production of legumes and in their isolation from root nodules.

Rhizobium Medium is recommended for isolation and cultivation of mannitol-positive *Rhizobium* species. It is also useful for the maintenance of *Rhizobium* species by adding extra 1% mannitol to the medium as specified by the American Type Culture Collection (1).

The medium is well buffered for pH changes and osmotic changes by presence of phosphate and sodium chloride salts. Yeast extract provides nitrogenous nutrients. Mannitol is the energy source while magnesium sulphate provides essential ions.

The inocula are transferred from agar slants into starter flasks containing Rhizobium Medium. After 4 days of growth, the culture from starter flasks is transferred into a small seed tank fermentor. At this stage, Rhizobium Medium is used for large scale production. *Rhizobium* may be isolated from the root system of the leguminous plant. The healthy, pinkish nodule on the tap root is carefully cut out. The nodule is surface sterilized for 5 minutes and then washed with solvents like ethanol etc. The nodule is then crushed with a sterile glass rod in a small aliquot of sterile water. Serial dilutions are subsequently made to get sparse and distinct colonies. The dilutions are plated on Rhizobium Medium and incubated for upto 4 days at 25-30°C (2).

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 2.0% Agar gel.

Colour and Clarity of prepared medium
Yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 3.18% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH
6.60-7.00

Cultural Response
M408: Cultural characteristics observed after an incubation at 25-30°C for upto 4 days.

Organism	Growth

Rhizobium japonicum ATCC luxuriant
10324
Rhizobium meliloti ATCC luxuriant
9930

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. ATCC Catalogue of Bacteria and Bacteriophages, 1992, 18th Ed., American Type Culture Collection, Rockville, MD.
2. Subba Rao N. S, Soil Microorganisms and Plant Growth- (Oxford and IBH Publishing Co.)

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