

Technical Data

Trichoderma harzianum Selective Agar Base

M1836

Intended Use:

Recommended for selective isolation of Trichoderma harzianum.

Composition**

| Ingredients | Gms / Litre |
|---------------------------------|-------------|
| Magnesium sulphate heptahydrate | 0.200 |
| Dipotassium hydrogen phosphate | 0.900 |
| Ammonium nitrate | 1.000 |
| Potassium chloride | 0.150 |
| Glucose(Dextrose) | 3.000 |
| Rose Bengal | 0.150 |
| Agar | 20.000 |

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 25.30 grams (the equivalent weight of dehydrated medium per litre) in 960 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 and aseptically add the rehydrated contents of one vial of Trichoderma harzianum Selective Supplement (FD276). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Trichoderma harzianum is an efficient biocontrol agent that is commercially produced to prevent development of several soil pathogenic fungi. Different mechanisms have been suggested as being responsible for their biocontrol activity, which include competition for space and nutrients, secretion of chitinolytic enzymes, mycoparasitism and production of inhibitory compounds. (1,2). Nevertheless, the biocontrol activity of *T. harzianum* could be affected by environmental, that include among others, the presence of plant nutrients at the field level (1). Also, the isolates of *Trichoderma harzianum* attack the commercial mushroom *Agaricus bisporus* colonize spawned compost and result in substantial yield reduction. This medium was based on the formulation of T.harzianum selective medium (THSM) (6). Glucose (Dextrose) in the medium serve as a source of energy as well as carbohydrate source and Dipotassium hydrogen phosphate buffers the medium. Magnesium Sulphate act as a source of ions and sulphates. Ammonium nitrate provides source of nitrogen. Rose Bengal is a selective agent that inhibits bacterial growth and restricts the size and height of colonies of more rapidly growing moulds. Care should be taken not to expose this medium to light, since photodegradation of Rose Bengal yields compound that are toxic to fungi.

Type of specimen

Soil samples.

Specimen Collection and Handling

For soil samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (4). 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. Some strains may show poor growth due to nutritional variations.

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

Light yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% Agar gel

Colour and Clarity of prepared medium

Light pink coloured clear to slightly opalescent gel forms in Petri plates.

Cultural Response

Cultural characteristics observed after incubation at 25-30°C for 5 to 6 days.

| Organism | Inoculum (CFU) | Growth |
|--|----------------------------|------------------------|
| Trichoderma harzianum Escherichia coli ATCC 25922 (00013*) | 50 -100 10 ⁴ | luxuriant inhibited |
| Staphylococcus aureus Subsp. aureus ATCC 25923 (00034*) | 104 | inhibited |

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°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 (3,4).

Reference

1. Angela Roco., (2001), In vitro biocontrol activity of *Trichoderma harzianum* on Alternaria alternata in the presence of growth regulators. Plant Pathology. 4:2

2. Haram, S.; Schickler, H.; Oppenheim, A. and Chet, I. (1996). Differential expression of *Trichoderma harzianum* chitinases during mycoparasitism. Phytopathology 86:980-985.

3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

4. 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.

5. Subba Rao N. S., 1977, Soil Microorganisms and Plant Growth, Oxford and IBH Publishing Co., New Delhi.

⁶.Williams, J., Clarkson, J.M., Mills, P.R., and Cooper, R.M. (2003) A Selective Medium for Quantitative Reisolation of *Trichoderma harzianum* from *Agaricus bisporus* Compost

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