PHM004 **Phyto Casein Hydrolysate Yeast Extract Agar Base**

Semi selective medium for the detection of *Clavibacter michiganensis subsp michiganensis* on seeds of tomato.

Composition **:

Ingradients	**Grams/Litre**
Yeast extract	2.00
Casein hydrolysate	4.00
Glucose, anhydrous	10.00
Magnesium sulphate, anhydrous	0.15
Boric acid	1.00
Ammonium chloride	1.00
Tris HCl	1.20
Agar	18.00
Final pH (at 25°C)	7.4

**Formula adjusted standard to suit the performance parameter.

Direction.:

Suspend 37.35 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize both the solutions separately by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50 °C. Aseptically add the contents of one vial of CNP supplement (PHS003). Mix well and pour into sterile Petri plates.

Principle and Interpretation

Clavibacter michiganensis subsp. *michiganensis* is a Gram-positive bacterium that causes wilting and cankers, leading to severe economic losses in commercial tomato production worldwide. Bacterial canker is one of the most destructive tomato diseases. Symptoms include necroses on leaves , stems and fruits during flowering of the plants.(1)

It can be recovered on any general microbiological media such as nutrient agar, however specialized medium such as Phyto Casein Hydrolysate Yeast Extract Agar Base are more advantageous.(2). It is comparatively a less selective medium

Casein hydrolysate and yeast extract supplies nitrogeneous compounds. Glucose serves as a source of carbohydrate. Boric acid helps in selectivity of the medium. Tris HCL helps in buffering of the medium.

Quality Control :

Appearance of powder :
Cream to yellow coloured, homogeneous, free flowing powder.
Gelling :
Firm, comparable with 1.8% Agar gel.
Colour and Clarity of prepared medium
Yellow coloured opalescent gel with white precipitate forms in Petri plates.
Reaction:
Reaction of 3.77% w/v aqueous solution is pH 7.4 at 25°C
Cultural Response:
Cultural characteristics observed with added CNP supplement (PHS003), after an incubation at 25-30°C for 6-7 days.

Organism (ATCC)	**Growth**	**Colony characteristics**
Clavibacter michiganensis subsp michiganensis	Luxuriant	Yellow, mucoid, glistening
Escherichia coli (25922)	inhibited	-

References:

1. Bradbury, J.F.(1986) Guide to plant pathogenic bacteria. CAB International Wallingford, UK.
2. Fatmi, M., Schaad, N.W. 1988. Semiselective agar medium for isolation of *Clavibacter michiganensis* subsp. *michiganensis* from naturally infected tomato seeds.

Storage and Shelf-life :
Store below 30°C and the prepared medium at 2 - 8°C. Use before expiry date on the label.

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