PHM006 **Phyto Lactose Trehalose Agar Base**

Semi-selective medium for the detection *Xanthomonas hortorum pv carotae* in carrot.

Composition **:

Ingredients	**Grams/Litre**
Yeast extract	0.50
Potassium dihydrogen phosphate	1.2
Di-potassium hydrogen phosphate	1.2
Ammonium chloride	1.00
Lactose monohydrate	10.00
Trehalose monohydrate	4.00
2-Thiobarbituric acid	0.20
Agar	18.00
Final pH (at 25°C)	6.6

**Formula adjusted standard to suit the performance parameter

Direction.

Suspend 35.1 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize the medium 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 CNTB supplement-1 (PHS005). Mix well and pour into sterile Petri plates .

Principle and Interpretation

The causative agent of bacterial leaf blight of carrot is *Xanthomonas hortorum* pv. *carotae* (also known as *Xanthomonas campestris* pv. *carotae*) causes significant yield losses. For routine testing of carrot seed a combination of two semi-synthetic media, Phyto Lactose Trehalose Agar Base/ Phyto Xanto Carota Agar Base and Phyto Lactose Trehalose Agar Base/ Phyto peptone Bromide Agar Base .is recommended (1).

This medium is a modification of the KM-1 medium (2). This medium is more sensitive and has a greater reproducibility as compared to other media.

Yeast extract serves as a nutritional source. Phosphates buffers the medium. Nystatin in the medium is used to inhibit the growth of fungi. Lactose monohydrate and trehalose monohydrate are the fermentable carbohydrates which are an additional nutitional source. This medium contains lowered concentration of tobramycin (2mg) since some strains of *Xanthomonas hortorum* pv. *carotae* are sensitive to concentration of tobramycin sulphate greater than 4 mg.

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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 forms in Petri plates
Reaction
Reaction of 3.51% w/v aqueous solution is pH 6.6 at 25°C.
Cultural Response:

Cultural characteristics observed with added CNTB supplement-1 (PHS005), after an incubation at 30-32°C for 5-6 days.

Organism (ATCC)	Growth	Colony characteristics
Xanthomonas hortorum pv carotae	Luxuriant	Cream to yellow or light brown or peach yellow, glistening, round and about 2-4 mm in diameter
Staphylococcus aureus (25923)	Inhibited	-

References:

1. Detection of *Xanthomonas hortorum* pv. *carotae* on *Daucus carota.* International Rules for seed testing 7-020, 2006.
2. Kim, H.K., Sasser, M. and Sands, D.C. 1982. Selective medium for *Xanthomonas hortorum pv translucens.* Phytopathology72:936.

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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