PHM016 **Phyto Sucrose Peptone Agar Base**

Selective medium for the detection of *Pseudomonas savastanoi pv. phaseolicola and Pseudomonas syringae* on seeds of bean.

Composition **
:

Ingredients	**Grams/Litre**
Peptone special	5.00
Di-potassium hydrogen phosphate	0.50
Magnesium sulphate anhydrous	0.13
Sucrose	20.00
Agar	20.00

Final pH (at 25°C) 7.4

**Formula adjusted standard to suit the performance parameter

Direction.:

Suspend 45.63 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. Cool to 45-50 °C and aseptically add the rehydrated contents of one vial of CNVB Supplement (PHS009). Mix well and pour into sterile Petri plates.

Principle and Interpretation

Pseudomonas syringae pv. *syringae* and *P. s.* pv. *phaseolicola* are the causal agents of brown spot and halo blight respectively and can reduce yield and quality (4). Several methods have been used for the detection of halo blight and brown spot pathogens in seed including plating seed soak liquid onto the medium B (3).

This medium is formulated based on the formulation of Medium MSP (2), which is a modification of sucrose peptone agar (1). This new medium permits better recovery of *P. s.* pv. *phaseolicola* inhibiting most of the saprophytic bacteria commonly associated with bean seed. Colonies of *P.s.* pv. *phaseolicola* were 3 mm in diameter, circular raised, glistening and light yellow coloured colonies.

This medium selectively eliminates most of the saprophytic bacteria however *P.s.* pv. *phaseolicola* can be easily differentiated on this medium due to the levan and acid from sucrose in the presence of bromothymol blue (2).

Peptone special serves as a source of nitrogen compounds. Phosphate buffers the medium. Sucrose is the fermentable carbohydrate. Bromothymol blue in the supplement serves as an indicator. Cephalexin monohydrate, vancomycin and nystatin in the supplement serves as selective agent.

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Quality Control :
Cream to yellow coloured, homogeneous, free flowing powder.
Gelling
Firm, comparable with 1.5% Agar gel.
Colour and Clarity of prepared medium
Blue coloured slightly opalescent gel forms in Petri plates

Cultural Response:
Cultural characteristics observed with added CNVB Supplement (PHS009), after an incubation at 25-30°C for 3-7 days ..

Organism (ATCC)	Growth	Colour of the Colony
P.s. pv. *phaseolicola*	luxuriant	light yellow
Pseudomonas syringae pv. *syringae*	luxuriant	light yellow
Staphylococcus aureus (25923)	inhibited	-
Saccharomyces cerevisiae (9763)	inhibited	-

References:
1.Hayward. A..C. 1960. A method for characterizing *Pseudomonas solanacearum.* Nature (London) 186:405-406
2.Mohan, S.K., and Schaad, N.W. 1987.An improved Agar Plating Assay for Detecting *Pseudomonas syringae* pv. *syringae* and *P.s.* pv. *phaseolicola* in Contaminated Bean Seed. Phytopathology 77 :139-1395.
3.Taylor. J.D. 1970., Dudley, C.L., and Presly, L.1979. Studies of halo blight seed infection and disease transmission in dwarf beans. Ann. Appl. Biol. 66:29-36.
4.Webster D.M., Atkin, J.D., and Cross J.E, 1983. Bacterial blights of snap beans and their control. Plant Disease 67: 935-939.

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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