

Crop:Bean, Leek, Pea, TomatoDisease:Bacterial brown spot (bean)Pathogen:Pseudomonas syringae pv. syringae<br/>Pseudomonas syringae pv. porri<br/>Pseudomonas syringae pv. pisi<br/>Pseudomonas syringae pv. tomato



Pseudomonas syringae pv. syringae (Pss) is the causal organism of bacterial brown spot of beans. This bacterium is seed borne and therefore its detection on seeds is important. KBBC medium is a rather selective medium to detect Pss on seeds of beans. This medium is based on King's B Medium (K5165), however in KBBC Medium boric acid (1.5 g/liter), cephalexin and nystatin are added. Nystatin is used to control fungi. As an alternative, cycloheximide, a more potent fungicide, can be used. KBBC is much more selective than MSP (M5167) and in general the recovery of Pss is smaller on KBBC than on MSP. Pspha, unlike Pss, will not grow on KBBC. Therefore, the chance of detection of Pss is higher when both complementary media are used. Detection of Pss is performed by the dilution plating of bacterial extract on KBBC and MSP. Then Pss-suspected isolates are transferred to KB medium. Finally, the identification of suspected colonies can be performed by a pathogenicity assay or PCR. Colonies of Pss on KBBC are 3-4 mm in diameter, flat, circular, translucent, creamy white and show blue fluorescence under UV light. This medium can also be used for the detection of seed borne Ps porri, Ps pisi and Ps tomato on seed of resp. leek, pea and tomato.

COMPOSITION OF MEDIA K5120: KBBC MEDIUM

COMPOUND	GRAM/LITER
Agar	15.0
Di-potassium hydrogen phosphate ( $K_2HPO_4$ )	1.5
Boric acid (H <sub>3</sub> BO <sub>3</sub> )	1.5
Magnesium sulphate anhydrous (MgSO4 anhydrous)	0.73
Proteose Peptone	20.0

METHOD

- Dissolve 38.7 grams of ingredients in distilled water and adjust volume to 970 ml.
- Add 30 ml glycerol (50%) and mix.
- Adjust pH to 7.2.
- Autoclave the solutions (121 °C, 15 psi, 15 minutes).
- Prepare sterile antibiotic solutions and add the following amounts per liter medium:
  - 80 mg cephalexin monohydrate (C0110)
  - 35 mg nystatin (N0138) or 100 mg cycloheximide (C0176)
- Allow medium to cool down to ca. 45  $^\circ\text{C}-$  50  $^\circ\text{C}$  and add antibiotics to the solution.
- Mix gently to avoid air bubbles and pour plates (15-20 ml per 9.0 cm plate).

## Reference:

Mohan, S.K. and Schaad, N.W. 1987. An improved agar plating assay for detecting *Pseudomonas syringae pv. syringae* and *Pseudomonas syringae pv. phaseolicola* in contaminated bean seed. Phytopathology 77: 1390-1395.

## **K5120 KBBC MEDIUM**

K5120.1000

1 kg

For prepared and ready to use plates of this medium contact:Tritium MicrobiologieTel : 040-2051615Rooijakkersstraat 6Fax : 040-20513955652 BB EindhovenEmail : info@tritium-microbiologie.nlThe NetherlandsEmail : info@tritium-microbiologie.nl