MEDIA FOR PHYTOPATHOLOGY

Duchefa Biochemie B.V. produces an extensive range of phytopathology media and media used in seed health testing. Since production takes place in our own laboratories, Duchefa Biochemie B.V. is also able to manufacture custom made media according to laboratory specifications. Obviously, strict secrecy is guaranteed.

POWDERED MEDIA

Powdered media are extremely hygroscopic and must be protected from atmospheric moisture. Be sure the glass bottle containing the powdered medium is carefully closed after opening. Otherwise the remaining contents will deteriorate.
Store the dry medium at 2-8°C and keep well closed.

Preparing the media in a concentrated form is not recommended.
Some salt complexes may precipitate in a concentrated solution.

CUSTOM MADE MEDIUM

As a manufacturer of powdered media Duchefa Biochemie B.V. has the ability to produce almost any medium desired. Many of our relations are using custom made media fitting to their own specific purposes, that are produced by Duchefa Biochemie B.V. If you are interested to have your own medium, please contact us or send the Custom Made Medium form.

1. **Name:** Please mention your full name, address, fax and telephone number, so we can contact you if anything proves to be unclear.
2. **Name** and/or **Product number** of the custom-made medium
3. **Formulation:** The formulation of the medium will be stated in mg/l or molarity. To prevent possible mistakes we prefer to have the concentration in both ways.
   Please be accurate in your description, for instance: magnesium sulphate anhydrous or magnesium sulphate heptahydrate.
4. **Quantity:** To guarantee absolute homogeneity a minimal quantity per production of one kilogram custom made medium (or it's equivalent in litres) is required.
5. **Delivery Schedule:** Most custom made media will be supplied within two weeks. Larger quantities can be dispatched in portions if desired.
6. **Declaration of discretion:** Before sending us your formulation Duchefa Biochemie B.V. is prepared to send you a declaration in which absolute secrecy will be assured. After receipt of the undersigned declaration simply send your formulation. Please contact us if such a declaration is required.

PRICES

The prices of most custom-made media are equal to the prices of our standard media. Favourable discounts will be granted on bulk quantities. However, additions of specific components to the media could have their influence on the price. Please indicate the details on the custom-made medium form and send it by mail, fax or e-mail to:

DUCHEFA BIOCHEMIE B.V.
We will contact you after receipt.

DISCLAIMER

Although described in literature as selective media for certain phytopathological micro-organisms Duchefa Biochemie B.V. strongly recommends that the enduser tests, each medium for its selective properties and nutritional requirements growth of mentioned micro-organisms. The use of positive controls and negative controls during the cultivation of pathogenic micro-organisms is strongly recommended. Duchefa B.V. does not accept any liability for the outcome of any test by using the phytopathology media as produced by Duchefa Biochemie B.V.

BEAN

Pseudomonas syringae pv. syringae	KBBC MSP MT
Pseudomonas savastanoi pv. phaseolicola	mKB MSP MT
Xanthomonas axonopodis pv. phaseoli	MT mXCP1 PTSA

BRASSICA

Xanthomonas campestris pv. campestris	mCS20ABN mFS
Xanthomonas campestris pv. armoraciae	mCS20ABN mFS

CARROTS

Xanthomonas campestris pv. carotae	mD5A mKM mTBM

LEEK

Pseudomonas syringae pv. porri	PSM KBBC

PEA

Pseudomonas syringae pv. pisi	SNAC KBBC

PEPPER

Xanthomonas campestris pv. vesicatoria	mTMB MXV CKTM

TOMATO

Clavibacter michiganensis subsp. michiganensis	mSCM D2ANX
Pseudomonas syringae pv. tomato	KBBC KBZ
Xanthomonas campestris pv. vesicatoria	mTMB MXV CKTM

BACTERIAL MEDIUM

bacteria	KB YDC CDA CDB

FUNGAL MEDIUM

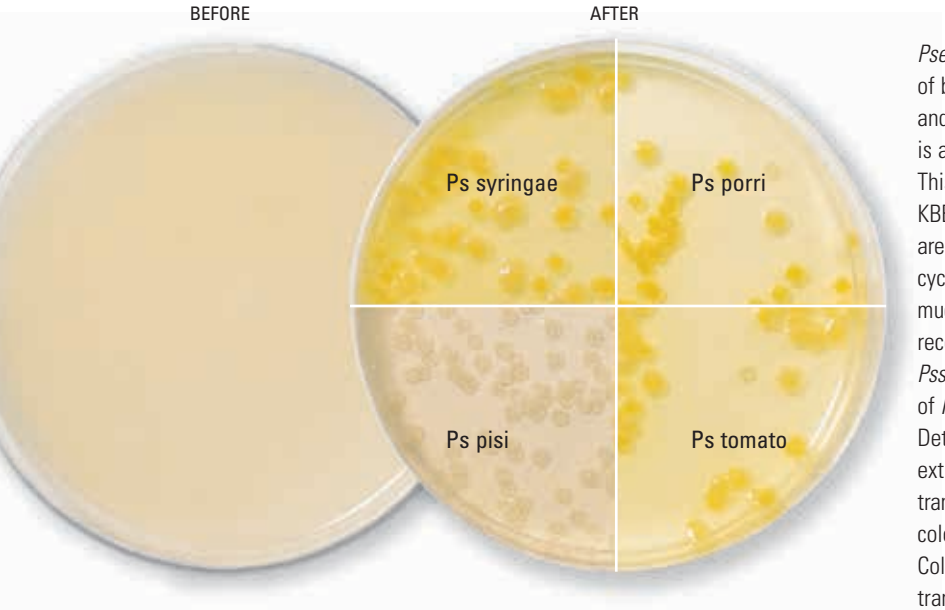
fungi	MA CDA CDB

Phytopathology

PHYTOPATHOLOGY

K5120 KBBC Medium

Crop:	**Bean, Leek, Pea, Tomato**
Disease:	**Bacterial brown spot (bean)**
Pathogen:	***Pseudomonas syringae* pv. *syringae***
	Pseudomonas syringae* pv. *porri
	Pseudomonas syringae* pv. *pisi
	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_3BO_3)	1.5
Magnesium sulphate anhydrous ($MgSO_4$ 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 °C – 50 °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

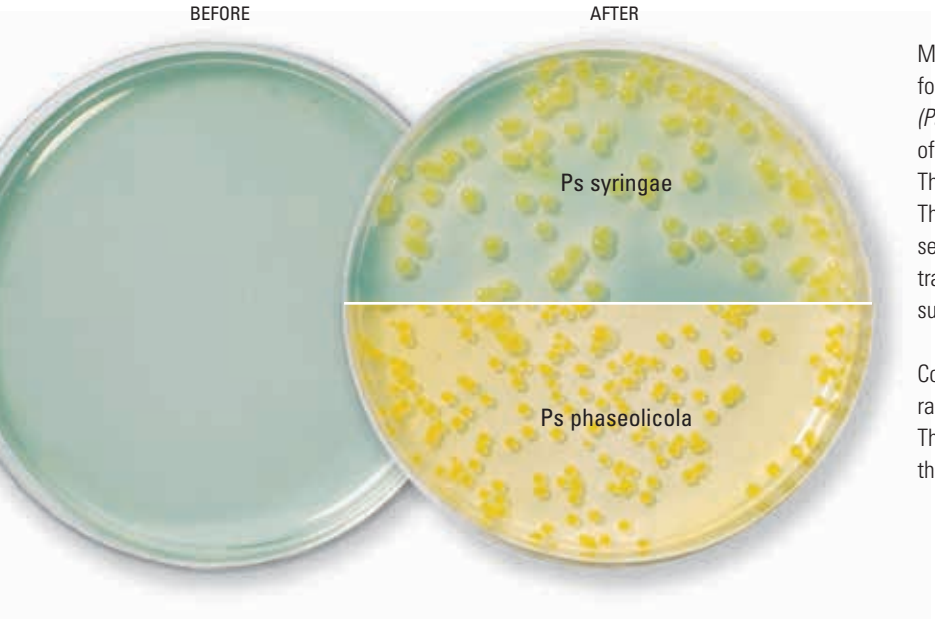
M5167
MSP Medium

Crop: **Bean *(Phaseolus vulgaris)***

Disease: **Bacterial brown spot and halo blight**

Pathogen: ***Pseudomonas syringae* pv. *syringae***

Pseudomonas savastanoi* pv. *phaseolicola



MSP (Modified Sucrose Peptone) medium is a suitable medium for the detection of *Pseudomonas savastanoi* pv. *phaseolicola (Pspha) and Pseudomonas syringae* pv. *syringae (Pss).* Addition of bromothymol blue gives this medium a blue appearance. The color of bacterial colonies is influenced by this compound. The assay starts with dilution plating of bacterial extract from seeds on MSP. Then suspected colonies from MSP can be transferred to King's B Medium (K5165). Finally, the identity of suspected isolates is confirmed by a pathogenicity test or PCR.

Colonies of *Pspha* and *Pss* are ca. 3 mm in diameter, circular, raised, globose, glistening and light yellow with a denser center. The medium around *Pspha* colonies turns light yellow after three days of incubation.

COMPOSITION OF MEDIA M5167: MSP MEDIUM

COMPOUND	GRAM/LITER
Agar	20.0
Di-potassium hydrogen phosphate (K_2HPO_4)	0.5
Peptone special	5.0
Magnesium sulphate anhydrous ($MgSO_4$ anhydrous)	0.13
Sucrose	20.0

METHOD

- Dissolve 45.6 grams of ingredients in distilled water and adjust volume to 1000 ml.
- Adjust pH to 7.4.
- Autoclave the solution (121 °C, 15 psi, 15 minutes).
- Prepare sterile solutions and add the following amounts per liter medium:
  80 mg cephalexin monohydrate (C0110)
  35 mg nystatin (N0138)
  10 mg vancomycin HCl (V0155)
  15 mg bromothymol blue
- Allow medium to cool down to ca. 45 °C – 50 °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.

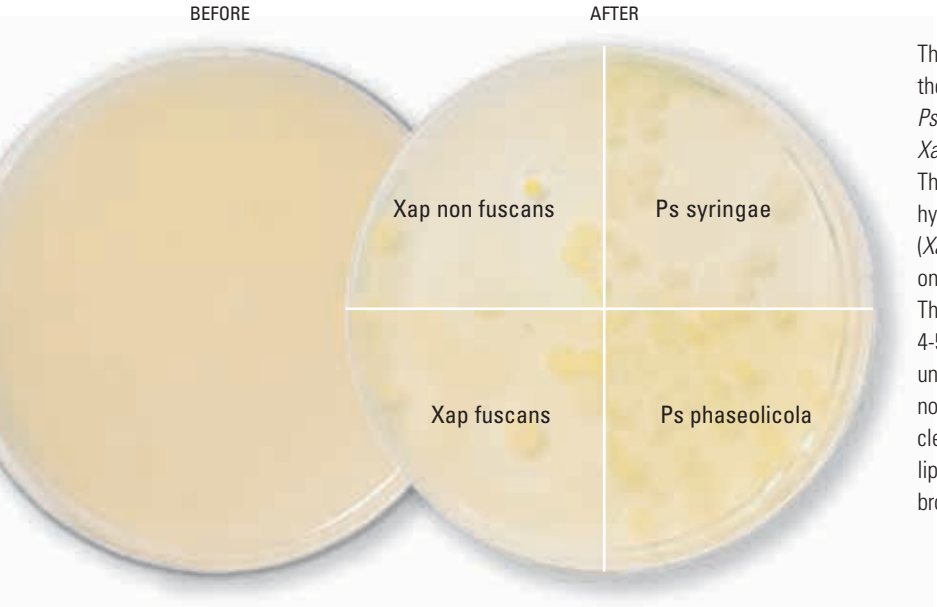
M5167 MSP MEDIUM	
M5167.1000	1 kg

M5133
MT Medium

Crop: **Bean *(Phaseolus vulgaris)***

Disease: **Bacterial brown spot, common blight and halo blight**

Pathogen: ***Pseudomonas syringae* pv. *syringae***
Pseudomonas savastanoi* pv. *phaseolicola
Xanthomonas axonopodis* pv. *phaseoli



The MT (Milk-Tween) Medium is a semi-selective medium for the detection of *Pseudomonas syringae* pv. *syringae* (*Pss*), *Pseudomonas savastanoi* pv. *phaseolicola* (*Pspha*) and *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) in bean seed. The medium relies on the ability of the micro-organisms to hydrolyze casein. Suspected isolates are transferred to YDC (*Xap*) or KB (*Pss* and *Pspha*). Finally, the identity of suspected colonies is determined by PCR or a pathogenicity test.
The colonies of *Pspha* and *Pss* are cream white, flat circular, 4-5 mm in diameter and produce a blue fluorescent pigment under UV light. *Xap* colonies (3 – 3.5 mm in diameter) are yellow, non fluorescent and typical two zones surround colonies: a bigger, clear zone of casein hydrolysis and a smaller zone of Tween 80 lipolysis. *Xap* var. fuscans (1 – 2 mm in diameter) produces a brown pigment within 5 days.

COMPOSITION OF MEDIA M5133: MT MEDIUM

COMPOUND	GRAM/LITER
Proteose Peptone	10.0
Calcium chloride anhydrous ($CaCl_2$ anhydrous)	0.25
Tyrosine	0.5
Agar	15.0

METHOD

- Dissolve 25.7 grams of ingredients in distilled water and adjust volume to 800 ml.
- Dissolve 10 ml Tween 80 in distilled water and adjust volume to 100 ml.
- Dissolve 10 g of skim milk powder in 100 ml distilled water.
- Autoclave the solutions separately (121 °C, 15 psi for 15 minutes).
- Prepare sterile antibiotic solutions and add the following amounts per liter medium:
  80 mg cephalexin monohydrate (C0110)
  35 mg nystatin (N0138)
  10 mg vancomycin HCl (V0155)
- Allow medium to cool down to ca. 45 °C – 50 °C and add the Tween, skim milk powder and antibiotics solutions.
- Mix gently to avoid air bubbles and pour plates (20 ml per 9.0 cm plate).

Reference:
Goszczynska and Serfontein, 1998 "Milk-Tween agar, a semiselective medium for isolation and differentiation of *Pseudomonas syringae pv. syringae, Pseudomonas syringae pv. phaseolicola and Xanthomonas axonopodis pv. phaseoli* ",
Journal of Microbiological Methods 32: 65-72.

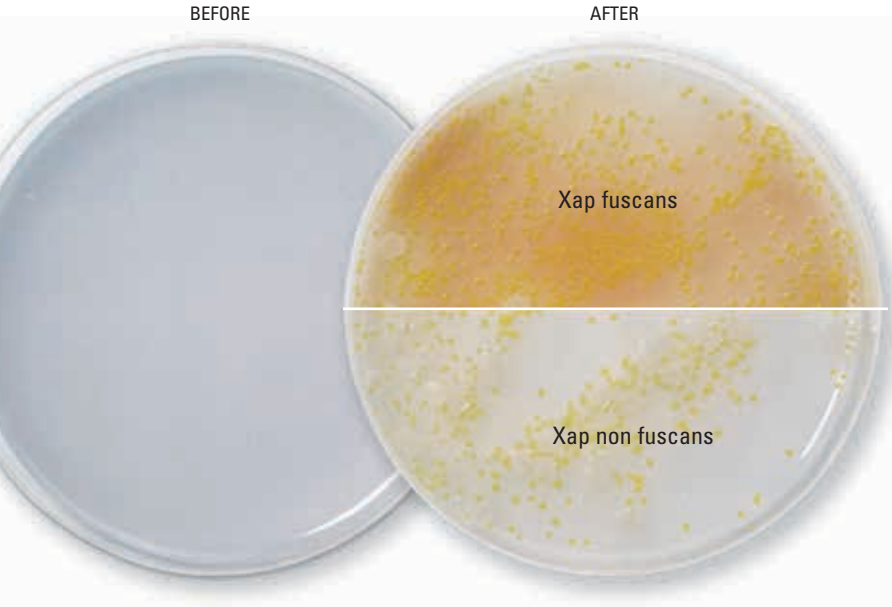
M5133 MT MEDIUM	
K5133.1000	1 kg

X5121
mXCP1 Medium

Crop: **Bean *(Phaseolus vulgaris)***

Disease: **Common blight**

Pathogen: ***Xanthomonas axonopodis* pv. *phaseoli***



The mXCP1 (modified Xanthomonas Campestris pv. Phaseoli) medium is a semi-selective medium for the detection of *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) in bean seed. Both the fuscans and non-fuscans type of *Xap* grow on mXCP1. However the production of the fuscous pigment only becomes visible after a relatively long incubation. Modification of the medium was necessary because of poor recovery of isolates of the *Xap* var. fuscans type. Recognition of putative *Xap* colonies relies on the ability of the *Xanthomonas axonopodis* pv. *phaseoli* to hydrolyze starch. The colonies of *Xanthomonas axonopodis* pv. *phaseoli* on the mXCP1 plate are surrounded by a clear zone of starch hydrolysis.
Detection of *Psp* and *Xap* is often performed in combi-assay. *Xap* is detected by dilution plating of bacterial extract from seeds on mXCP1. Then suspected colonies from mXCP1 should be transferred to YDC. Finally, the identity of suspected isolates is confirmed by a pathogenicity test or PCR.
Xap colonies are yellow mucoid, convex and surrounded by a clear zone of starch hydrolysis. Colonies of var. fuscan are distinguished by brown pigmentation.

COMPOSITION OF MEDIA X5121: mXCP1 MEDIUM

COMPOUND	GRAM/LITER
Peptone special	10.0
Potassium bromide (KBr)	10.0
Calcium chloride anhydrous ($CaCl_2$ anhydrous)	0.25
Agar	20.0
Soluble Starch	20.0
Crystal Violet	0.0015

METHOD

- Dissolve 60.2 grams of the ingredients in distilled water and adjust volume to 900 ml.
- Dissolve 10 ml Tween 80 in distilled water and adjust volume to 100 ml.
- Autoclave the solutions (121 °C, 15 psi, 15 minutes).
- Prepare sterile antibiotic solutions and add the following amounts per liter medium:
  10 mg cephalexin monohydrate (C0110)
  3 mg 5-fluorouracil (F0123)
  0.1 mg tobramycin sulphate (T0153)
  35 mg nystatin (N0138)
- Allow medium to cool down to ca. 45 °C – 50 °C, mix solutions and add antibiotics.
- Mix gently to avoid air bubbles and pour plates (15-20 ml per 9.0 cm plate).
- Store plates for 4 days at 4° C to improve visibility of starch hydrolysis.

Reference:
McGuire,R.G., Jones, J.B. and Sasser, M. 1986. Tween media for semiselective isolation of *Xanthomonas campestris pv. vesicatoria* from soil and plant material. Plant Dis. 70: 887 - 891

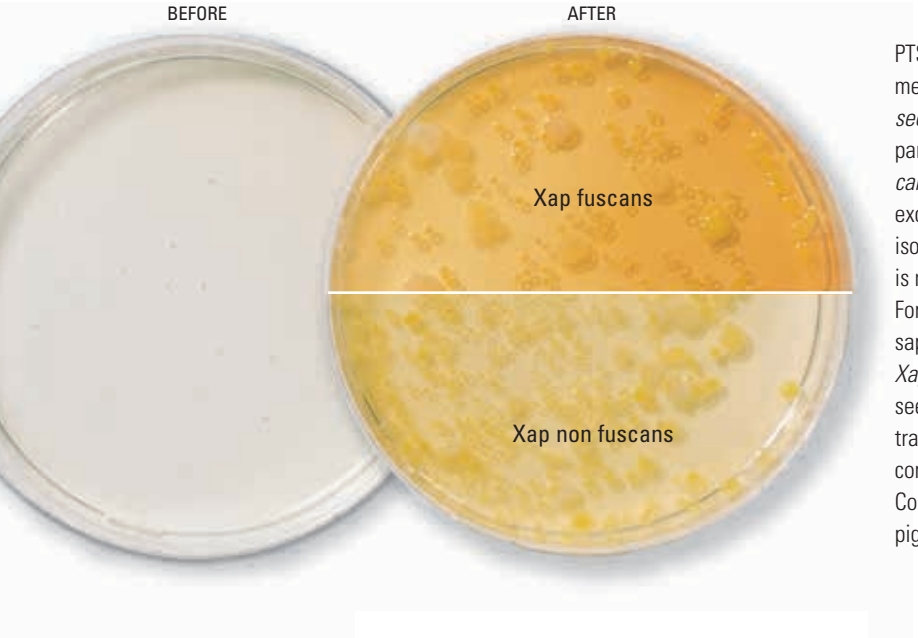
X5121 mXCP1 MEDIUM	
X5121.1000	1 kg

P5135
PTSA Medium

Crop: **Bean *(Phaseolus vulgaris)***

Disease: **Common blight**

Pathogen: ***Xanthomonas axonopodis* pv. *phaseoli***



PTSA (Peptone Tyrosine Sodium chloride Agar) is a semi-selective medium for the detection of *Xanthomonas axonopodis* pv. *phaseoli* in bean seed. The medium is not very selective in comparison with mXCP1, but especially colonies from the var. *fuscans* are easily recognized on this medium because of their excessive production of visible brown pigment. The non-fuscans isolates of *Xap* grow well on PTSA medium but their recognition is much more difficult due to the lack of pigment production.
For relatively clean seed lots, PTSA medium is useful, but for saprophyte-rich samples mXCP1 is much more suitable.
Xap is detected by dilution plating of bacterial extract from seeds on PTSA. Then suspected colonies from PTSA should be transferred to YDC. Finally, the identity of suspected isolates is confirmed by a pathogenicity test or PCR.
Colonies of *Xap* var. fuscans are distinguished by brown pigmentation.

COMPOSITION OF MEDIA P5135: PTSA MEDIUM

COMPOUND	GRAM/LITER
Peptone special	10.0
L-tyrosine	1.0
Soluble starch	2.0
Sodium chloride (NaCl)	5.0
Agar	15.0

METHOD

- Dissolve 33.0 grams of ingredients in distilled water and adjust volume to 1000 ml.
- Autoclave the solution (121 °C, 15 psi, 15 minutes).
- Allow medium to cool down to ca. 45 °C – 50 °C.
- Mix gently to avoid air bubbles and pour plates (15-20 ml per 9.0 cm plate).

Reference:
Van Vuurde J.W.L., Van den Bovenkamp, G.W. and Birnbaum, Y. 1983. Immunofluorescence microscopy and enzyme-linked immunosorbent assay as potential routine tests for the detection of *Pseudomonas syringae pv. phaseolicola and Xanthomonas campestris pv. phaseoli* in bean seeds. Seed Sc. & Technol. 11: 547 -559

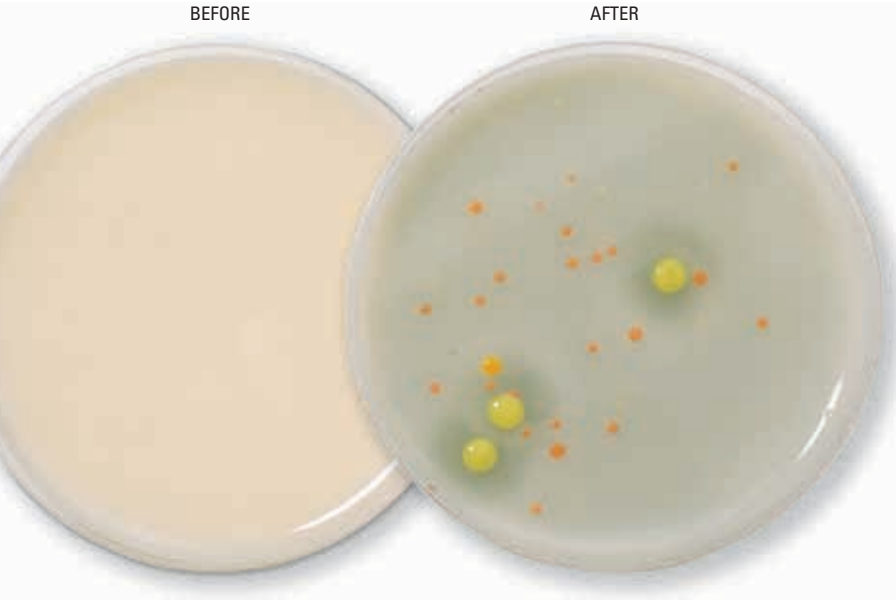
P5135 PTSA MEDIUM	
P5135.1000	1 kg

C5122
mCS20ABN Medium
(extra phosphate and Agar)

Crop: **Brassica**

Disease: **Black rot and bacterial leaf spot**

Pathogen: ***Xanthomonas campestris* pv. *campestris* and *Xanthomonas campestris* pv. *armoraciae***



CS20ABN has been developed by Chang et al. to isolate *Xanthomonas campestris* pv. *campestris (Xcc)* from crucifer seeds. The original medium recipe allowed the quick isolation of most isolates of *Xcc*. However, the recovery of some isolates of *Xcc* was poor due to pH-dependent sensitivity to neomycin. In the modified version, the pH is lowered to 6.5 by the addition of extra potassium dihydrogen phosphate.
This modification improved the recovery of some neomycin-sensitive isolates considerably.
Contaminated seed lots can be detected by dilution plating of the bacterial extract on mCS20ABN and mFS. Suspected isolates are then transferred to YDC. Finally, the identity of the suspected isolates can be determined by a pathogenicity test using brassica seedlings.
The colonies of *Xcc* and *Xanthomonas campestris* pv. *armoraciae* are yellow, mucoid and surrounded by a zone of starch hydrolysis.

COMPOSITION OF MEDIA C5122: mCS20ABN MEDIUM

COMPOUND	GRAM/LITER
Agar	18.0
Soluble starch	25.0
Soya Peptone	2.0
Tryptone	2.0
Potassium dihydrogen phosphate (KH_2PO_4)	2.8
Di-ammonium hydrogen phosphate ($(NH_4)_2HPO_4$)	0.8
Magnesium sulphate anhydrous ($MgSO_4$ anhydrous)	0.1952
L-glutamine	6.0
L-histidine	1.0
Glucose monohydrate	1.0

METHOD

- Dissolve 58.8 grams of ingredients in 900 ml distilled water.
- Adjust pH to 6.5 and adjust volume to 1000 ml.
- pH should be 6.5 and not higher!
- Autoclave the solution (121 °C, 15 psi, 15 minutes).
- Prepare sterile antibiotic solutions and add the following amounts per liter medium:
  35 mg nystatin (N0138)
  40 mg neomycin (M0135)
  100 mg bacitracin (B0106)
- Allow medium to cool down to ca. 45 °C – 50 °C and add antibiotics.
- Mix gently to avoid air bubbles and pour plates (15-20 ml per 9.0 cm plate).
- Store plates for 4 days at 4˚ C to improve visibility of starch hydrolysis.

Reference:
Chang, C.J., Donaldson, R., Crowley, M, and Pinnow, D. 1991. A new semiselective medium for the isolation of *Xanthomonas campestris pv. campestris*. Phytopathology 81:449-453.

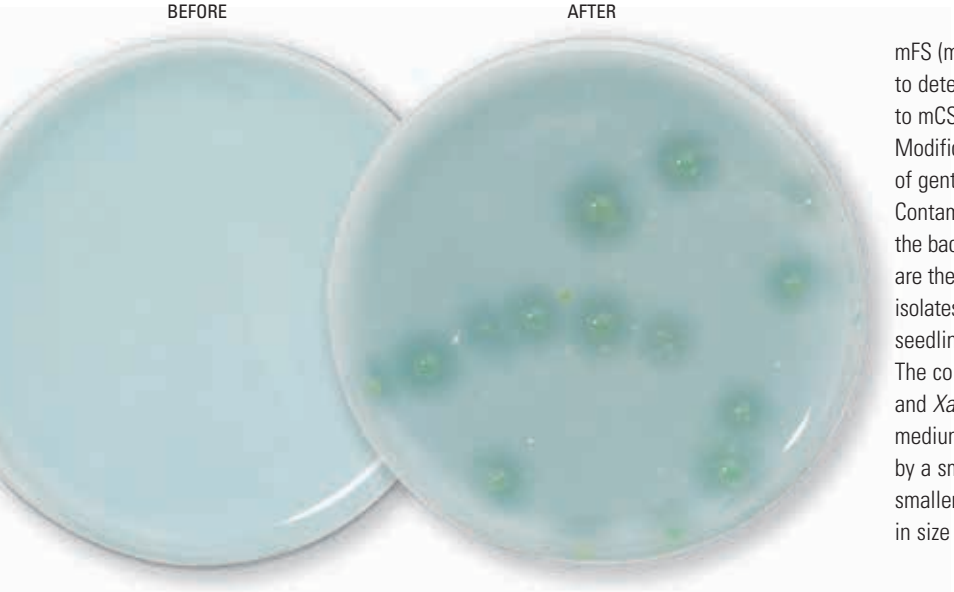
C5122 mCS20ABN MEDIUM	
C5122.1000	1 kg

F5123
mFS Medium

Crop: **Brassica**

Disease: **Black rot and bacterial leaf spot**

Pathogen: ***Xanthomonas campestris* pv. *campestris***
Xanthomonas campestris* pv. *armoraciae



mFS (modified Fieldhouse Sasser medium) has been developed to detect black rot in brassica. This medium is complementary to mCS20ABN (C5122) due to some alternative antibiotics. Modifications concern the addition of extra starch and omission of gentamycin.
Contaminated seed lots can be detected by dilution plating of the bacterial extract on mCS20ABN and mFS. Suspected isolates are then transferred to YDC. Finally, the identity of the suspected isolates can be determined by a pathogenicity test using brassica seedlings.
The colonies of *Xanthomonas campestris* pv. *campestris (Xcc)* and *Xanthomonas campestris* pv. *amoraciae* (*Xca*) on mFS medium are pale green to transparant, mucoid and surrounded by a small zone of starch hydrolysis. Colonies are in general smaller than on mCS20ABN and may show remarkable variation in size and may be visible only after 5-6 days.

COMPOSITION OF MEDIA F5123: mFS MEDIUM

COMPOUND	GRAM/LITER
Soluble starch	25.0
Yeast Extract	0.1
Di-potassium hydrogen phosphate (K_2HPO_4)	0.8
Potassium dihydrogen phosphate (KH_2PO_4)	0.8
Potassium nitrate (KNO_3)	0.5
Magnesium sulphate anhydrous ($MgSO_4$ anhydrous)	0.0488
Agar	15.0

METHOD

- Dissolve 42.2 grams of ingredients in distilled water and adjust volume to 950 ml and adjust pH to 6.8.
- Add 1.5 ml methyl green (1 % aq.) and adjust volume to 1000 ml with distilled water.
- Autoclave the solution (121 °C, 15 psi, 15 minutes).
- Prepare the following sterile solutions of vitamins, amino acids and antibiotics per liter medium:
  35 mg nystatin (N0138)
  3 mg D-methionine (M0715)
  1 mg pyridoxine-HCl (P0612)
  50 mg cephalexin monohydrate (C0110)
  30 mg trimethoprim (T0154)
- Allow medium to cool down to ca. 45 °C – 50 °C and add solutions.
- Mix gently to avoid air bubbles and pour plates (15-20 ml per 9.0 cm plate).
- Store plates for 4 days at 4° C to improve visibility of starch hydrolysis.

Reference:
Yuen, G.Y., Alvarez, A.M., Benedict, A.A., and Trotter, K.J. 1987. Use of monoclonal antibodies to monitor the dissemination of *Xanthomonas campestris pv. campestris*. Phytopathology 77:366-370.

F5123 mFS MEDIUM	
F5123.1000	1 kg

D5124
mD5A Medium

Crop: **Carrot *(Daucus carota)***

Disease: **Bacterial leaf blight**

Pathogen: ***Xanthomonas hortorum* pv. *carotae***

mD5A (modified D-5 Agar medium) is used to detect seed borne *Xanthomonas campestris* pv. *carota (Xccar),* the causal organism of bacterial blight of carrots. Contaminated seed lots can be detected by dilution plating of the bacterial extract on mD5A and another semi-selective medium. Suspected isolates are then transferred to YDC. Finally, the identity of the suspected isolates can be determined by PCR. Colonies of *Xccar* on mD5A medium look straw-yellow, glistening, round, smooth, convex and are 2–3 mm in diameter.

COMPOSITION OF MEDIA D5124: mD5A MEDIUM

COMPOUND	GRAM/LITER
Agar	15.0
Sodium dihydrogen phosphate (NaH_2PO_4)	0.9
Di-potassium hydrogen phosphate (K_2HPO_4)	3.0
Magnesium sulphate anhydrous ($MgSO_4$ anhydrous)	0.15
Ammonium chloride (NH_4Cl)	1.0

METHOD

- Dissolve 20.1 grams of ingredients in distilled water and adjust volume to 900 ml and adjust pH to 6.4.
- Dissolve 10.0 grams of D-cellobiose in distilled water and adjust volume to 100 ml.
- Autoclave the solutions separately (121 °C, 15 psi, 15 minutes).
- Prepare the following sterile amino acids and antibiotics solutions and add the following amounts per liter medium:
  5 mg L-glutamic acid (G0707)
  1 mg L-methionine (M0715)
  35 mg nystatin (N0138)
  10 mg cephalexin monohydrate (C0110)
  10 mg bacitracin (B0106)
- Allow medium to cool down to ca. 45 °C – 50 °C and add solutions.
- Mix gently to avoid air bubbles and pour plates (15-20 ml per 9.0 cm plate).

Reference:
Kuan, T.L., Minsavage, G.V. and Gabrielson, R.L. 1985. Detection of *Xanthomonas campestris pv. carotae* in carrot seed. Plant disease 61758-61760.
Cubeta, M.S. and Kuan, T.L. 1986 Comparison of MD5 and XCS media and development of MD5A medium for detection of *Xanthomonas hortorum p.v. carotae* in carrot seed, Phythopathology 76: 1109 (Abstract)

D5124 mD5A MEDIUM	
D5124.1000	1 kg

K5125 mKM Medium

Crop: **Carrot *(Daucus carota)***

Disease: **Bacterial leaf blight**

Pathogen: ***Xanthomonas hotorum* pv. *carotae***

mKM medium (modified KM-1 medium) is used to detect *Xanthomonas hortorum* pv. *carotae (Xccar).* Contaminated seed lots can be detected by dilution plating of the bacterial extract on mD5A and another semi-selective medium. Suspected isolates are then transferred to YDC. Finally, the identity of the suspected isolates can be determined by PCR. The colonies of *Xccar* on mKM plates are light-yellow cream, light brown to peach yellow, glistening, round and about 2 – 4 mm in diameter.

COMPOSITION OF MEDIA K5125: mKM MEDIUM

COMPOUND	GRAM/LITER
Agar	18.0
Potassium dihydrogen phosphate (KH_2PO_4)	1.2
Di-potassium hydrogen phosphate (K_2HPO_4)	1.2
Ammonium chloride (NH_4Cl)	1.0
Lactose monohydrate	10.0
Threhalose anhydrous.	4.0
2-Thiobarbituric acid	0.2
Yeast Extract	0.5

METHOD

- Dissolve 36.1 grams of the ingredients in distilled water and adjust volume to 1000 ml and adjust pH to 6.6.
- Autoclave the solution (121 °C, 15 psi, 15 minutes).
- Prepare sterile antibiotic solutions and add the following amounts per liter medium:
  35 mg nystatin (N0138)
  10 mg cephalexin monohydrate (C0110),
  50 mg bacitracin (B0106)
  2 mg tobramycin sulphate (T0153)
- Allow medium to cool down to ca. 45 °C – 50 °C and add antibiotics.
- Mix gently to avoid air bubbles and pour plates (15-20 ml per 9.0 cm plate).

Reference:

Kim, H.K., Sasser, M. and Sands, D.C. 1982. Selective medium for *xanthomonas hortorum pv. translucens* Phytopathology 72:936. (Abstn)

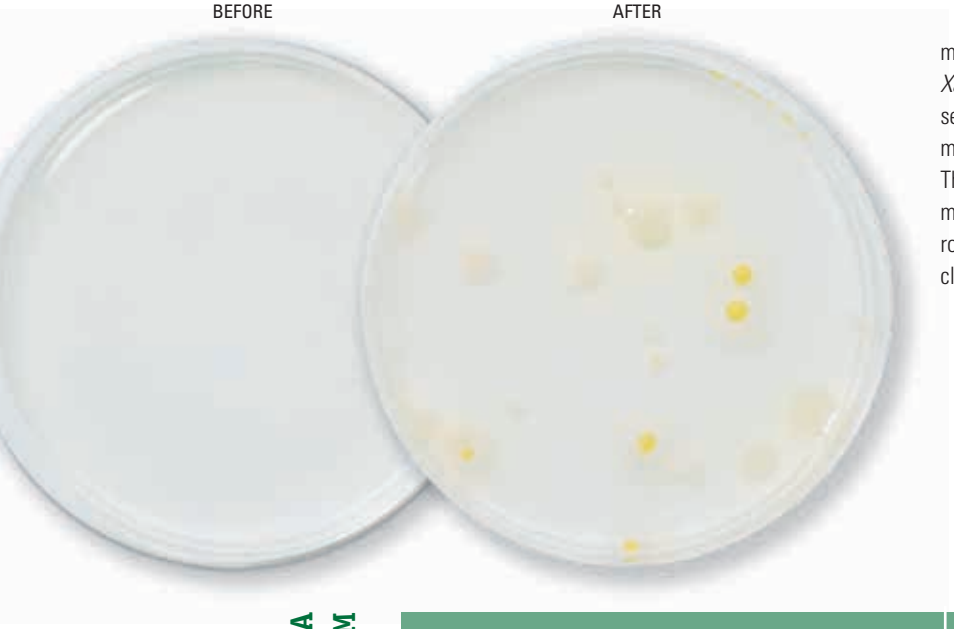
K5125 mKM MEDIUM	
K5125.1000	1 kg

T5132
mTBM Medium

Crop: **Carrot (Daucus carota)**

Disease: **Bacterial leaf blight**

Pathogen: ***Xanthomonas hortorum pv. carotae***



mTBM Medium (modified TBM medium) is used to detect *Xanthomonas hortorum* pv. *carotae* (*Xccar*). Other semi-selective media for *Xanthomonas campestris* pv. *carotae* are mKM Medium (K5125) and mD5A Medium (D5124).
The colonies of *Xanthomonas hortorum* pv. *carotae* on mTBM plates are white or yellow or white-yellow, glistening round, convex with entire margins and surrounded by a large clear zone of casein hydrolyses.

COMPOSITION OF MEDIA T5132: mTBM MEDIUM

COMPOUND	GRAM/LITER
Agar	15.0
Boric acid (H_3BO_3)	0.3
Potassium bromide (KBr)	10.0
Peptone	10.0

METHOD

- Dissolve 35.3 grams of ingredients in distilled water and adjust volume to 800 ml and adjust pH to 7.4.
- Dissolve 10 ml of Tween 80 indistilled water and adjust to 100 ml.
- Dissolve 10 g of skim milk powder in distilled water and adjust volume to 100 ml.
- Autoclave the solutions separately (121 °C, 15 psi, 15 minutes).
- Prepare sterile antibiotic solutions and add the following amounts per liter medium:
  20 mg nystatin (N0138)
  65 mg cephalexin monohydrate (C0110)
  12 mg 5-fluorouracil (F0123)
- Allow solution to cool down to ca. 45 °C – 50 °C and mix the solutions.
- Mix gently to avoid air bubbles and pour plates (20 ml per 9.0 cm plate).

Reference:
McGuire, R.G., Jones, J.B. and Sasser, M. 1986. Tween medium for semiselective isolation of Xanthomonas hortorum pv vesicatoria from soil and plant material. Plant Dis. 70; 887 – 891.

T5132 mTBM MEDIUM	
T5132.1000	1 kg

PHYTOPATHOLOGY • SEED HEALTH TESTING

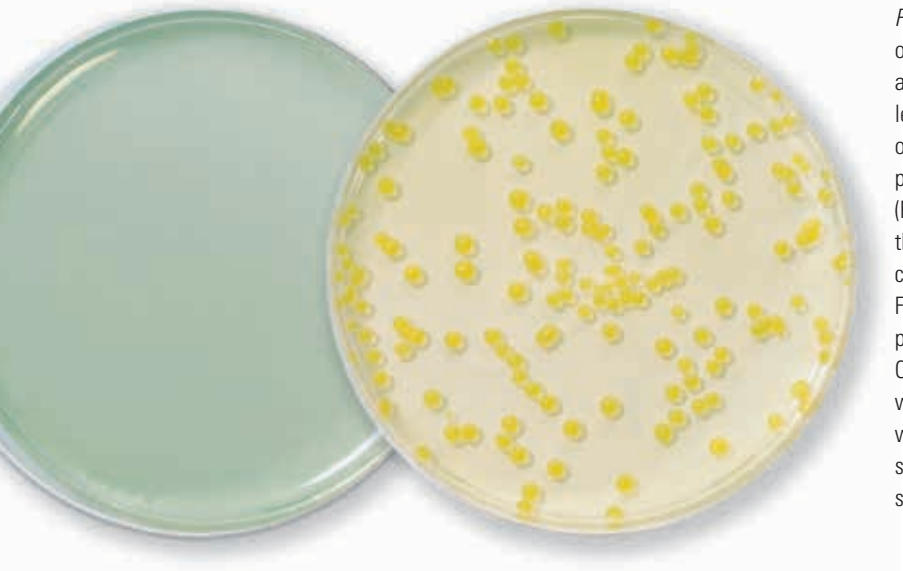
P5134
PSM Medium

Crop: **Leek**

Disease: **Bacterial blight of leek**

Pathogen: ***Pseudomonas syringae* pv. *porri***

BEFORE AFTER



Pseudomonas syringae pv. *porri* (*Pspo*) is the causal organism of bacterial blight of leek. This pathogen can be seed-borne and therefore the testing of seeds of leek is common. Seeds of leek can be saprophyte-rich and this might disguise the presence of *Pspo*. Detection of this bacterium is performed by dilution plating on highly selective media such as KBBC and PSM (Pseudomonas Syringae Medium). Putative *Pspo* colonies are then transferred to KB. Thereafter the identity of the suspected colonies is determined by immunofluorescence microscopy. Finally, the identity is determined by a *Pspo*-specific PCR or a pathogenicity assay using seedlings of leek.
On PSM the colonies of *Pspo* are 2-4 mm in diameter, circular with smooth edge, translucent, creamy-yellow to transparant white. Note that the color of *Pspo* colonies is rather variable since the accumulation of bromothymol blue per colony is strongly dependent on the total number of colonies per plate.

COMPOSITION OF MEDIA P5134: PSM MEDIUM

COMPOUND	GRAM/LITER
Sucrose	20.0
Peptone special	5.0
Di-potassium hydrogen phosphate (K_2HPO_4)	0.5
Magnesium sulphate anyhydrous ($MgSO_4$)	0.13
Agar	20.0

METHOD

- Dissolve 45.6 grams of ingredients in 970 ml distilled water, adjust pH to 7.5 and adjust volume to 990 ml.
- Add 1 gram of boric acid to 10 ml of distilled water.
- Autoclave the solutions separately (121 °C, 15 psi, 15 minutes).
- Prepare sterile solutions and add the following amounts per liter medium:
  80 mg cephalexin monohydrate (C0110)
  35 mg nystatin (N0138)
  10 mg vancomycin HCl (V0155)
  15 mg bromothymol blue
- Allow medium to cool down to ca. 45 °C – 50 °C and add boric acid and antibiotic solutions to mixture of the ingredients.
- Mix gently to avoid air bubbles and pour plates (15-20 ml per 9.0 cm plate).

Reference:
Koike, S.T., Barak, J.D., Henderson, D.M., and Gilbertson, R.L. 1999. Bacterial blight of leek: A new disease in California caused by *Pseudomonas syringae*. Plant Dis. 83:165-170.

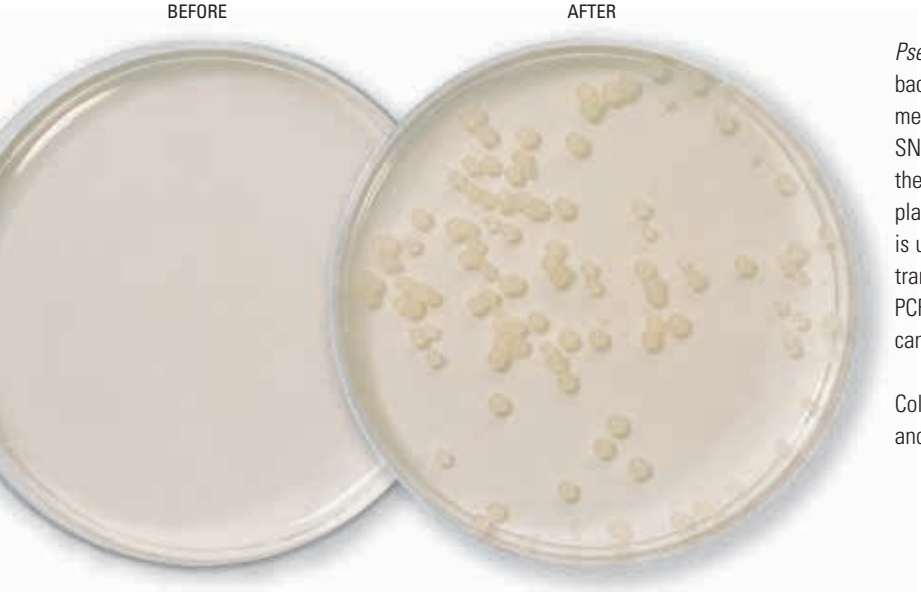
P5134 PSM MEDIUM	
P5134.1000	1 kg

S5130
SNAC Medium

Crop: **Pea**

Disease: **Bacterial blight of pea**

Pathogen: ***Pseudomonas syringae* pv. *pisi***



Pseudomonas syringae pv. *pisi* (*Pspi*) is the causal organism of bacterial blight of pea. The use of clean seeds is an important measure for controlling this disease. SNAC is derived from the SNA medium. The selectivity of the medium was increased by the addition of boric acid and antibiotics. In general dilution plating on semi-selective medium such as SNAC and/or KBBC is used for the detection of *Psp*. Then suspected colonies are transferred to KB. Through immunofluorescence microscopy, PCR or a pathogenicity assay the identity of suspected isolates can be confirmed.

Colonies of *Pspi* on SNAC are white to transparent mucoid and dome-shaped.

COMPOSITION OF MEDIA S5130: SNAC MEDIUM

COMPOUND	GRAM/LITER
Tryptone	5.0
Peptone	3.0
Sodium chloride (NaCl)	5.0
Sucrose	50.0
Agar	15.0

METHOD

- Dissolve 75.0 grams of ingredients in distilled water and adjust volume to 990 ml.
- Add 1 gram of boric acid to 10 ml of distilled water.
- Autoclave the solutions separately (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)
- Allow medium to cool down to ca. 45 °C – 50 °C and add boric acid and antibiotic solutions.
- Mix gently to avoid air bubbles and pour plates (15-20 ml per 9.0 cm plate).

Reference:
Franken, A.A.J.M., and van den Bovenkamp, G.W. 1990. The application of the combined use of immunofluorescence microscopy and dilution plating to detect *Pseudomonas syringae pv. pisi* in pea seeds. In proceedings of the 7th ICPP pp. 871-875.

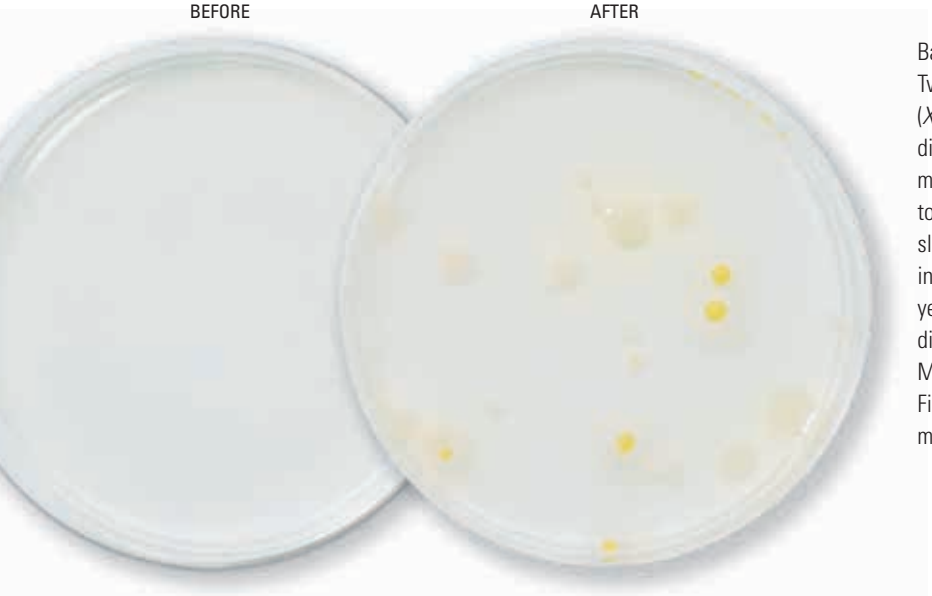
S5130 SNAC MEDIUM	
S5130.1000	1 kg

T5126
mTMB Medium

Crop: **Pepper *(Capsicum annuum)***
Tomato *(Lycopersicon lycopersicum)*

Disease: **Bacterial spot**

Pathogen: ***Xanthomonas campestris* pv. *vesicatoria***
Xanthomonas vesicatoria



Bacterial spot is an important bacterial disease of peppers. Two different bacteria, *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) and *Xanthomonas vesicatoria* (*Xv*) can incite this seed borne disease. mTMB (modified Tween Medium B) is a semi-selective medium for detection of *Xcv* and *Xv* on seeds of pepper and tomato. The colonies of *Xcv* and *Xv* on mTMB plates are yellow, slightly mucoid, mounded and round. *Xcv* utilizes Tween 80 and in 3-7 days a white crystalline halo usually forms around the yellow colony. Contaminated seed lots can be detected by dilution plating of the bacterial extract on CKTM, mKM or MXV. Suspected isolates are then transferred to YDC. Finally, the identity of the suspected isolates can be determined by a pathogenicity test or PCR.

COMPOSITION OF MEDIA T5126: mTMB MEDIUM

COMPOUND	GRAM/LITER
Agar	15.0
Potassium bromide (KBr)	10.0
Boric acid (H_3BO_3)	0.1
Calcium chloride anhydrous ($CaCl_2$ anhydrous)	0.25
Peptone	10.0

METHOD

- Dissolve 35.3 grams of ingredients in distilled water and adjust volume to 900 ml.
- Dissolve 10 ml of Tween 80 in distilled water and adjust volume to 100 ml.
- Autoclave the solutions separately (121 °C, 15 psi, 15 minutes).
- Prepare sterile antibiotic solutions and add the following amounts per liter medium:
  65 mg cephalexin monohydrate (C0110)
  12 mg 5-fluorouracil (F0123)
  0.2 mg tobramycin sulphate (T0153)
  100 mg cycloheximide (C0176)
- Allow medium to cool down to ca. 45 °C – 50 °C, mix the solutions and add antibiotics.
- Mix gently to avoid air bubbles and pour plates (15-20 ml per 9.0 cm plate).

Reference:
McGuire, R.G., Jones, J.B., and Sasser, M. 1986. Tween medium for semiselective isolation of *Xanthomonas campestris pv. veiscatoria* from soil and plant material. Plant Dis. 70:887-891.

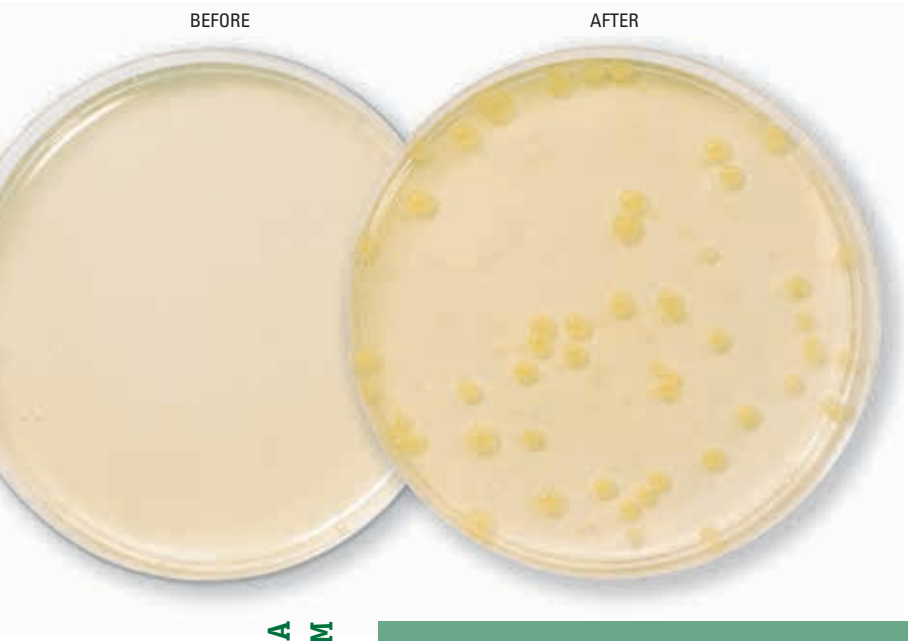
T5126 mTMB MEDIUM	
T5126.1000	1 kg

M5131
MXV Medium

Crop: **Pepper *(Capsicum annuum)***
Tomato *(Lycopersicon lycopersicum)*

Disease: **Bacterial spot**

Pathogen: ***Xanthomonas campestris* pv. *vesicatoria***
Xanthomonas vesicatoria



Bacterial spot is an important bacterial disease of peppers. Two different bacteria, *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) and *Xanthomonas vesicatoria* (*Xv*) can incite this seed borne disease. MXV medium is a semi-selective medium for detection of *Xcv* and *Xv* on seeds of pepper and tomato. The colonies of *Xcv* on MXV plates utilize Tween 80 and are yellow and mucoid. Contaminated seed lots can be detected by dilution plating of the bacterial extract on mTMB, CKTM or mKM. Suspected isolates are then transferred to YDC.
Finally, the identity of the suspected isolates can be determined by a pathogenicity test or PCR.

COMPOSITION OF MEDIA M5131: MXV MEDIUM

COMPOUND	GRAM/LITER
Agar	15.0
Potassium dihydrogen phosphate (KH_2PO_4)	0.8
Di-potassium hydrogen phosphate (K_2HPO_4)	0.8
Ammonium chloride (NH_4Cl)	1.0
Lactose	10.0
Threhalose	4.0
Thiobarbituric acid	0.1
Yeast Extract	0.5

METHOD

- Dissolve 32.2 grams of the ingredients in distilled water, adjust volume to 900 ml and adjust pH to 6.6.
- Dissolve 10 ml of Tween 80 in distilled water and adjust volume to 100 ml.
- Autoclave the solutions separately (121 °C, 15 psi, 15 minutes).
- Prepare sterile antibiotic solutions and add the following amounts per liter medium:
  32.5 mg cephalexin monohydrate (C0110)
  100 mg bacitracin (B0106)
  6 mg 5-fluorouracil (F0123)
  10 mg neomycin sulphate (M0135)
  0.2 mg tobramycin sulphate (T0153)
  100 mg cycloheximide (C0176)
- Allow medium to cool down to ca. 45 °C – 50 °C, mix the solutions and add antibiotics.
- Mix gently to avoid air bubbles and pour plates (15-20 ml per 9.0 cm plate).

Reference:
McGuire, R.G., Jones, J.B., and Sasser, M. 1986. Tween medium for semiselective isolation of *Xanthomonas campestris pv. veiscatoria* from soil and plant material. Plant Dis. 70:887-891.

M5131 MXV MEDIUM	
M5131.1000	1 kg

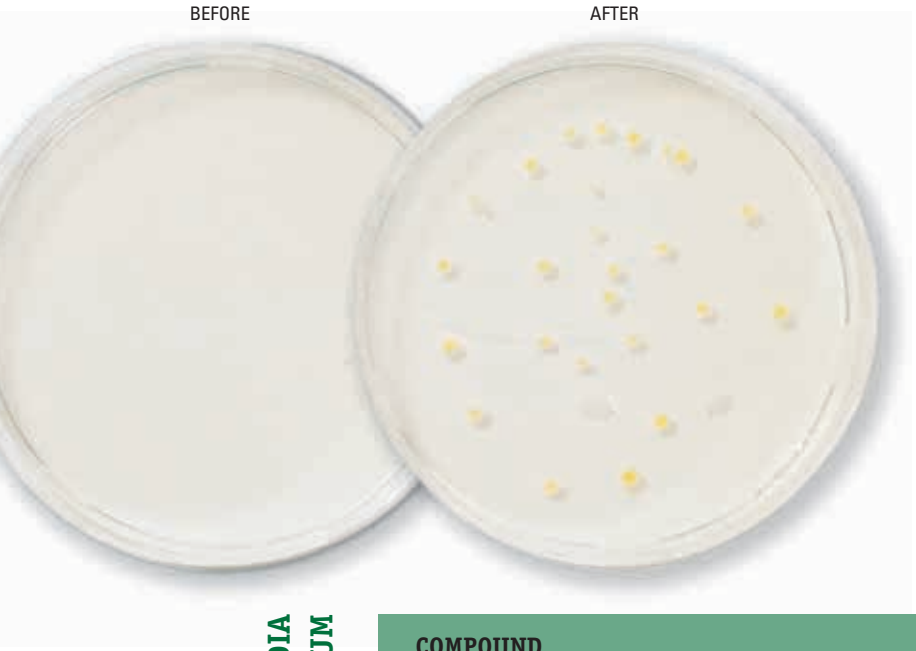
C5140 CKTM Medium

Crop: **Pepper (Capsicum annuum)**

Tomato (Lycopersicon lycopersicum)

Disease: **Bacterial spot**

Pathogen: ***Xanthomonas campestris* pv. *vesicatoria***



CKTM medium is a semi-selective medium, which is used in combination with modified TMB medium (T5126) or MXV medium (M5131) to detect *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) in seeds of pepper and tomato.
Xcv colonies on plates containing CKTM media are yellow, mucoid, mounded and round.

COMPOSITION OF MEDIA C5140: CKTM MEDIUM

COMPOUND	GRAM/LITER
Soya Peptone	2.0
Tryptone	2.0
Glucose anhydrous	1.0
L-glutamine	6.0
L-histidine	1.0
Di-ammonium hydrogen phosphate ($(NH_4)_2HPO_4$)	0.8
Potassium dihydrogen phosphate (KH_2PO_4)	1.0
Magnesium sulfate anhydrous ($MgSO_4$ anh)	0.2
Agar	15.0

METHOD

- Dissolve 29.0 grams of the ingredients in distilled water and adjust volume to 900 ml.
- Dissolve 10 ml of Tween 80 in distilled water and adjust volume to 100 ml.
- Autoclave the solutions separately (121 °C, 15 psi for 15 minutes).
- Prepare sterile antibiotic solutions and add the following amounts per liter medium:
  65 mg cephalexin monohydrate (C0110)
  12 mg 5–fluorouracil (F0123)
  0.4 mg tobramycin sulphate (T0153)
  100 mg cycloheximide (C0176)
  100 mg bacitricin (B0106)
  10 mg neomycin sulphate (M0135)
- Allow medium to cool down to ca. 45 °C – 50 °C, mix the solutions and add antibiotics.
- Mix gently to avoid air bubbles and pour plates (15-20 ml per 9.0 cm plate).

Reference:
Sijam, K., Chang, C.J. and Gitaitis, R.D. 1992. A medium for differentiation tomato and pepper strains of *Xanthomonas campestris pv. vesicatoria*. Canad. J. Plant Pathol. 90: 208-213.

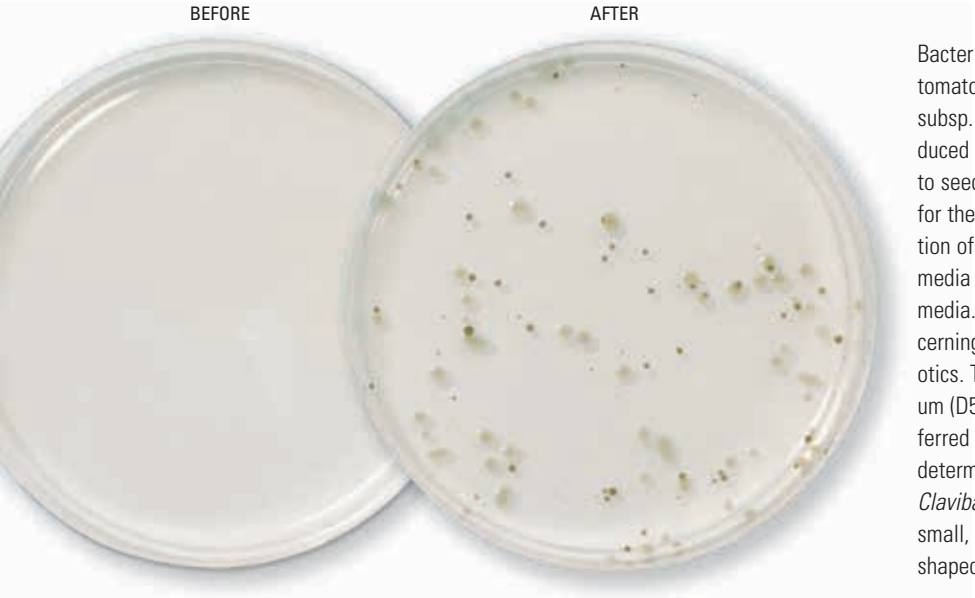
C5140 CKTM MEDIUM	
C5140.1000	1 kg

S5127
SCM Medium

Crop: **Tomato *(Lycopersicon lycopersicum)***

Disease: **Bacterial canker**

Pathogen: ***Clavibacter michiganensis* subsp. *michiganensis***



Bacterial canker is the most important bacterial disease of tomato. The causal organism is *Clavibacter michiganensis* subsp. *michiganensis (Cmm)* and this bacterium can be introduced by contaminated seeds. For the detection of *Cmm,* tomato seeds are first soaked in buffer. Then a stomacher is used for the release of bacteria from the seeds. After the concentration of the bacteria, dilution plating on two semi-selective media is performed. SCM medium is such a semi-selective media. Actually, there are several modifications in use concerning the used carbon source, LiCl and the addition of antibiotics. This medium is used in combination with D2ANX medium (D5128). After dilution plating suspected isolates are transferred to YDC. Finally the identity of suspected isolates is determined by a pathogenicity test or PCR. The colonies of *Clavibacter michiganensis* subsp. *michiganensis* on SCM are small, light to dark grey, glistening, fluidal and often irregularly shaped.

COMPOSITION OF MEDIA S5127: mSCM MEDIUM

COMPOUND	GRAM/LITER
Agar	18.0
Potassium dihydrogen phosphate (KH_2PO_4)	0.5
Di-potassium hydrogen phosphate (K_2HPO_4)	2.0
Magnesium sulphate anhydrous ($MgSO_4$ anhydrous)	0.122
Boric acid (H_3BO_3)	1.5
Yeast Extract	0.1
Sucrose	10.0

METHOD

- Dissolve 32.2 grams of ingredients in distilled water, adjust volume to 1000 ml and adjust pH to 7.3.
- Autoclave the solution (121 °C, 15 psi, 15 minutes).
- Prepare sterile solutions and add the following amounts per liter medium:
  100 mg nicotinic acid (N0611)
  30 mg nalidixic acid (N0134)
  100 mg cycloheximide (C0176)
  10 mg potassium tellurite (1 ml of 1% tellurite solution)
- Allow medium to cool down to ca. 45 °C – 50 °C and add antibiotics.
- Mix gently to avoid air bubbles and pour plates (15-20 ml per 9.0 cm plate).

Reference:

Fatmi, M. and Schaad, N.W. 1988. Semiselective agar medium for isolation of *Clavibacter michiganense subsp.* michiganense from tomato seeds. Phytopathology 78:121-126.

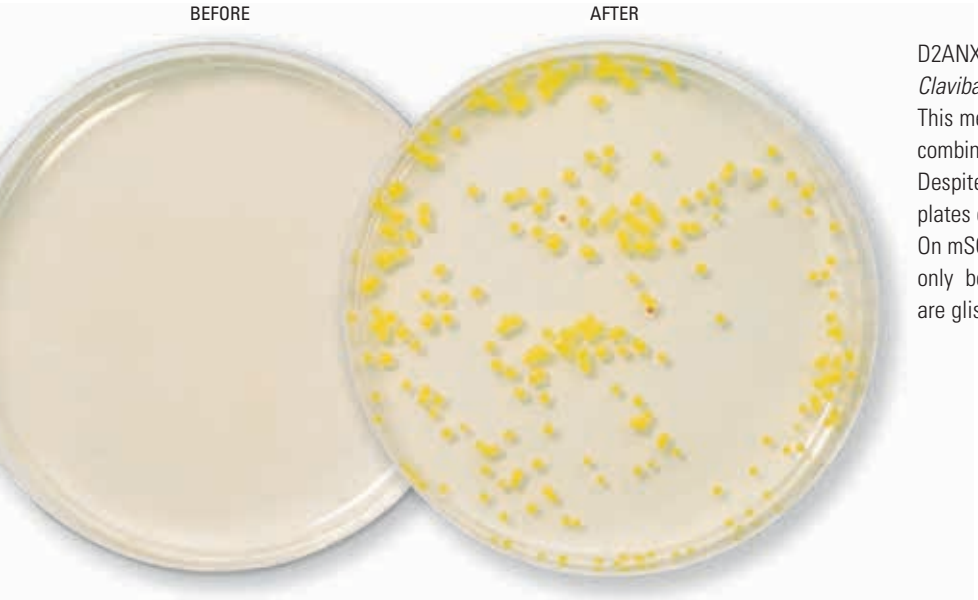
S5127 SCM MEDIUM	
S5127.1000	1 kg

D5128
D2ANX Medium

Crop: **Tomato *(Lycopersicon lycopersicum)***

Disease: **Bacterial canker**

Pathogen: ***Clavibacter michiganensis* subsp. *michiganensis***



D2ANX is a semi-selective medium, which is used to detect *Clavibacter michiganensis* subsp. *michiganensis (Cmm).* This medium, with a relatively low selectivity, is often used in combination with the more selective mSCM medium (S5127). Despite the slow growth of *Cmm* colonies the evaluation of plates can already be performed after 6-7 days of incubation. On mSCM, the growth is more slow and *Cmm* colonies can only be seen after about 9-10 days. On D2ANX, *Cmm* colonies are glistening, yellow and mucoid.

COMPOSITION OF MEDIA D5128: D2ANX MEDIUM

COMPOUND	GRAM/LITER
$MgSO_4$ anhydrous	0.15
Glucose anhydrous	10.0
Yeast Extract	2.0
Agar	18.0
Tris HCl	1.2
Boric acid (H_3BO_3)	1.0
Ammonium chloride (NH_4Cl)	1.0
Casein hydrolysate	4.0

METHOD

- Dissolve 37.3 grams of ingredients in distilled water, adjust volume to 1000 ml and adjust pH to 7.4.
- Autoclave the solution (121 °C, 15 psi, 15 minutes).
- Prepare sterile antibiotic solutions and add the following amounts per liter medium:
  28 mg nalidixic acid (N0134)
  100 mg cycloheximide (C0176)
  10 mg polymixin B sulphate (P0145)
- Allow solutions to cool down to ca. 45 °C – 50 °C and add antibiotics.
- Mix gently to avoid air bubbles and pour plates (15-20 ml per 9.0 cm plate).
- R: 36/37/38

Reference:
Kado, C.I., and Heskett, M.G. 1970. Selective media for the isolation of *Agrobacterium, Corynebacterium, Erwinia, Pseudomonas* and *Xanthomonas*. Phytopathology 60:969-976.

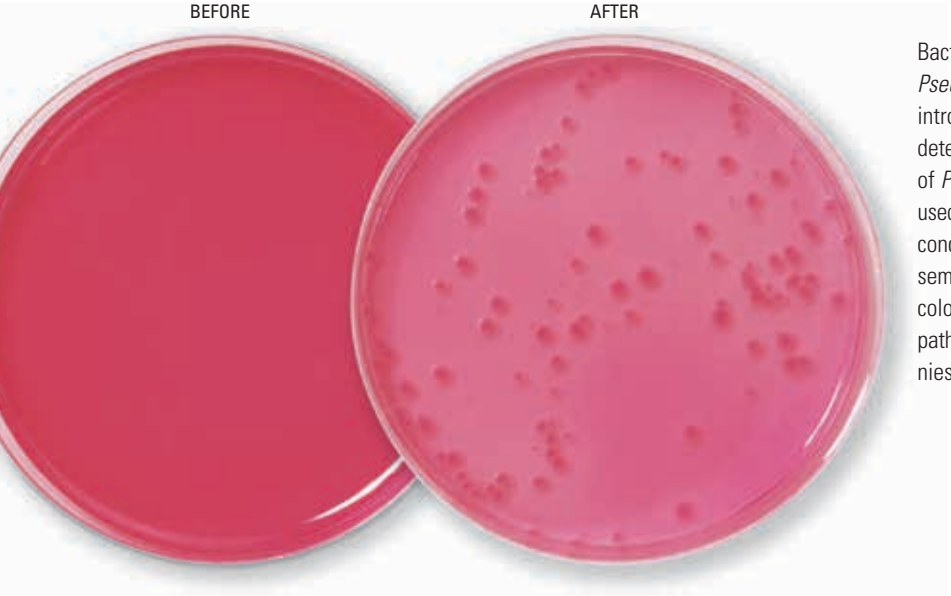
D5128 D2ANX MEDIUM	
D5128.1000	1 kg

K5129
KBZ Medium

Crop: **Tomato**

Disease: **Bacterial speck**

Pathogen: ***Pseudomonas syringae* pv. *tomato***



Bacterial speck of tomatoes is caused by the bacterium *Pseudomonas syringae* pv. *tomato (Pst).* The bacterium can be introduced by the use of *Pst*-contaminated seeds. Therefore, detection of *Pst* in seeds of tomato is common. For the detection of *Pst,* seeds are first soaked in buffer. Then a stomacher is used for the release of bacteria from the seeds. The bacteria are concentrated by centrifugation. Then dilution plating on two semi-selectice media KBZ and KBBC is performed. Suspected colonies are transferred to KB and finally identified by PCR or a pathogenicity assay. *Pst* forms small, flat and pink-colored colonies on KBZ after ca. 5 days.

COMPOSITION OF MEDIA K5129: KBZ MEDIUM

COMPOUND	GRAM/LITER
Agar	15.0
Di-potassium hydrogen phosphate (K_2HPO_4)	1.5
Magnesium sulphate anhydrous ($MgSO_4$ anhydrous)	0.73
Proteose	20.0

METHOD

- Dissolve 37.2 grams of ingredients in distilled water, adjust volume to 960 ml and adjust pH to 7.5.
- Prepare 30 ml of 50 % glycerol.
- Dissolve 1.5 g boric acid in 10 ml distilled water.
- Autoclave the solutions separately (121 °C, 15 psi, 15 minutes).
- Prepare sterile solutions and add the following amounts per liter medium:
  160 mg cephalexin monohydrate (C0110)
  1,4 mg triphenyltetrazoliumchloride
  100 mg cycloheximide (C0176)
  18 mg paraosanilin
- Allow medium to cool down to ca. 45 °C – 50 °C, mix the solutions and add antibiotics.
- Mix gently to avoid air bubbles and pour plates (15-20 ml per 9.0 cm plate).

Reference:

King, E.O. Ward, M.K. and Raney, D.E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. 44:301-307.

K5129 KBZ MEDIUM	
K5129.1000	1 kg

K5165 KB Medium

Medium: **General bacterial medium**

Purpose: **Subculturing of numerous bacterial species**

BEFORE AFTER

KB (King's B) is a non-selective medium and used to subculture suspected isolates. Addition of antibiotics such as cephalexine will make the medium (mKB) suitable for the detection of several Pseudomonads such as *Pseudomonas syringae* pv. *syringae* and *Pseudomonas savastonoi* pv. *phaseolicola* (see photo).

King's B medium is amongst others used for detection and subculturing of fluorescent pseudomonads from seeds and plants. Pathovars of *Pseudomonas syringae* produce a blue fluorescent pigment that becomes visible under UV light.

COMPOSITION OF MEDIA K5165: KB MEDIUM

COMPOUND	GRAM/LITER
Agar	15.0
Di-potassium hydrogen phosphate (K_2HPO_4)	1.5
Magnesium sulphate anhydrous ($MgSO_4$ anhydrous)	0.73
Proteose	20.0

METHOD

- Dissolve 37.2 grams of ingredients in distilled water, adjust volume to 980 ml and adjust pH to 7.5.
- Add 20 ml of 50% glycerol.
- Autoclave the solution (121 °C, 15 psi, 15 minutes).
- Allow medium to cool down to ca. 45 °C – 50 °C.
- Mix gently to avoid air bubbles and pour plates (15-20 ml per 9.0 cm plate).

- **Optional: addition of 50 mg cephalexin and 35 mg nystatin per liter to allow selectivity for pseudomonads (mKB).**

Reference:

King, E.O. Ward, M.K. and Raney, D.E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. 44:301-307.

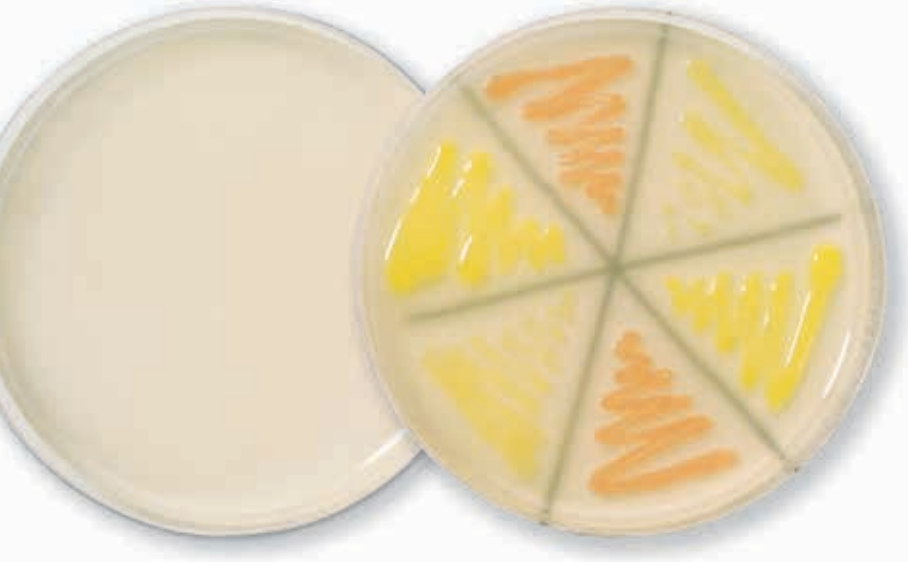
K5165 KB MEDIUM	
K5165.1000	1 kg

Y5136
YDC Medium

Medium: **General bacterial medium**

Purpose: **Subculturing bacteria such as xanthomonads and clavibacters**

BEFORE AFTER



YDC (Yeast extract-dextrose-$CaCO_3$) medium is a non-selective media. YDC is used amongst others for subculturing suspected xanthomonads (yellow) and clavibacters (orange) after dilution on semi-selective media (see photo).

COMPOSITION OF MEDIA Y5136: YDC MEDIUM

COMPOUND	GRAM/LITER
Yeast Extract	10.0
Calcium carbonate ($CaCO_3$)	20.0
Agar	15.0
Glucose anhydrous	20.0

METHOD

- Dissolve 65.0 grams of ingredients in distilled water, adjust volume to 1000 ml and adjust pH to 6.9.
- Autoclave the solution (121 °C, 15 psi, 15 minutes).
- Allow medium to cool down to ca. 45 °C – 50 °C.
- Mix gently to avoid air bubbles and pour plates (15-20 ml per 9.0 cm plate).
- During pouring of medium mix the $CaCO_3$ thoroughly.

Reference:
Wilson, E.E. Zeitoun, F.M. Fredrickson, D.L. 1967. Bacterial phloem canker, a new disease of Persian walnut trees. Phytopathology 57:618-621.

Y5136 YDC MEDIUM	
Y5136.1000	1 kg

C1715
Czapek Dox Agar, CDA

Medium: **General fungal and bacterial medium**

Purpose: **Cultivation of fungi and bacteria**

BEFORE AFTER

Czapex Dox Agar medium is used for the cultivation of those fungi and bacteria that are able to utilize sodium nitrate as the sole source of nitrogen.

COMPOSITION OF MEDIA C1715: CZAPEK DOX AGAR,CDA

COMPOUND	GRAM/LITER
Agar	12.0
Ferrous sulphate	0.01
Magnesium glycerophosphate	0.5
Potassium chloride	0.5
Potassium sulphate	0.35
Sodium nitrate	2.0
Sucrose	30.0

METHOD

- Dissolve 45.5 grams of ingredients in distilled water and adjust volume to 1000 ml.
- The final pH has to be 6.8 ± 0.2.
- Autoclave the solution (121 °C, 15 psi, 15 minutes).
- Allow medium to cool down to ca. 45 °C – 50 °C.
- Mix gently to avoid air bubbles and pour plates (15-20 ml per 9.0 cm plate).

Reference:

Tuite, J. 1969. Plant pathological methods - fungi and bacteria. Burgess publishing co., Minneapolis, MN. 293 pp.

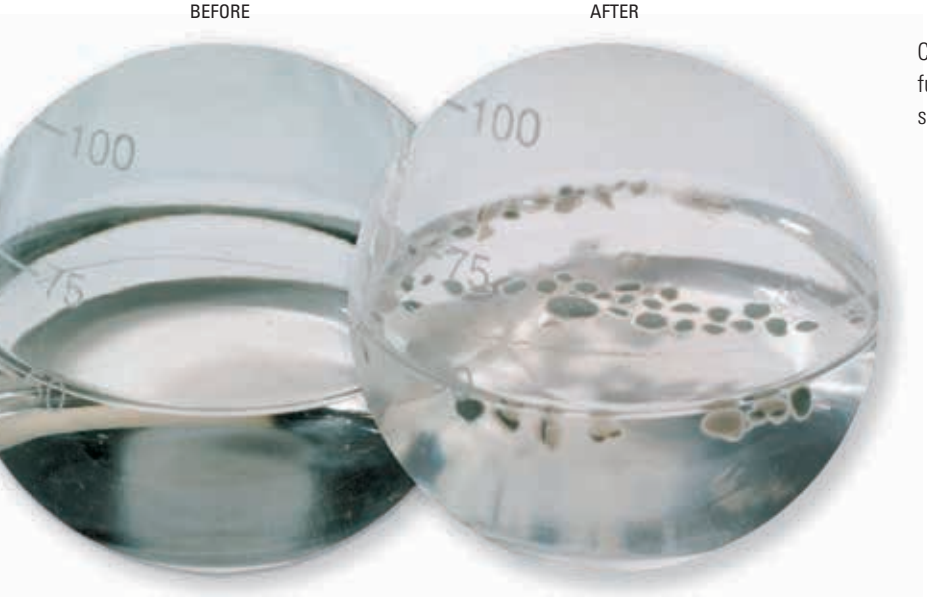
C1715 CZAPEK DOX AGAR, CDA	
C1715.0100	100 g
C1715.0500	500 g
C1715.1000	1000 g

C1714
Czapek Dox Broth, CDB

Medium: **General fungal and bacterial medium**

Purpose: **Cultivation of fungi and bacteria**

BEFORE AFTER



Czapex Dox Broth medium is used for the cultivation of those fungi and bacteria that are able to utilize sodium nitrate as the sole source of nitrogen.

COMPOSITION OF MEDIA C1714: CZAPEK DOX BROTH,CDB

COMPOUND	GRAM/LITER
Ferrous sulphate	0.01
Magnesium glycerophosphate	0.5
Potassium chloride	0.5
Potassium sulphate	0.35
Sodium nitrate	2.0
Sucrose	30.0

- Dissolve 33.4 grams of ingredients in distilled water and adjust volume to 1000 ml.
- The final pH has to be 6.8 ± 0.2.
- Autoclave the solution (121 °C, 15 psi, 15 minutes).
- Allow medium to cool down.

Reference:

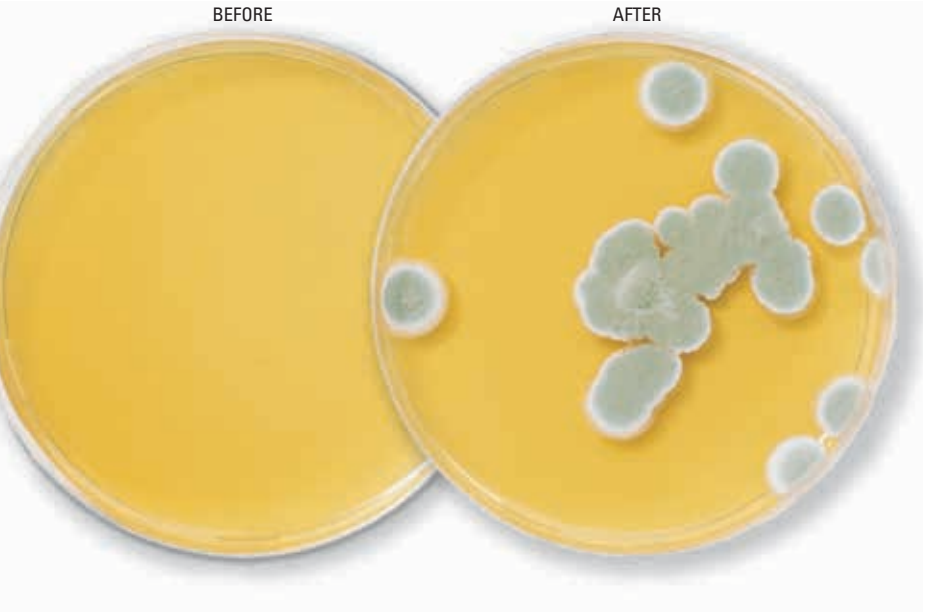
Tuite, J. 1969. Plant pathological methods - fungi and bacteria. Burgess publishing co., Minneapolis, MN. 293 pp.

C1714 CZAPEK DOX BROTH, CDB	
C1714.0500	500 g
C1714.1000	1000 g

L1719
Malt Agar, MA

Medium: **General fungal medium**

Purpose: **Culturing of fungi**



Malt Agar medium is a non-selective multipurpose medium for cultivation of numerous fungi. Lowering the pH of the medium below 5.5 results in the inhibition of bacteria and permits good recovery of yeasts and moulds. Growth of bacteria can be reduced by the addition of antibiotics.

COMPOSITION OF MEDIA L1719 MALT AGAR, MA

COMPOUND	GRAM/LITER
Agar	30.0
Malt extract	15.0

METHOD

- Dissolve 45 grams of ingredients in distilled water and adjust volume to 1000 ml.
- Autoclave the solution (121 °C, 15 psi, 15 minutes).
- Allow medium to cool down to ca. 45 °C – 50 °C.
- Mix gently to avoid air bubbles and pour plates (15-20 ml per 9.0 cm plate).

Reference:
Tuite, J. 1969. Plant pathological methods - fungi and bacteria. Burgess publishing co., Minneapolis, MN. 293 pp.

L1719 MALT AGAR, MA	
L1719.0100	100 g
L1719.0500	500 g
L1719.1000	1 kg

B1713

Bacteria Screening Medium 523

Medium: **General bacterial medium**

Purpose: **Cultivation of bacteria**

COMPOSITION OF MEDIA B1713: BACTERIA SCREENING MEDIUM 523

COMPOUND	GRAM/LITER
Casein hydrolysate	8.0
Magnesium sulphate heptahydrate	0.15
Potassium phospate monobasic	2.0
Yeast Extract	4.0
Sucrose	10.0
Agar	8.0

METHOD

- Dissolve 32.15 grams of ingredients in distilled water and adjust volume to 1000 ml.
- Autoclave the solution (121 °C, 15 psi, 15 minutes).
- Allow medium to cool down to ca. 45 °C – 50 °C.
- Mix gently to avoid air bubbles and pour plates (15-20 ml per 9.0 cm plate).

Reference:
Viss, et al., In Vitro Cell. Dev. Biol., 27P, **42** (1991)

B1713 BACTERIA SCREENING MEDIUM 523	
B 1713.0100	100 g
B 1713.0500	500 g
B 1713.1000	1 kg

L1716

Leifert and Waites Sterility Test Medium

Medium: **General bacterial medium**

Purpose: **Sterility test medium for bacteria**

In the Duchefa Biochemie's Leifert and Waites Sterility Test, Medium Beef extract 3.0 g/l has been replaced by 7,0 g/l Meat extract to obtain a more clear and stable medium.

COMPOSITION OF MEDIA L1716: LEIFERT AND WAITES STERILITY TEST MEDIUM

COMPOUND	GRAM/LITER
Meat Extract	7.0
Glucose	5.0
MS medium + vitamins	2.2
Peptone	4.0
Sodium chloride	2.0
Sucrose	15.0
Yeast Extract	10.0

METHOD

- Dissolve 45.2 grams of ingredients in distilled water and adjust volume to 1000 ml.
- Autoclave the solution (121 °C, 15 psi, 15 minutes).
- Allow medium to cool down to ca. 45 °C – 50 °C.
- Mix gently to avoid air bubbles and pour plates (15-20 ml per 9.0 cm plate).

Reference:
Leifert, et al., J. Applied Bacteriology, 67, 353-361 (1989)

L1716 LEIFERT AND WAITES STERILITY TEST MEDIUM	
L 1716.0100	100 g
L 1716.0500	500 g
L 1716.1000	1 kg

L1718

Luria Broth Agar, Miller

Medium: **General bacterial medium**

Purpose: **Cultivation of bacteria**

COMPOSITION OF MEDIA L1718: LURIA BROTH AGAR, MILLER

COMPOUND	GRAM/LITER
Sodium chloride	0.5
Tryptone	10.0
Yeast Extract	5.0
Agar	15.0

METHOD

- Dissolve 30.5 grams of ingredients in distilled water and adjust volume to 1000 ml.
- Autoclave the solution (121 °C, 15 psi, 15 minutes).
- Allow medium to cool down to ca. 45 °C – 50 °C.
- Mix gently to avoid air bubbles and pour plates (15-20 ml per 9.0 cm plate).

L1718 LURIA BROTH AGAR, MILLER

L 1718.0100	100 g
L 1718.0500	500 g
L 1718.1000	1 kg

L1717

Luria Broth Base, Miller

Medium: **General bacterial medium**

Purpose: **Cultivation of bacteria**

COMPOSITION OF MEDIA L1717: LURIA BROTH BASE, MILLER

COMPOUND	GRAM/LITER
Sodium chloride	0.5
Tryptone	10.0
Yeast Extract	5.0

METHOD

- Dissolve 16.5 grams of ingredients in distilled water and adjust volume to 1000 ml.
- Autoclave the solution (121 °C, 15 psi, 15 minutes).
- Allow medium to cool down.

L1717 LURIA BROTH BASE, MILLER	
L 1717.0100	100 g
L 1717.0500	500 g
L 1717.1000	1 kg

Cat. nr.	Description of medium	Pathogen	ANTIBIOTICS (mg per liter medium)										
			Against gram positive, like Clavibacter				Against gram negative like Pseudomonas en Xanthomonas.					Antifungal	
			Bacitracin	Cephalexin monohydrate	Vancomycin HCl	Trimethoprim	Nalidixic acid	Neomycin sulphate	Polymixin B sulphate	Tobramycin sulphate	5-Fluorouracil	Cycloheximide	Nystatin
			B0106	C0110	V0155	T0154	N0134	M0135	P0145	T0153	F0123	C0176	N0138
K5120	KBBC	Pseudomonas syringae pv. syringae, pv. porri, pv. pisi, pv. tomato		80									35
M5167	MSP	Pseudomonas savastanoi pv. phaseolicola, Pseudomonas syringae pv. syringae		80	10								35
M5133	MT	Pseudomonas syringae pv. syringae Pseudomonas savastonoi pv. phaseolicola Xanthomonas axonopodis pv. phaseoli		80	10								35
X5121	mXCP1	Xanthomonas axonopodis pv. phaseoli		10						0.1	3		35
P5135	PTSA	Xanthomonas axonopodis pv. phaseoli	No antibiotics added										
C5122	mCS20ABN	Xanthomonas campestris pv. campestris Xanthomonas campestris pv. armoraciae	100					40					35
F5123	mFS	Xanthomonas campestris pv. campestris Xanthomonas campestris pv. armoraciae		50		30							35
D5124	mD5A	Xanthomonas campestris pv. carotae	10	10									35
K5125	mKM	Xanthomonas campestris pv. carotae	50	10						2			35
T5132	mTBM	Xanthomonas campestris pv. carotae		65							12		20
P5134	PSM	Pseudomonas syringae pv. porri		80	10								35
S5130	SNAC	Pseudomonas syringae pv. pisi		80									35
T5126	mTMB	Xanthomonas campestris pv. vesicatoria Xanthomonas vesicatoria		65						0.2	12	100	
M5131	MXV	Xanthomonas campestris pv. vesicatoria Xanthomonas vesicatoria	100	32,5				10		0.2	6	100	
C5140	CKTM	Xanthomonas campestris pv. vesicatoria	100	65				10		4	12	100	
S5127	mSCM	Clavibacter michiganensis subsp. michiganensis					30					100	
D5128	D2ANX	Clavibacter michiganensis subsp. michiganensis					28		10			100	
K5129	KBZ	Pseudomonas syringae pv. tomato		160								100	
K5165	mKB	Used for culturing pseudomonas		50									35
K5165	KB	Used for culturing bacteria	No antibiotics added										
Y5136	YDC	Used for culturing bacteria like xanthomonas and clavibacters	No antibiotics added										
P1721	Potato Dextrose Agar, PDA	General fungal medium	No antibiotics added										
L1719	Malt Agar	General fungal medium	No antibiotics added										
P1722	Potato Dextrose Broth, PDB	General fungal medium	No antibiotics added										
C1715	CDA	General fungal and bacterial medium	No antibiotics added										
C1714	CDB	General fungal and bacterial medium	No antibiotics added										