ISOLATION OF *Fusarium oxysporum f. sp. cubense*: OBTAINING SINGLE SPORE CULTURES

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INISAV

Culture media more frequently used

Carnation Leaf Agar (CLA)
(Natural medium)

Procedure:

1) Leaf of non-treated (with pesticides) carnation plants are harvested and cut in fragments of 5-8 mm.
2) Leaf are dried in a oven at 70° C for 3-4 hours until they become fragile
3) Sterilize in plates or bags by gamma irradiation at 2.5 megarads.
4) Place sterile carnation leaf fragments in Petri plates of 2% (20 g/L) of water agar. (Five per plates).
5) Conidia were produced in 7-10 days. Very important for diagnosis based in morphological features. Allow store cultures by a year.

Preparation of Carnation Leaf Agar

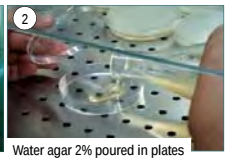
Carnation leaf fragments of 5-8 mm

Dry at 70ºC for 3-4 hours

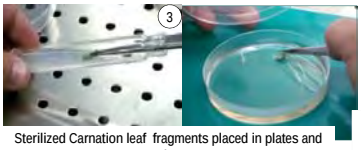
Sterlization by gamma irradiation at 2.5 megarads

Preparation of Carnation Leaf Agar

Biological safety cabinet



Water agar 2% poured in plates



Sterilized Carnation leaf fragments placed in plates and tubes

Plates and tubes with sterilized Carnation leaf fragments ready for inoculation

Culture media more frequently used

Water Agar

- ✓ Agar 20g
- ✓ Distilled water until 1 L

Autoclave at 121 °C for 20 min.

Recommend for isolation.

Agar papa dextrosa (PDA)

- Potatoes (peel) 200 g
- Dextrose 20 g
- Agar 20g
- Distilled water until 1 L.

Recommended to establish grown rate, colony morphology and pigmentation.

Not recommended for morphological characterization or strain conservation for further studies.

Modified Komada medium (K2)
(Su *et al*., 1978)

- D-Galactose 10.0 g
- L-Asparagine 2.0 g
- KH_2PO_4 1.0 g
- KCl 0.5 g
- $MgSO_4 \cdot 7H_2O$ 0.5 g
- FeNa EDTA 10.0 mg
- Agar 20.0 g
- Distilled water 900 mL

Adjust pH to 3.8 with 10% of phosphoric acid. Add 100 mL of filtration sterilized solution (≈ 50°C) of :

1. Streptomycin Sulphate 0.3 g .
2. Oxgall 0.5 g
3. $N_{a2}B_4O_7$. 0.5 g
4. PCNB (75% PH) 0.9 g

Plates are inoculated with a 0.5 ml diluted soil suspension.

Culture media more frequently used

Carrot Agar

- Carrots (peel and washed) 400 g
- Distilled water 500 ml

1) Boil for 1 hour in distilled water.

- Homogenize in a blender
- Filter through fine sieve
- Add distilled water 500 mL
- Agar 20g

2) Autoclave at 121 °C for 20 min.(very important in order to destroid resistant spores).

3) Pour abundantly (15-17 mL for plates with 60 mm diammeter) because incubation period can reach six weeks

Recommended for sexual crosses in order to generate teleomorph state

Agar Spezieller Nährstoffarmer (SNA).

- KH_2PO4 1 g
- KNO_3 1 g
- $MgSO_4 \cdot 7H2O$ 0.5 g
- KCl 0.5 g
- Glucosa 0.2 g
- Sacarosa 0.2 g
- H_20 destilada 1L

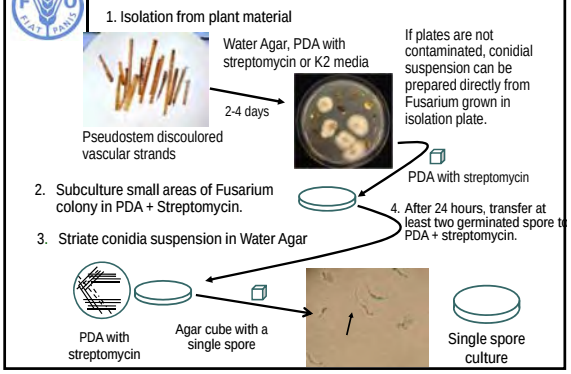
If piaces of Whatman # 1 filter paper are added, sporulation are stimulate.

Recommended to achieve a stable conidial production and chlamydospore formation.

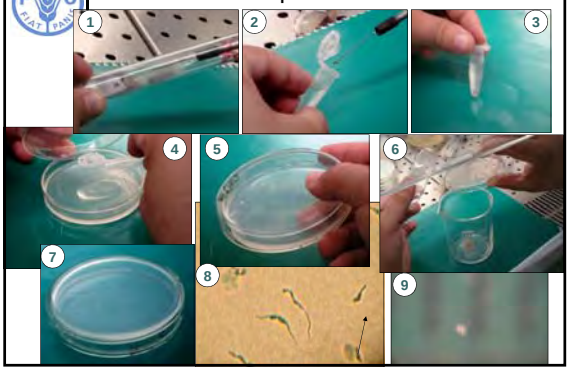
Isolation of *Foc*.

- ✓ Isolations were made when vascular strands were dry.
- ✓ Place small sections 3-6 mm of discoulored vascular strand in Water Agar, weak PDA (25% strength) + streptomycin sulphate (100 ppm); or in K2 medium
- ✓ Colonies shows up in a period of 2-4 days.

Single spore culture through striated



Single spore culture through conidia suspension dilution



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5. Fusarium normal grown, (must be visible in 4-5 days):

- ✓ Chose one single spore culture to represent each isolate and discharge the rest.
- ✓ Assign a unique accession number.
- ✓ Subculture in CLA; it become in the source of VCG and volatile production test, DNA ,for conservation purposes and for other CLA cultures if liophylization is required.