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

Luis Pérez Vicente
Alicia Batlle
Einar Martínez

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

1. Carnation leaf fragments of 5-8 mm
2. Dry at 70°C for 3-4 hours
3. Sterilization by gamma irradiation at 2.5 megarads
Preparation of Carnation Leaf Agar

1. Biological safety cabinet
2. Water agar 2% poured in plates
3. Sterilized Carnation leaf fragments placed in plates and tubes
4. Plates and tubes with sterilized Carnation leaf fragments ready for inoculation

Preparation of Carnation Leaf Agar

- Sterilized Carnation leaf fragments placed in plates and tubes
- 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
  - KH2PO4 1.0 g
  - KCl 0.5 g
  - MgSO4·7H2O 0.5 g
  - FaHk BETA 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. Na2B4O7 0.5 g
    4. PCNB (75% PH) 0.9 g
  - Plates are inoculated with a 0.5 ml diluted soil suspension.

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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.
  2) Homogenize in a blender
  3) Filter through fine sieve
  4) Add distilled water 500 ml.
  5) Agar 20g
  2) Autoclave at 121°C for 20 min (very important in order to destroy resistant spores).
  3) Pour abundantly (15-17 mL for plates with 60 mm diameter) because incubation period can reach six weeks
- Recommended for sexual crosses in order to generate teleomorph state

Agar Spezieller Nährstoffarmer (SNA)

- KH2PO4 1 g
- KNO3 1 g
- MgSO4·7H2O 0.5 g
- KCl 0.5 g
- Glucosa 0.2 g
- Sacarosa 0.2 g
- H2O destilada 1L
- If places 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 show up in a period of 2-4 days.

Single spore culture through striated

1. Isolation from plant material
   - Pseudostem discoulored vascular strands
   - If plates are not contaminated, conidial suspension can be prepared directly from Fusarium grown in isolation plate.
2. Subculture small areas of Fusarium colony in PDA + Streptomycin.
4. After 24 hours, transfer at least two germinated spore to PDA + streptomycin.

Single spore culture through conidial suspension dilution
CONT.

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 lyophilization is required.

1 Filter paper culture
1 Culture in rice
1 PD broth if DNA analysis are required
1 plate with CLA
3 plates of KPS to generate mutants for VCGs determination