METHODS FOR STORAGE OF CULTURES of *Fusarium oxysporum*

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1. Storage in filter paper.

   **Method.**
   - Whatman #1 (5 cm of diameter) filter papers discs are sterilized in autoclave in glass Petri plates.
   - Discs are then aseptically placed on surface PDA ¼ strength media in Petri plates.
   - Fusarium isolates to be stored are grown in CLA for 7-10 days.
   - *F. oxysporum* mycelial plugs (3 mm diameter) are then placed on the sterile filter paper and allowed to grow for 7-10 days in the plates until the mycelial growth completely covers the filter paper dishes.
   - The filter paper with the fungal growth is raised from the agar, and placed on another sterile piece of paper filter and allowed to dry for one day.
   - After dry, it is cut into small pieces (5 mm diameter) and placed in cryovials.
   - Cryovials are marked with the isolate number and stored at 5°C until used.

   **Storage period recommended:** 3 months-1 year

2. Storage in slants with Carnation leaves agar (CLA).

   **Method.**
   - Fresh carnation leaves not treated with agrochemicals are cut in small pieces of 8 × 3 mm before placed to dry in an oven at 70°C until dry.
   - When dry, leaf fragments are placed in containers appropriate for Gamma radiation (i.e.; glass or polystyrene containers with caps or Petri dishes sealed with Parafilm). Note that Gamma radiation will degrade plastics after repeated exposures.
   - Containers are placed in a Gamma cell and irradiated at a total rate of 2.5 Mega Rad.
   - The sterile pieces are stored in a refrigerator at 4°C until ready to be used.
   - Prepare water agar (AA), dissolving 20 g agar in a 1L of distilled water and sterilize in autoclave at 20°C for 20 min.
   - After autoclaving, aliquots of 10 ml of water agar are taken and filled in 25-50 ml sterile bottles or tubes in a lamina flux bench.
   - The bottles are placed lying-down with support in a tray at a 45° angle until culture media slants solidify.
- A piece of carnation leaf is placed on agar surface. The isolate is then placed close to the edge of the carnation leaf piece in the water agar and allowed to grow at 25°C for a week.
- All cultures are clearly marked with the number of isolate and stored at 5°C until ready to use.

*Storage period recommended: 3 months -2 years.*

3. **Deep freezing.**

*Method.*
- First, a glycerol stock solution is prepared and sterilized in autoclave
- *F. oxysporum* is cultured for 7-10 days in ¼ strength PDA at 25°C
- 10 mL of 15% glycerol is pipetted over the fungal growth in a Petri plate in a sterile air flow bench cabin. The spores and some hyphae are released gently with a sterile and cool scalpel
- Aliquots of 1mL are pipette into 2 mL cryovials tubes.
- Each one of the cryovials is carefully marked and stored in cryoconservation boxes at -80°C.
- When it is necessary to recover the isolate, small amounts of the freeze solution in the cryovial are scratched with a scalpel and placed in a culture media.

*Storage period recommended: Until 5 years.*

4. **Storage in soil.**

*Method:*
- The soil is primarily sterilized in small bottles or tubes.
- Cultures are then grown in ¼ strength PDA plates for 7-10 days.
- Distilled water (20 mL) is poured on each culture under an air lamina flow bench cabinet; spores are gently released with a sterile scalpel or spatula.
- 10 mL of spore suspension is transferred aseptically from Petri plate cultures to the soil in the glass bottles and tubes.
- All glass tubes and bottles are clearly labelled with isolate number and stored at room temperature.
- The isolate is recovered placing a small amount of soil in culture media.

*Storage period recommended: Up to 5 years.*
5. **Lyophilization of *Fusarium* cultures.**


- Isolates for lyophilisation are grown in CLA or PDA in Petri plates for 7-10 days. In CLA the fungus colonize leaves and sporulate.

- Some fractions of leaves or small pieces of mycelia and spores are transferred aseptically to each one of five 5 mL sterile glass vials that are tagged with the isolate number.

- An aliquot of 0.5 mL of sterile skim milk is added to each vial.

- Vials are plugged with gum plug caps with small channels that allows air evacuation.

- Vials are placed in a tray and frozen rapidly by pouring liquid N in the tray.

- A Lucita plaque is placed slightly higher than the used tray, on the upper part of the partially plugged tubes.

- A lyophilizer chamber is use for drying.
  - The tray is placed in the cold plate in the dry chamber and submitted to vacuum.
  - When refrigeration is complete, the heater is turned on while the samples are gradually drying
  - Vials are then sealed in vacuum, inflating the gum diaphragm of chambers on the tray which press down the Lucita plate and force the gum tubes to seal the vials.
  - After lyophilisation, vials are tapped, labelled and marked at -20°C.

*Storage period recommended: up to 20 years.*