ISOLATION AND IDENTIFICATION OF PATHOGENIC FUNGI IN LABORATORY

Graciela García Guzmán
Irma Acosta Calixto
Rosamond Coates
Juan Núñez Farfán
Martin Heil
Julio César Montero

Dirección General de Asuntos del Personal Académico

PAPIME
PE201717 / PE202919

ISOLATION OF FUNGI CAUSING THE SYMPTOMS (LEAF SPOTS)

MATERIALS:

Burners, 50 ml beakers, dissecting needles, scalpel, chronometer, markers, sterile paper, chloride (sodium hypochlorite), sterile distilled water, Petri dish with sterile PDA medium.

METHOD:

FUNGI CULTURE

1) Incisions will be made in leaves at the edge of the rotting area (with spots or dots) using a scalpel sterilized under the burner flame.

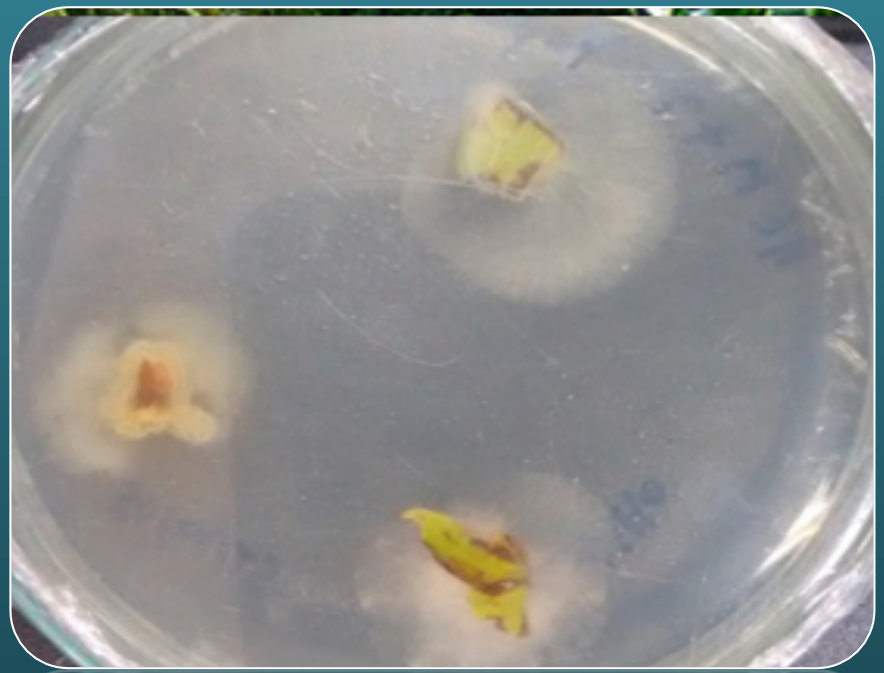
2) The samples will be placed in a solution of sodium hypochlorite at 65% during 2 minutes for sanitation.

3) The samples will be washed with sterile distilled water for 2 minutes.

4) Then, the samples will be placed on sterile paper to eliminate the excess of water.

5) Once dry, the samples will be cultivated in Petri dishes with PDA medium using a dissecting needles or scissors (previously sterilized under the burner flame).

6) Finally the Petri dishes will be left at room temperature or inside an incubator at 25°C until development and sporulation of the fungus is observed.



FUNGI CULTURE

PURIFICATION OF FUNGI CULTURES

Once the fungi emerge from the plant tissues:

- A small portion of the mycelium is obtained with a previously sterilized dissecting needle and transferred to Petri dishes with PDA medium (all are sterile).
- This process is carried out as many times as necessary in order to obtain pure fungi cultures.
- Then, the strains of fungi are purified by making monosporic cultures.

FUNGI DETERMINATION

Finally, the determination of the fungi will be based on its colonial morphology and the microscopic observation of preparations of the obtained microorganisms, using taxonomic identification keys.

PATHOGENICITY TESTS

Once the fungi have been isolated and determined, pathogenicity tests will be carried out in order to verify that the isolated fungi are indeed the causal agents of the observed damage.

METHOD:

1) Healthy plants are produced from seeds under greenhouse controlled conditions.

2) A suspension of mycelium and spores will be prepared from the purified fungi.

3) The experimental unit will consist of a leaf of the plant with five repetitions and control leaves.

4) The leaves of plants will be disinfected with alcohol at 70% and inoculated with the suspension of the purified organisms, using the following methods:

- BY WOUND: a small incision or superficial scrape of the leaf blade will be made, and using a dissecting needle the fungus will be inoculated directly.

- BY CONTACT: small pieces of cotton with the mycelium-spores suspension will be placed on a selected leaf for 48 hours.

5) Once the leaves are inoculated, they will be covered in plastic bags during 48 hours to prevent the fungus from being swept away.

6) Finally, observations every 24 hours will be made until the symptoms are detected and the isolation and determination of the species of the fungus in question will be carried out again.

7) For the control groups, the same method will be used, but instead of the mycelium-spore suspension sterile water will be used.