ISOLATION OF PHYTOPATHOGENIC FUNGI Version 2

Daisy Perez-Brito

Abstract

To control a disease it is essential to know its causal agent. A correct diagnosis provides a considerable amount of basic information to select correct control methods. An accurate diagnosis always starts with a representative sample of the damaged tissue, from which a portion is taken, to isolate the causal agent. In the case of phytopathogenic fungi, there are several isolation methods, depending on the tissue or substrate in which they are found. This protocol describes the isolation and purification of phytopathogenic fungi from leaves and fruits of different tropical crops.

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Protocol

Fungi Isolation

Step 1.

Cut diseased plant tissues, taken from the advanced margin of lesions, into small pieces (5 × 5 mm) with a scalpel.

Fungi Isolation

Step 2.

Disinfest by immersing them in 3% NaOCl solution.

REAGENTS
- 3% NaOCl solution by Contributed by users

Fungi Isolation

Step 3.

Rinse with sterile distilled water. (1/3)

Fungi Isolation

Step 4.

Rinse with sterile distilled water. (2/3)

Fungi Isolation

Step 5.

Rinse with sterile distilled water. (3/3)

Fungi Isolation

Step 6.

Transfer each small piece onto 90 x 15 mm Petri Dish containing Potato Dextrose Agar (PDA) medium.

Fungi Isolation

Step 7.

Incubate at room temperature (25°C) for 7 days.

Fungi Purification

Step 8.

Cut in the edge of each fungi colony, with a scalpel a mycelial segment of approximately 5 x 5 mm.

Fungi Purification

Step 9.

Transfer each small mycelial piece onto 90 x 15 mm Petri dish containing water-agar medium (2%).

REAGENTS

- water-agar medium (2%) by Contributed by users

Fungi Purification

Step 10.

Incubate at room temperature (25°C) for 5 days.

Fungi Purification

Step 11.

Observe the fungal colony on a stereo microscope and with an insulin syringe needle (29 gauge) cut one hyphae tip.

Fungi Purification

Step 12.

Transfer hyphae tip onto 90 x 15 mm Petri Dish containing Potato Dextrose Agar (PDA) medium.

Fungi Purification

Step 13.

Incubate at room temperature (25°C) for 7 days.