Aug 09, 2018 Version 2

ISOLATION OF PHYTOPATHOGENIC FUNGI V.2

PLOS One

DOI
dx.doi.org/10.17504/protocols.io.sgsebwe

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OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.sgsebwe

External link: 10.1007/s10658-012-0065-7

Protocol Citation: Daisy Perez-Brito 2018. ISOLATION OF PHYTOPATHOGENIC FUNGI. **protocols.io** https://dx.doi.org/10.17504/protocols.io.sgsebwe

Manuscript citation:
Rodríguez-García CM, Ruiz-Ruiz JC, Peraza-Echeverría L, Peraza-Sánchez SR, Torres-Tapia LW, Pérez-Brito D, Tapia-Tussell R, Herrera-Chalé FG, Segura-Campos MR, Quijano-Ramayo A, Ramón-Sierra JM, Ortiz-Vázquez E (2019) Antioxidant, antihypertensive, anti-hyperglycemic, and antimicrobial activity of aqueous extracts from twelve native plants of the Yucatan coast. PLoS ONE 14(3): e0213493. doi: 10.1371/journal.pone.0213493

Protocol status: Working
We use this protocol and it's working

Created: August 08, 2018

Last Modified: August 09, 2018

Protocol Integer ID: 14578

Keywords: Phytopathogenic fungi, Colletotrichum spp, Alternaria spp

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.

Attachments

DOCX

ISOLATION OF PHYTOPA...

15KB

Materials

STEP MATERIALS

- 3% NaOCl solution
- water-agar medium (2%)
- 3% NaOCl solution
- water-agar medium (2%)

Protocol materials

- water-agar medium (2%)
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Fungi Isolation

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

2 Disinfest by immersing them in 3% NaOCl solution.
3% NaOCl solution

3 Rinse with sterile distilled water. (1/3)

4 Rinse with sterile distilled water. (2/3)

5 Rinse with sterile distilled water. (3/3)

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

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

Fungi Purification

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

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

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

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

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

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