

Aug 09, 2018

Version 1

# **③** ISOLATION OF PHYTOPATHOGENIC FUNGI V.1

DOI

dx.doi.org/10.17504/protocols.io.sgrebv6

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DOI: https://dx.doi.org/10.17504/protocols.io.sgrebv6

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.sgrebv6

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Protocol status: Working

We use this protocol and it's working



Created: August 09, 2018

Last Modified: August 09, 2018

Protocol Integer ID: 14577

**Keywords:** Phytopathogenic fungi, Colletotrichum spp, Alternaria spp, isolation of phytopathogenic fungi, purification of phytopathogenic fungi, case of phytopathogenic fungi, phytopathogenic fungi, fungi, several isolation method, purification, fruits of different tropical crop, different tropical crop, isolation, leaf, disease

### **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**



# **Troubleshooting**



# **Fungi Isolation**

1

#### Command

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

new command

#### Command

Disinfested by immersing them in 3% NaOCI solution.

new command

#### Command

Rinsing with sterile distilled water three times 3.



#### Command

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

new command

#### Command

Incubated at room temperature (25°C) for 7 days 5.

new command

# **Fungi Purification**

2

## Command

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



#### Command

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

new command

#### Command

Incubated at room temperature (25°C) for 5 days. 3.

new command

#### Command

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



## Command

Transferred hyphae tip onto 90 × 15 mm Petri Dish containing 5. Potato Dextrose Agar (PDA) medium.

new command

## Command

Incubated at room temperature (25°C) for 7 days. 6.