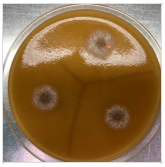

Dec 11, 2020

Rapid Protocol for Single Sporing *Ascochyta rabiei*

Melody Christie[1], Kevin Moore[2]
[1]Griffith University; [2]NSW DPI

1 Works for me This protocol is published without a DOI.

GRDC Ascochyta rabiei research program

Ido Bar
Griffith University

ABSTRACT

A rapid protocol for single sporing of *A. rabiei.* This protocol reduces the time required for single sporing to just 48 hours and allows rapid detection of contamination.

PROTOCOL CITATION

Melody Christie, Kevin Moore 2020. Rapid Protocol for Single Sporing Ascochyta rabiei. **protocols.io** https://protocols.io/view/rapid-protocol-for-single-sporing-ascochyta-rabiei-bqmnmu5e

CREATED

Dec 11, 2020

LAST MODIFIED

Dec 11, 2020

PROTOCOL INTEGER ID

45454

GUIDELINES

All work should be undertaken in a laminar flow and surfaces sterilized with 80% ethanol prior to commencement.

SAFETY WARNINGS

Ethanol is highly flammable, please use with cautious and wear appropriate PPE.

ABSTRACT

A rapid protocol for single sporing of *A. rabiei.* This protocol reduces the time required for single sporing to just 48 hours and allows rapid detection of contamination.

BEFORE STARTING

- Pour water agar plates (~ 2 cm thickness and containing antibiotic) 1-2 days before culturing to ensure plates are dry.
- Autoclave 20 ml glass bottles (McCartney) containing 10 mL MilliQ water with aluminum lids loosely on. Allow to cool to room temperature.

1 Pour water agar plates (~ 2 cm thickness and containing antibiotic) 1-2 days before culturing to ensure plates are dry.

Autoclave 20 ml glass bottles (McCartney) containing 10 ml milliQ water with aluminum lids loosely on. Allow to cool to

2 room temperature.

3 Add ~ 2 drops of Lactic acid **Contributed by users** (approximately **30 µl**) to each bottle.

4 Place a short segment of stem with lesion and visible pycnidia into bottle, tighten lid and shake to mix.

5 Incubate for **00:30:00** at room temperature (**25 °C**). 30m

6 Vigorously shake McCartney bottle to release spores into suspension (shake for **00:00:40**). 40s

7 Pour suspension on to prepared media and immediately pour off.

8 Seal petri dishes with parafilm and incubate at room temperature (**25 °C**).

9 Examine conidia development/spore germination between 24 - **48:00:00** incubation for single spore isolation. 2d

10 Adjust the focus point of the dissecting microscope to the surface of the media to find germinating spores.

11 A small piece of media with the target spore attached should be picked up using a sterilized needle and transferred to 90 mm V8 media plate (containing antibiotic). Place 3-4 individual spores on each plate evenly.

12 When placing spore on media produce a small cut as you set it down. This will concentrate any bacterial contamination inside the cut and make it easy to avoid during spore isolation (see attached image).

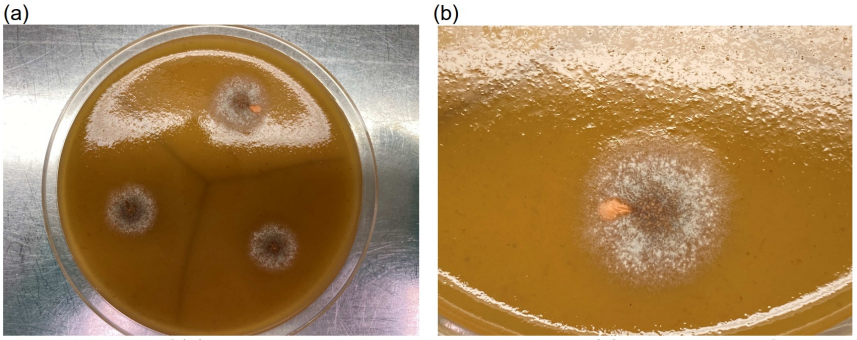

Figure 1. Image of (a) evenly spaced single spore isolates; and (b) concentration of bacterial contamination contained within a small cut in the media.