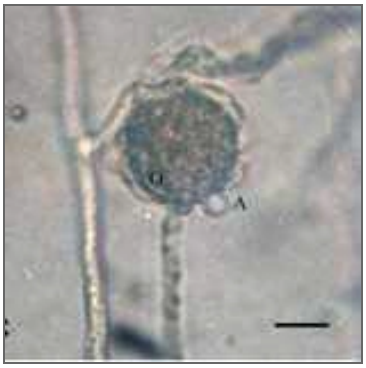

VERSION 3

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OPEN ACCESS

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Protocol status: In development
We are still developing and optimizing this protocol

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Pythium Zoospore Production Soaking Solution V.3

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ABSTRACT

Creation and test of soaking solutions to be used for large-scale zoospore production for *Pythium myriotylum.* This is modified from methods in:
Nyochembeng, L. M., Pacumbaba, R. P., & Beyl, C. A. (2002). Calcium Enhanced Zoospore Production of Pythium myriotylum in vitro. Journal of Phytopathology, 150(7), 396–398. https://doi.org/10.1046/J.1439-0434.2002.00759.X

PROTOCOL integer ID: 94530

Keywords: pythium, myriotylum, zoospore, soaking solutions, fungi, oospore

MATERIALS

Soaking Solutions:

- Verified Pythium myriotylum culture
- 1.5-2% WA or CMA plates, 90 mm (4 * replicates)
- RO/DI water
- 1 L beaker
- 4 x 500 mL autoclavable bottles and lids
- Calcium carbonate ($CaCO_{3)}$
- Sucrose
- Whatman #1 filter paper and funnel
- 1 N KOH – pH adjustment
- 1-5 N HCl – pH adjustment
- pH meter and standards
- Stir bars and stir plate
- Autoclave

Testing Zoospore Soaking Solutions:

- Haemocytometer
- Microscope 40X and slides
- Counter
- 0.08% Methylene blue

Preparation

1 Have mature colonies of verified *Pythium myriotylum* growing on CMA or 1.5-2% WA 90 mm plates. Colony maturity ~7-14 days, with visible oospores.

Note

Fungal growth rates may vary with ambient temperature and petri plate sizes (60 mm vs 90 mm), so wait until mycelium covers the petri plate and has visible oospores before proceeding. Oospores will form at the edges of the dish.

CITATION

Jones, B. L., & Woodard, K. E (1986). A Technique for Evaluating Peanut Germ Plasm for Resistance to Pythium myriotylum. Plant Disease, 70(11), 1038–1043.

LINK

https://doi.org/10.1094/PD-70-1038

Soaking Solutions

2 Make Soaking Solutions 1, 2, 3, and Control.

- Prep 4 x 1 L autoclavable bottles for each Soaking Solutions (1-3) and Control.

CITATION

Nyochembeng, L. M., Pacumbaba, R. P., & Beyl, C. A (2002). Calcium Enhanced Zoospore Production of Pythium myriotylum in vitro. Journal of Phytopathology, 150(7), 396–398.

LINK

https://doi.org/10.1046/J.1439-0434.2002.00759.X

2.1 **Soaking Solution #1**, [M] 0.01 Molarity (m) Ca++ (1 L):

- 1 L RO water at pH 7 in 1 L beaker
- Add 1 g CaCO3
- Filter through Whatman #1 filter paper using a funnel into a 1 L bottle.
- Add a stir bar to the bottle.
- Adjust pH from pH 8.5 to pH 10.5 to pH 7.0 with [M] 5 N HCl and [M] 1 Mass Percent KOH to duplicate the soaking solution in original methods.

Note

Normality is a measure of concentration that is equal to the gram equivalent weight of solute per litre of solution. Gram equivalent weight is a measure of the reactive capacity of a molecule.

The original methods use 1 N HCl, but it is sometimes too weak to induce pH changes unless large volumes are used.

2.2 **Soaking Solution #2**, [M] 0.01 Molarity (m) Ca++ + [M] 0.001 Molarity (m) sucrose (500 mL):

- Transfer 500 mL of SS#1 into a clean 500 mL bottle labeled SS #2.
- Add 171 mg sucrose to make [M] 0.001 Molarity (m) sucrose solution.

Note

Where **Mass (g) = Concentration (mol/L; M) x Volume (L) x Molecular Weight (g/mol)**
Sucrose (g) = 0.001 mol/L sucrose x 0.500 L x 342.3 g/mol
Sucrose (g) = 171.15 milligrams

- Check that pH is pH 7.

2.3 **Soaking Solution #3**, [M] 0.001 Molarity (m) sucrose (500 mL):

- Add 171 mg sucrose to make [M] 0.001 Molarity (m) sucrose solution in 500 mL RO water
- Check that pH is pH 7.

3 Autoclave for 20 minutes on liquid cycle. Remove from autoclave immediately to reduce water evaporation and concentration changes.

Protocol

NAME
Sterilizer (Consolidated)

CREATED BY
Nimalka Weerasuriya

PREVIEW

Testing Zoospore Production

1d

4 Take 30 mL of each Soaking Solution Soaking Solution Preparation onto surface of *Pythium myriotylum* plate cultures that have actively produced oospores.

5 Incubate under light at Room temperature for 24:00:00. 1d
Check for abundant sporangia that will appear after immersion.

6 After 1.5 up to 4 h every 30 minutes:

Take 80 µL soaking liquid from each immersion to a microcentrifuge tube with 20 µL 0.08% methylene blue. Gently invert to stain (or gently vortex) and immobilize zoospores.

Note

Methylene blue (0.1% stock):
- Dissolve 0.1 g methylene blue in 100 mL dH2O

Methyene blue (0.08% working solution):
- Mix 8 mL MB stock solution with 92 mL RO water in a labeled dropper bottle. This solution is used for the viability staining.

7 Take 10 µL of liquid into haemocytometer and examine under 40x to quantify.