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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.2

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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: 94524

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 (CaCO₃₎
- 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

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- 2.1 Soaking Solution #1, [M] 0.01 Molarity (m) Ca++ (1 L):
 - 🗸 1 L RO water at 🕞 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 பூ 8.5 to பூ 10.5 to பூ 7.0 with міть в N HCl and міть 1 N 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.

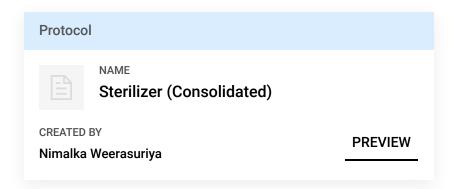
- 2.2 Soaking Solution #2, [M] 0.01 Molarity (m) Ca++ + [M] 0.001 Molarity (m) sucrose (500 mL):
 - Transfer 🔼 500 mL of SS#1 into a clean 500 mL bottle labeled SS #2.
 - Add 🗸 171 mg sucrose to make [M] 0.001 Molarity (m) sucrose solution.

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):

 - 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.



Testing Zoospore Production

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- Take 30 mL of each Soaking Solution Soaking Solution Preparation onto surface of *Pythium myriotylum* plate cultures that have actively produced oospores.
- Incubate under light at Room temperature for 24:00:00

 Check for abundant sporangia that will appear after immersion.

1d

6 After 1.5 up to 4 h every 30 minutes:



Take 4 80 µL soaking liquid from each immersion to a microcentrifuge tube with 4 20 µL 0.08% methylene blue. Gently invert to stain (or gently vortex) and immobilize zoospores.