



VERSION 2

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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

MATERIALS

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

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  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  8.5 to  10.5 to  7.0 with [M] 5 N HCl and [M] 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  7.

2.3

Soaking Solution #3,  0.001 Molarity (m) sucrose (500 mL):

- Add  171 mg sucrose to make  0.001 Molarity (m) sucrose solution in  500 mL RO water
- Check that pH is  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 .
Check for abundant sporangia that will appear after immersion.


1d

- 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.



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