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Pythium Zoospore Production Soaking Solution

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We are still developing and optimizing this protocol

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ABSTRACT

Creation of soaking solutions for *Pythium myriotylum* to be used for large-scale zoospore production.

MATERIALS

Soaking solutions:

- 1 L beaker
- 1 g CaCO₃
- Whatman #1 filter
- 1 N KOH – pH adjustment
- 1 N HCl – pH adjustment
- Sucrose

Testing Zoospore Culture:

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

Preparation

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

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 and 3:

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

pH is adjusted from pH 8.5 to pH 10.5 with [M] 1 N HCl to duplicate soaking solution in original methods (Pacumbaba, cite)

2.2

Soaking Solution #2, [M] 0.01 Molarity (m) Ca++ + [M] 0.001 Molarity (m) sucrose (500 mL):
 <missing info>

pH is adjusted to pH 7 with [M] 1 N HCl

Note

(1 M (mol/l) = 1 N for an acid that releases 1 proton* when dissolved in water)

2.3

Soaking Solution #3, [M] 0.001 Molarity (m) sucrose (500 mL):
 <missing info>

pH is adjusted to pH 7 with [M] 1 N HCl

3 Autoclave for 20 minutes liquid cycle

Protocol



NAME

Sterilizer (Consolidated)



CREATED BY



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PREVIEW

Testing Zoospore Production

1d



4 Take  30 mL of each Soaking Solution  [go to step #2 Soeaking Solution Preparation](#)
onto surface of 7-day cultures in plate.


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

1d

6 At 1.5 up to 4 h every 30 minutes:



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

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