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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

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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 $CaCO_3$
-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 $CaCO_3$

Filter through Whatman #1

pH is adjusted from pH 8.5 to pH 10.5 with [M] 1 Mass Percent 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 Mass Percent 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 Mass Percent 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 Soeaking Solution Preparation onto surface of 7-day cultures in plate.

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

6 At 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.