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Version 1

Pythium Zoospore Production Soaking Solution V.1

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Protocol status: In development

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

Troubleshooting



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.

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

LINK

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.

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

LINK




- 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 CaCO_3

Filter through Whatman #1




pH is adjusted from  8.5 to  10.5 with  1 Mass Percent HCl to duplicate soaking solution in original methods (Pacumbaba, cite)

- 2.2 **Soaking Solution #2,**  0.01 Molarity (M) Ca++ +  0.001 Molarity (M) sucrose (500 mL):
<missing info>

pH is adjusted to  7 with  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,**  0.001 Molarity (M) sucrose (500 mL):
<missing info>

pH is adjusted to  7 with  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 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.

Citations

Step 1

Jones, B. L., & Woodard, K. E. A Technique for Evaluating Peanut Germ Plasm for Resistance to *Pythium myriotylum*

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

Step 2

Nyochembeng, L. M., Pacumbaba, R. P., & Beyl, C. A. Calcium Enhanced Zoospore Production of *Pythium myriotylum* in vitro

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