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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 q CaCO3
- -Whatman #1 filter
- -1 N KOH pH adjustment
- -1 N HCI pH adjustment
- -Sucrose

Testing Zoospore Culture:

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

Troubleshooting



Preparation

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):

Add 4 1g CaCO₃

Filter through Whatman #1



pH is adjusted from 🕞 8.5 to 🕞 10.5 with [м] 1 Mass Percent HCI to duplicate soaking solution in original methods (Pacumbaba, cite)

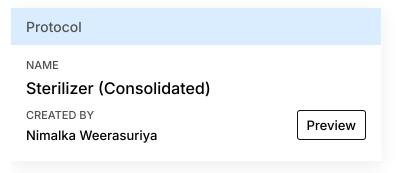
2.2 Soaking Solution #2, [M] 0.01 Molarity (M) Ca++ + [M] 0.001 Molarity (M) sucrose (500 mL): <missing info>

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 (р 7 with [м] 1 Mass Percent HCl

3 Autoclave for 20 minutes liquid cycle



Testing Zoospore Production

1d

- 4 Take 4 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 4 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.

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