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Protoplast Isolation from Zymoseptoria tritici

Forked from Protoplast Isolation from Zymoseptoria tritici

Haseena Khan[1], Megan Mcdonald[1], Peter Solomon[1]

[1]Australian National University

Megan Mcdonald: * Current address University of Birmingham

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Solomon Lab - Australian National University | Zymoseptoria community protocols (STBnet)

Megan Mcdonald

ABSTRACT

This is a basic protocol for protoplasting the fungal pathogen *Zymoseptoria tritici*

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FORK NOTE

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BEFORE STARTING

Prepare the following solutions:

Stock solutions (do not filter sterilise)

A. 50 mL 1.2 M $MgSO_4.7H_2O$ (14.8 g / 50mL)

B. 20 mL 1.2M Sorbitol (4.32 g / 20 mL)

C. 1 M Tris pH 7.5

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D. 1 mL 1M $CaCl_2$ (0.147 g / mL)

Glucanex solution (1.2M MgSO4, 10mM Phosphate buffer pH 5.8)

(Glucanex) Lysing Enzymes from Trichoderma harzianum
by Sigma Aldrich Catalog #L1412-10G

Add 0.04g NaH_2PO_4 to 20ml 1.2M $MgSO_4.7H_2O$ and adjust pH to 5.8 using NaOH drop by drop. A precipitate will form after each drop but will disappear after stirring for a couple of minutes. Once pH is correct, make up volume to 25ml with remaining 1.2M $MgSO_4.7H_2O$ solution. Filter sterilise and store at room temperature.

Add 0.35g glucanex to the solution on the morning of the transformation and shake well to resuspend. Do not filter sterilize after adding glucanex.
NOTE:
Always make fresh solutions prior to experiment.

Protoplast overlay solution 600 mM Sorbitol, 10 Tris pH 7.5
5 mL per prep
Add 2.5 mL of soln B to 2.45 mL milliQ water and 50 ul soln C. Filter sterilise.
1 M Sorbitol-tris solution **1 M Sorbitol, 10 Tris pH 7.5**
5 mL per prep
Add 4.17 mL soln B to 780 ul milliQ water and 50 uL soln C. Filter sterilse.
STC buffer , 1.2 M Sorbitol, 10 mM $CaCl_2$, 10 Tris pH 7.5
10 mL (~10 mL required per prep)
Add 9.8 mL soln B to 100 uL soln C and 100 uL soln D. Filter sterilise.

Inoculum

1 Prepare a culture of *Zymoseptoria tritici* by inoculation of 100ul dense spore suspension into 500 ml Potato Dextrose Broth (PDB), incubate culture at 22°C with 130 rpm shaking.

You can also take 50ul from a dense spore suspension and inoculate 100ml PDB.

Spore Collection

2 Collect the spores on fourth day of culture. Pour 50 ml of Zymo culture into a 50 mL falcon tube and centrifuge at 4000 rpm for 10 minutes at 4°C. Pour off supernatant.

Repeat with a second 50 mL of Zymo culture.

3 Resuspend the pellet in 50ml of sterile water by shaking the tube.
Centrifuge (same conditions as step 2). Discard the supernatent.

4 Resuspend the pellet in 40 ml of 1 M Sorbitol by shaking and Centrifuge at 4000 rpm for 10 minutes at 4°C. Pour off the supernatant.

5 Add 0.35 g glucanase enzyme to 25 ml of filter sterilized glucanex solution and add it to the pellet. Shake the tube gently to dissolve the pellet and pour in a sterile plastic petri dish.

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6 Incubate for 1 hour 40 minutes at 28°C (avoid shaking).

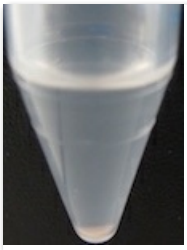
7 Pour the digested sample from petri plate into a 50 ml falcon tube very carefully and overlay with 5 ml of 600 mM Sorbitol + 10 mM Tris (pH 7.5) very gently. Don't mix.
NOTE: Use a 1000 ml pipette tip for overlay and pour it very gently to the wall of falcon tube just above the digested solution.

8 Centrifuge at 4000 g for 15 minutes at 4°C. After centrifugation three layers will appear.

Collect the interface carefully (without touching the bottom layer) with 1000ml pipette in a 15ml falcon tube and add an equal amount of 1M Sorbitol + 10 mM Tris (pH 7.5) to the top. Mix gently by tilting the tube slowly.

9 **Keep the tubes on ice ONWARDS**.
Pour the whole solution over an autoclaved cotton wool in a sterile syringe with no piston and allow it to drip slowly, collect in a 15 ml falcon tube.

10 Centrifuge at 1500 g for 5 minutes at 4°C and protoplasts will settle down in the bottom.
Remove the supernatant with 1000 ml pipette without disturbing the pellet.



11 Wash the pellet by suspending in 1 ml of STC (1.2 M Sorbitol, 10 mM $CaCl_2$, 10 mM Tris pH 7.5), Spin it down at 1500 g for 5 min at 4°C. Remove the supernatant carefully.

Gently resuspend the protoplasts in 500 ul of STC buffer by tapping the tube with fingers, take 10ul of protoplast solution and count in a hemocytometer. Dilute to a final concentration of $1´10^6$ protoplasts/ml with STC buffer.
Use 100-200 ul of these freshly isolated protoplasts for transformation.

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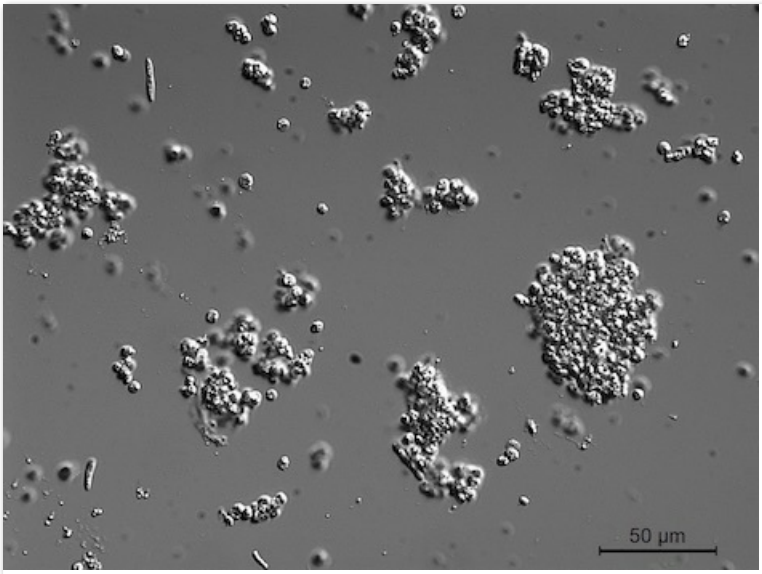

Test to confirm Protoplasts

12 After Protoplasting, put a drop of the protoplast suspension on a slide and examine under a microscope. After visualizing round protoplasts, add a drop of sterile water to the side of the slide without disturbing the coverslip. Once it spreads through the slide, all the protoplasts will disappear because they burst open due to the change in isotonic solution. Spores or structures that stay intact or swell, will be intact cells or debris.

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