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Protoplast method for Chromosome prep with Zymoseptoria tritici

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Zymoseptoria communit...

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Abstract

Preparation of Zymos*eptoria tritici* isolates for extraction of chromosomes via pulsed-field electrophoresis

Pat Martinez 1990 (Bruce McDonald lab) (Simplified from Cooley, et al, Curr. Genet. 13:383-389.)

Prepare spores

1 Grow single-spore isolate in 40 ml **yeast-sucrose broth**, **without chloramphenicol**, until cells are in log phase, (5-8 days at room temperature) in shaker at 150-200 rpm. **Do not grow cells past log phase!** In stationary phase, protoplasts do not form as easily.

Transfer culture to sterile 50 ml Falcon tube. Harvest spores and mycelium by centrifuging at top speed in IEC clinical centrifuge for 5 minutes. Pour off supernatant.

Make protoplasts

2 Add 40 ml of 600 mM MgSO4 pH 5.8 (sterile) and resuspend cells completely in solution. Harvest washed cells by centrifuging at top speed for 5 minutes in IEC clinical centrifuge.

3 Resuspend pellet in 20 ml of a 1.2 M MgSO4 pH 5.8 solution containing 3.0 mg/ml of Novozyme 234 (this solution must be filter sterilized, mix Novozyme with 1.2 M sltn and vortex to mix before filter sterilizing). Sterilize by forcing solution slowly through 5 ml syringe-mounted Nalgene filter. Transfer spore-Novozyme solution to a 40 ml glass Corex centrifuge tube.

4 Incubate at 30^{o} C for 2 hours without agitation. Check for formation of protoplasts after two hours, and again at 15 minute intervals. **Do not agitate tube any more than necessary!**

Harvest protoplasts

5 Once protoplasts have formed, centrifuge in IEC clinical at top speed for 3 minutes. Should find that the majority of pinkish cells are floating on top of liquid; these are protoplasts and some undigested spores. Pipette away cloudy, brown liquid underneath protoplast layer and save top layer.

6 To wash cells, add 20 ml of 1.2 M sorbitol and resuspend cells. Harvest cells by spinning at top speed, IEC clinical for 5 minutes. Discard supernatent and save pellet of protoplasts. Repeat this wash step again (**two washes total**). After second wash, resuspend protoplasts in 0.4 ml of 1.0 M sorbitol and quantify protoplast concentration with hemocytometer (probably will need to make 1:10 dilution of protoplast prep). Add 1.2 M sorbitol to achieve protoplast concentration of between 5-10 × 10^{8} protoplasts per ml.

7 Add equal volume of **2.2%** low melting point (LMP) agarose (in TE) to protoplasts and mix well but gently. Keep solution liquid in 37^{o} C water bath. Tape closed one end of plug molds to receive protoplast solution. Use pasteur pipette to pipette protoplast-LMP

solution into each well of plug mold (push pipette end to bottom of well and work upward as protoplast solution enters well) and allow to solidify for **two hours** at 4^{o} C.

8 Push out plugs with flame-blunted pasteur pipette into a 15 ml Falcon tube containing 10 ml lysis buffer (1% sarkosyl, 450 mM EDTA, 1 mg/ml proteinase K). Incubate tubes at 50^{o} C for 48 hours, replacing lysis buffer once after 24 hours.

9 Rinse plugs in 500 mM EDTA and store in 500 mM EDTA at 4^{o} C