Spore production for Phytophthora infestans

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Abstract

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Protocol

Step 1.

Take agar cubes / rains from glycerol stock and place 3-4 pieces on agar plates

When using another plate, cut small cubes (approx 0.5x0.5 cm) and place 3-4 upside down on the agar plate

For longer term cultivation use RyeA agar

For sporulation use RyeB agar

PROTOCOL

. Rye B Agar

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Step 1.1.

Soak 60 g of rye grain in distilled water for 24 hours at room temperature. This is done in a small tray so that water just covers grain. Cover tray tightly with aluminum foil.

Step 1.2.

Next day, pour supernatant off germinated grain and put aside.

Step 1.3.

Boil rye grain (do not blenderize) for one hour in enough additional distilled water to cover the grain (about 1 inch above). Do this in a 2 liter beaker with foil to cover top. Check water level regularly, add more water if needed.

Step 1.4.

Filter through 4 thicknesses of cheese cloth, squeezing gently to remove residual liquid. Discard cheese cloth and grain

Step 1.5.

Combine the supernatants.

Step 1.6.

Add 15g of Agar, 20g of sucrose and 0.05g of ß-Sitosterol. Adjust volume to 1l.

Step 1.7.

Autoclave at 15 psi for 20 min

Step 2.

Grow plates for about 7 days in the dark, untill the plate is fully covered

Optimum temperature is about 18-20°C

Step 3.

Move plates to at 16/8 light/dark cycles for about 2-3 days to induce sporulation

Step 4.

Harvest sporangia by adding several ml ice cold water to the plates and scraping of the sporangia with a pipette tip

Step 5.

Aspirate all liquid and store in fridge for 3-6 hours to induce hatching of the spores

Step 6.

Pipette off the upper layers (spores swim up, debris sink) and check spore density under microsope