Phytophthora Storage Methods

Water

1.) Fill large screwcap test tubes approximately ¾ full with dH_2O. If making tubes for *P. infestans*, add a pinch of rye grain to the tube. If making tubes for other *Phytophthora* species, in addition to the tubes, place dry hemp seed in a beaker and cover with foil for sterilization. Cap the tubes lightly. Autoclave the tubes (and hemp seed if needed) for 30 minutes on the liquid cycle.
2.) After cooling, if making tubes for other *Phytophthora*, aseptically add a few sterile hemp seeds to each tube. Sterile hemp seeds are added to the water tubes after autoclaving because hemp seeds sterilized in water can discolor the water, making evaluation for contamination difficult.
3.) From an actively growing plate, use the wide end of a sterile Pasteur pipet to punch out 4-5 plugs/tube. Aseptically transfer the plugs to the tube. Cap and seal with parafilm. Make at least 2 tubes/isolate, 4 if the isolate is particularly important.
4.) Label the tube with the species, isolate name, and date entered into the tube.

Agar Slant

1.) Prepare 1 liter of rye A agar (for *P. infestans* only – see recipe below; make lima bean agar for other *Phytophthora* species) and autoclave for 30 minutes on the liquid cycle. After cooling slightly (should still be liquid), use a pipet to transfer approx. 8-10 mL of agar to medium screwcap test tubes (Fisher Catalog no. 14-959-25C). Tubes should be approx. 1/2 – 2/3 full. Cap lightly and reautoclave for 30 minutes on liquid cycle.
2.) In the laminar flow hood, arrange an incline on which to lean tube racks (in our lab this is typically accomplished using a bundle of metal tubes taped together, but anything that can be used to prop the rack up will do). Upon completion of the autoclave cycle, lean the tubes on the incline so that the agar slants slightly (approx. 45 degree angle). Allow to cool. Once cool, tighten caps and store in fridge until use.
3.) From an actively growing plate, use the wide end of a sterile Pasteur pipet to punch out 1 plug/tube. Aseptically transfer the plug to the tube, active growth side against the media. Cap and seal with parafilm. Make at least 2 tubes/isolate, 4 if the isolate is particularly important.
4.) Allow the tubes to incubate until mycelium has covered the surface of the slant. Aseptically pipet sterile light mineral oil into the tube until the oil is at least 1 cm above the top of the slant to prevent drying out. The oil should have been sterilized twice in the past 24 hours before applying to slants.
NOTE: If the culture has a lot of aerial growth that's trapping air under the oil it is a good idea to wait a day or two before placing in storage to allow the oil to settle so it can be topped off if needed. The agar needs to be completely covered. Any exposed agar can act as a wick and draw moisture out of the slant over time, even if the rest of the slant is covered.
5.) Seal with parafilm and label the tube with the species, isolate name, and date entered into the tube.

Rye A Agar

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.
2. Next day, pour supernatant off germinated grain and put aside.
3. Place grain in a blender, add distilled water (about 1 inch above grain) and blend on high for 2 minutes. Cook in water bath for 1 hour at 68ºC. Don't modify extraction time or temperature.
4. Filter through 4 thicknesses of cheese cloth squeezing gently to remove residual liquid. Discard cheese cloth and grain sediment.
5. Combine original supernatant (liq. poured off grain at the beginning) with filtrate. (At this point the preparation can be frozen for use later).
6. Add 20g sucrose, 15g Bacto Agar then adjust volume to 1 liter.

7. Autoclave for 30 minutes.

Reference: Caten, C. E. and J. L. Jinks. 1968. Spontaneous variability of single isolates of *Phytophthora infestans*. I. Cultural variation. Can. J. Bot. 46: 329-348.

Cryostorage

1.) Prepare sterile 10% DMSO by adding 5 mL of sterile DMSO to a 50mL falcon tube and adding sterile dH_2O to the 50mL mark. Keep tube in the dark when not in use.
2.) From an actively growing plate, use the wide end of a sterile Pasteur pipet to punch out 4-5 plugs/tube. Aseptically transfer the tubes to a sterile cryovial. Make at least 2 tubes/isolate, 4 if the isolate is particularly important.
3.) Add 1mL 10% DMSO using aseptic technique. Cap tightly and label on both the top and side of the vial with the isolate name and date entered. Place immediately on ice.
4.) Transfer tubes to a -80˚C freezer and freeze for 1 hour. The tubes can stay at -80 for slightly longer if needed, but try to get the samples into the cryotank on the same day, as extended storage at -80 can potentially affect viability.
5.) Using a box with ice, quickly transfer tubes to the cryotank. Make a note of the location of each tube to be entered into the cryostorage database.

Storage Recovery

1.) For water tubes, pour the contents of a tube into a sterile Petri dish. Use sterile forceps to transfer plugs to pea broth for recovery. A new tube will need to be made to replace the tube used.
2.) For slants, use a sterile tool, such as a small metal spatula, to remove a piece of agar from the slant and place in pea broth. The slant can be reused if clean and if material is still present.
3.) For cryostorage, after removal from the tanks, place the tube on ice and allow to thaw on ice slowly (approx. 30-60 min). After thawing, use sterile forceps to extract 1-2 plugs from the tube and place into pea broth. The remaining plugs can be refrozen and returned to cryostorage. When returning the tube to cryostorage, follow the same procedure as for freezing a new tube (keep on ice, transfer to a -80°C freezer for approximately 1 hour, then return to the tank).