

Culturing *Batrachochytrium dendrobatidis*

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Cultures of *Batrachochytrium* will be sent on nutrient agar in sealed Petri plates or in 1 % T broth in screw-capped centrifuge tubes. Upon receipt, transfer 2 mm² chunks of culture to several containers of 1% tryptone broth to serve as stock cultures.

Stock cultures of *Batrachochytrium* remain viable longer when grown in broth. Place 75 mL of broth into 150 mL screw-capped flasks--nothing sacred about size here--use larger or smaller containers depending on what is available. Because of the danger of contamination, I use screw-capped vessels and keep duplicates. Incubate at 23° C or below. For long-term (at least 3 months) storage place at 5° C after growth is evident. When growing to produce zoospores for inoculum or other purposes, grow on 1 % tryptone agar medium.

Production of zoospores: Grow *Batrachochytrium* in broth until clumps of thalli are visible to the eye. Use a sterilized Pasteur pipette to add about 1/2 to 3/4 mL of this broth culture to tryptone agar in 9 cm culture dishes. Leave inoculated dishes open in laminar flow hood until the added broth is dry; replace covers on dishes and either seal plates with Parafilm® or place in plastic sleeve. Incubate in an incubator set between 15 and 23° C; the potential harvest period is longer if plates are kept in plastic sleeves at 15° C. After 7-10 days look for active zoospores around the periphery of fungal colonies by inverting dishes on the stage of a compound microscope and examining with the 10 X objective.

Harvest zoospores: Flood plates with about 2-3 mL of sterile distilled water and decant after about 30 minutes to collect zoospores. Zoospore concentration can be measured by optical density or by counting a measured amount on a hemocytometer. Zoospores may stay motile (thus infective) for up to 24 hours, however, most encyst before 24 hrs.

Biosafety: This organism can be lethal to amphibians. Although thorough drying can probably kill it, leave nothing to chance. Autoclave all materials that contain or have come into contact with the fungus before disposal. If *Batrachochytrium* is used to inoculate amphibians, kill and incinerate or fix all exposed animals after the experiment. Be sure that cages, water, and other material in cages are disinfected at the end of experiments. Do not dump potentially contaminated material in the trash or down the drain without first treating it (heat or bleach) to kill the fungus.

Recipes

Tryptone agar

10 g tryptone
10 g agar
1000 mL distilled water

Tryptone broth

10 g tryptone
1000 mL of distilled water