Recommended Culture Method for *Pseudogymnoascus destructans*

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for
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Samples for Culture:

Pseudogymnoascus destructans can be isolated from multiple substrates in caves as well as from bats outside of caves.

1. Swab Samples:
    - If you intend to use the same samples for PCR, use only sterile synthetic-fiber tipped swabs (not cotton) on synthetic applicator sticks (not wood). If the samples will be used only for culture, sterile cotton-tipped swabs on wooden applicators can be used.
    - Rub the swab tip over the substrate of interest several times
    - Store each swab individually in sterile containers if not used immediately for culture.
    - Swabs not used immediately should be stored at -20C or colder until used.

2. Direct from Substrate
    - The substrate of interest also can be placed directly on an agar surface.
    - This is not recommended for bat wing samples. It is recommended that bat wings be swabbed. I found swabbing more effective for isolating *P. destructans* from bat wings than direct culture of wing tissue. The sections of bat wing placed on agar surfaces inevitably became overgrown with other fungal species, such as *Mucor* and *Penicillium*, and no *P. destructans* could be obtained.

Selection of Culture Medium:

- Use DPYA culture medium with ox gall and sodium proprionate (see formula in Appendix 1)

Dextrose-peptone-yeast extract Agar (DPYA): Makes 1L	
Glucose (dextrose)	5.0 g
Peptone	1.0 g
Yeast extract	2.0 g
NH_4NO_3	1.0 g
K_2HPO_4	1.0 g
$MgSO_4 \cdot 7H_2O$	0.5 g
$FeCl_3 \cdot 6H_2O$	0.01 g
Oxgall	5.0 g
Sodium propionate	1.0 g
Chlortetracycline	30 mg
Streptomycin	30 mg
Agar	20 g
Distilled water	1000 ml

I have found DPYA more effective for isolating *P. destructans* than Sabouraud-Dextrose agar, which tends to be overgrown with *Mucor*, *Mortierella*, and *Penicillium* spp. As well as low sugar, oxgall and sodium propionate restrict the growth of some rapidly spreading fungi, while the chlortetracycline and streptomycin discourage bacteria. If DPYA is unavailable, other low-sugar mediums can be substituted.

Culture Procedure:

- Streak the swab tip over the whole agar surface to spread out the spores
- Replace the plate cover and seal the agar plate with parafilm.
- Incubate plates at 5-10°C.

Pseudogymnoascus destructans colonies should appear within 2 weeks, if present.

Verify Growth of *Pseudogymnoascus destructans*

- Verification should be done by PCR analysis at a laboratory which uses the technique described in:

Muller, LK, JM Lorch, DL Lindner, M O'Connor, A Gargas and DS Blehert. 2013. Bat white-nose syndrome: a real-time TaqMan polymerase chain reaction test targeting the intergenic spacer region of Geomyces destructans. Mycologia 105:253-259.

- Culture morphology will help identify *P. destructans* colonies. (See photographs below.) *P. destructans* colonies will first appear white but turn gray/green with age. Often, but not always, a clear/yellowish exudate will appear near the center of isolates. A detailed description of spore morphology can be found in:
  Gargas, A., M.T. Trest, M. Christensen, T.J. Volk, and D.S. Blehert. 2009. *Geomyces destructans* sp. nov. associated with bat white-nose syndrome. Mycotaxon 108: 147-154.

Fig. 1: *Pseudogymnoascus destructans* on DPYA (without oxgall and sodium propionate) with exudate clearly shown

Fig. 2: As *P. destructans* colonies age, the exudate will disappear leaving behind a honeycomb appearance.

Fig. 3: Young white *P. destructans* colonies on DPYA (with oxgall and sodium propionate) from a bat wing swab streaked across half of the agar surface. Note the *Mucor* at the top of the photo growing from a section of bat wing. Other fungal species are also visible (e.g. dark growth).

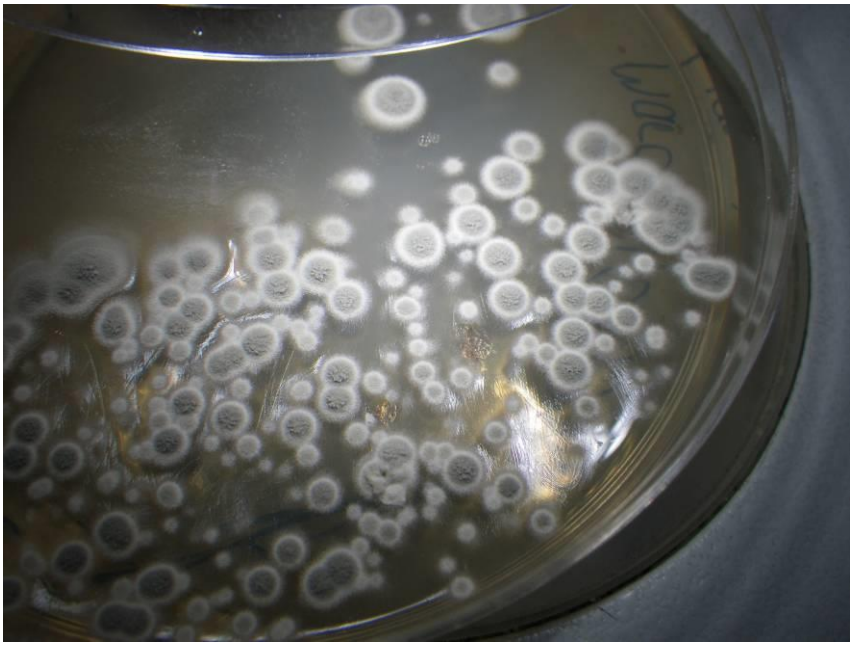

Fig. 4: Older grey *P. destructans* colonies on DPYA (with oxgall and sodium propionate) from a bat wing swab streaked across half of the agar surface.