

**Purifying shrimp chitin for bait** (from Karling 1945)

Decalcify shells in cold 1% hydrochloric acid for 1 wk; change acid several times.

Wash in distilled water

Soak in KOH for 10 d to remove protein and other organics

Wash in water

Soak in ethyl alcohol for 3—7 d; change alcohol 1—2 times

**Collecting pollen for bait**

In spring monitor male cones of evergreens (in the North) or sweetgum trees (in the South) for pollen discharge. On a day when the pollen is being shed use a large plastic bag to enclose a group of male cones, shake the branch and the pollen will pour out of the cones into the bag. Repeat as many times as necessary to get the amount of pollen you will need for several years. If pollen is being released, don't wait until the next day to collect, do it immediately. Take the pollen to the lab and leave bag open for several weeks to allow the pollen to dry. Then place pollen in glass vial or other container with a tight lid.

I prefer spruce pollen because it is larger than pine pollen; consequently chytrids that grow on spruce pollen are larger than the same species grown on pine pollen. Larger thalli make observation and isolation easier.

**Media**

Note that recipes are for 500 mL, which makes between 20 and 23 Petri dishes of medium. The most frequently used media in our lab are the first three below. For isolation media we add **Penicillin G** (200 mg/L) and **Streptomycin sulfate** (200 - 500 mg/L) after autoclaving, and before pouring agar into Petri dishes. We weigh antibiotic powder as aseptically as possible, add it to the autoclaved medium and swirl to mix. Although earlier workers used glass distilled water, we have had no problem with house distilled.

**Barr's PmTG**

Peptonized milk 0.5 g

Tryptone 0.5 g

Glucose 2.5 g

5 g agar for plates, 6 g for tubes

Distilled water 500 mL

**mPmTG**

Peptonized milk 0.2 g

Tryptone 0.2 g

Glucose 1.0 g

Distilled water 500 mL

5 g agar for plates, 6 g for tubes

**Cellobiose-dilute soluble starch (Cd)**

Peptonized milk 0.1 g  
Tryptone 0.2 g  
Cellobiose 1.0 g  
Soluble starch 1.0 g  
5 g agar for plates, 6 g agar for tubes

Other media include:

**½ strength Emerson's YpSs**

Soluble starch 10.0 g  
Yeast extract 0.5 g  
K<sub>2</sub>HPO<sub>4</sub> 0.5 g  
MgSO<sub>4</sub>·7H<sub>2</sub>O 0.25 g  
Dist water 1000 mL

***Harpochytrium* broth**

Tryptone 10 g  
Glucose 3.2 g  
Dist water 1000 mL

**Koch's K-1 agar**

Peptone 0.3 g  
Yeast extract 0.2 g  
Glucose 0.6 g  
Agar 5 g  
Dist water 500 mL

**Lima Bean medium**

(especially for *Gonapodya* spp.)  
Frozen Lima Beans 1 package  
(Boil 1/2 hour in 1000 mL distilled water; filter through cheese cloth, and replenish water to 1 liter).  
Add 10 g agar for agar medium

**M<sub>3</sub> chytrid agar:**

17 g Difco Cornmeal Agar  
5 g glucose  
5 g soluble starch  
1 g peptone  
1 g yeast extract  
1L Distilled water

**Murray & Lovett code = M & L**

(try this for difficult chitinophiles)

Glucose	0.6 g
n-acetyl glucosamine	0.3 g
Asparagine	0.25 g
Tryptone	1.0 g
Agar	7.0 g
Machlis' soln	50 mL
Distilled water	450 mL

**10X Machlis' solution**

NH <sub>4</sub> NO <sub>3</sub> (5 X 10 <sup>-3</sup> M)	1 g
CaCl <sub>2</sub> (5 X 10 <sup>-4</sup> M)	0.18 g
MgSO <sub>4</sub> (5 X 10 <sup>-4</sup> M)	0.15 g
Na <sub>2</sub> HPO <sub>4</sub> (5 X 10 <sup>-3</sup> M)	3.35 g
KH <sub>2</sub> PO <sub>4</sub> (5 X 10 <sup>-3</sup> M)	1.7 g

**P-S isolating agar (C.E. Miller)**

30 g Difco agar
0.05 g glucose
0.05 g peptone
0.05 g yeast extract
0.5 g streptomycin sulfate
0.5 g penicillin G
1 L Distilled water

**PYG**

Peptone	0.6 g
Yeast extract	0.6 g
Glucose	3 g
Agar	5 g
Dist water	500 mL

**Tnag**

Tryptone	0.8 g
n-acetyl glucosamine	0.4 g
agar	5 g
water	500 mL

**TYG**

Tryptone	1.25 g
Yeast Extract	0.625 g
Dextrose	1.5 g
Distilled water	500 mL