

## **Nonsporulating Mycelia:**

### Method #1

Use clarified rye broth

Set up stationary cultures:

--in petri plates (normally use 100 x 20 or 100 x 25 plates; these are deeper than normal).

--or can use flasks (1 liter glass flask, for example). However, this is not advised since sometimes it is harder to harvest the tissue.

--fill 100 mm plates with ca. 30 mls of rye broth: the idea is to have no more than 6 mm of liquid.

--inoculate with sporangia: ca  $10^4$ /ml.

--let grow ca. 3-4 days at 18C. Sporangia will germinate during the first 1-2 days.

Eventually the mycelia will become vacuolated and float; the next step (which can occur very quickly) is sporulation. Therefore, you MUST check it each day to check on its progress. Also, you MUST harvest before aerial hyphae are present.

--harvest by either pouring culture into a Buchner funnel containing Whatman Type 54 paper, using vacuum. (it helps to try to pour the mycelia into one spot). (alternatively, can usually pick up all of the mycelia with forceps and place on the paper in a small area; this makes it easier to recover the tissue).

### Method #2.

Lay a sterilized polycarbonate membrane (85 mm) on top of rye agar.

Spread  $10^4$  to  $10^5$  sporangia per 100 mm plate.

Let grow at 18C for ca. 2-3 days. As above, must monitor closely to check for aerial hyphae. This is much trickier to do on the membrane, vs. liquid culture.

### General advice

After harvesting, I recommend that you look at the tissue under the microscope to make sure that there are no spores (or at least very few spores). Make sure that you distinguish "new" spores from the spores used for the inoculum (not all may have necessarily germinated)

## **Sporulating mycelia.**

Do one of the above, but let tissue grow longer (typically 7 days?).

Before harvesting, check a tuft of tissue under the microscope to ensure that there are lots of sporangia. Ideally, it would be good to quantitate this (can put a tuft of known surface area in a small tube containing water of known volume; vortex briefly to detach sporangia; count under a haemocytometer).