

## Isolating from Basidiomycetes

Cultures of Basidiomycetes may be started from spores, basidiocarp tissues, or mycorrhizal roots.

### Spores:

To collect the spores, sever the cap from the stem of a fresh, cleaned basidiocarp. Place the cap, or portion of the cap, gill side down on a piece of white paper or a cleaned microscope slide. If the specimen is partially dried, add a couple drops of water to aid in spore release. To minimize evaporation and disturbance from air currents, place a beaker or Petri dish over the cap. After several hours, a spore print will be present. If you have made the print on paper, cut it out, fold it in half, seal it in an airtight container and label the specimen. If the print was made on a microscope slide, place another slide over the spores and seal the edges with tape to prevent contamination. These methods will allow you to store the spore prints until you are ready to culture. A quick way to establish cultures from basidiocarps using spores is to attach a small piece of the cap (with gills) to the inside lid of a Petri dish of agar medium using petroleum jelly. Place the dish on a slant so that the spores are showered down across the agar inside of massed up in a spore print. This makes it easier to transfer spores, and the spores are more likely to germinate than if they are in a mass.

To culture from spores, sterilize an inoculating loop or other transfer tool. Scrape some spores off the spore print and streak across the surface of agar medium (potato sucrose agar, rabbit food agar, MEA). When starting a new culture from spores, it is best to inoculate at least three media dishes to improve the chances of getting successful germination.

In general, fresh basidiocarps will yield the best spores for germinating. However, if you are using an older basidiocarp, or an older spore print, it may still be possible to germinate the spores if they are rehydrated first. Spores can be soaked in sterilized water to rehydrate them. Scrape the spores from the spore print and transfer to a sterile test tube filled with sterile distilled water. Seal the tube and let stand for 6-12 hours. Draw up several ml of spore suspension with a sterile pipette and inoculate several plates with one or two drops of suspension.

Not all spores will germinate, and others will have very poor germination. For those that do not germinate readily, Watling (1977) lists the following techniques:

1. Place the spores of the fungus on a piece of sterilized membrane or cellophane directly onto a culture of the same or closely related agaric. When the spores germinate, pick them off and transfer to media.
2. Some spores germinate when they are close to contaminating yeasts or bacteria. It may be beneficial to introduce some contaminants into the culture with the spores.
3. A range of media with vegetable or fruit extracts have been useful in inducing spore germination. Watling recommends chick-pea agar and prune agar.
4. For coprophilous and other dark-spored agarics, pre-treatment in an air oven at 60 C for 1 hour or 37 C overnight before plating out often induces germination.
5. Many coprophilous fungi require gut enzymes to induce germination. Treatment of the spores by washing in an aqueous solution of pancreatin has been shown to be useful in inducing germination in some fungi.

6. A 0.01% aqueous solution of furfural added to the molten agar prior to pouring into the Petri dish has been found to give encouraging results. Washing the spores in a 0.01% solution of furfural prior to streaking out on agar has also been successful.

### **Other tissues:**

Tissue cultures must be taken from basidiocarps within 24-48 hours after collection. If the specimens are too old or too mature, a pure culture will be extremely difficult to isolate.

Since the entire basidiocarp is composed of compressed mycelia, a culture can be obtained from any part of the mushroom. The cap, the upper region of the stem and the area where the gill plate joins the underside of the cap are the best locations for excising tissue free of contaminants. The stem is often colonized by maggots and other insects, which bring with them their own set of contaminating microorganisms.

It may be possible with some specimens to peel back the 'skin' (cuticle) from the upper surface of the cap and obtain clean tissue from the underlying flesh. This is particularly true with specimens of *Suillus*, *Gomphidius*, and other mushrooms with slimy cuticles. Wipe the surface of the mushroom with a cotton swab soaked in 70% EtOH and remove any dirt or damaged tissue. Break the cap or stem, exposing the internal tissue. Working quickly to reduce chances of contamination, remove a small piece of tissue and transfer to the center of an appropriate medium. Do at least five transfers in this manner. If successful, growth should be present in 3-7 days.

With more delicate fungi, the stipes can be either plated out directly onto the agar or washed in vials of water (up to 12 times in small quantities of sterile water in vials), cut into segments and then plated out. Dipping the tougher tissues in 10% NaOCl for a few seconds also gives adequate results. This technique also will work for rhizomorphs. Many agarics and boletes produce veils which protect the developing spore-producing layer. Fragments of this veil from an actively growing basidiocarp can be treated as delicate stems and plated out directly onto agar, or if more leathery, washed and chopped into pieces before plating.

In general, many basidiomycetes grow well in culture at room temperature. Initially you may want to try potato dextrose agar and potato sucrose agar for the isolations. MEA and rabbit food agar are also good media for basidiomycetes.

### **Mycorrhizal roots:**

For ectotrophic mycorrhizal fungi, the most common mycorrhizae associated with woody plants, the short roots of the tree are washed several times in distilled water and cut into portions and plated out directly. Isolation from roots can be accomplished simply by dissecting the roots into two parts in sterile water, the outer cortex and inner stele. These are either plated out directly, or incorporated into nutrient agar after additional fragmentation. Dispersal in the agar is accomplished by swirling the plate containing the molten agar and root fragments. Ectotrophic mycorrhizal fungi are often deficient in thiamine, so a glucose/ammonium tartrate medium should be used if growth is poor. Many ectomycorrhizal fungi, for example *Cortinarius* species, will produce rhizomorphs in culture.

Endomycorrhizal fungi (VAM), the mycorrhizae common on herbaceous plants, cannot be cultured.

### **References**

Stamets, P. and J. S. Chilton. 1983. *The Mushroom Cultivator. A Practical Guide to Growing Mushrooms at Home.* Agarikon Press, Olympia, WA. 415 pp.

Watling, R. 1977. *How to Identify Mushrooms to Genus V. Cultural and Developmental Features.* Mad River Press, Eureka, CA. 169 pp.