

Chapter 6

SPAWN MAKING: A JOB ONLY FOR WELL-TRAINED PERFECTIONISTS

Logically, this should be the first chapter, since we must have spawn before mushrooms can be grown. However, most mushroom growers buy their spawn. Spawn making is a business that does not mix well with mushroom growing. It should always be a completely separate facility at least several kilometers (miles) from any mushroom growing facility.

Spawn making requires the utmost cleanliness and care. It must be cleaner than any area used for human surgery. All clothes must be cleaned every day. Shoes must be used only for the area where the spawn is produced, and **never** worn into any area that is not absolutely clean. All workers heads should be covered so that nothing can fall from hair into the spawn. Workers should wear surgical masks. Workers who smoke or are exposed to smoke must take a shower before they enter the spawn-making area. Workers must also take a shower and wash thoroughly after any exposure to any other

74 OYSTER MUSHROOM CULTIVATION

fungi or mushroom material. All of the surfaces in the rooms should be hard. Ceiling, walls and floor must be washed regularly, preferably every day. All air must be filtered with High Efficiency Particulate Air (HEPA) filters.

There is some danger in making spawn, particularly if new cultures are started from mushrooms. It is reported that a number of amateurs have contracted serious mycoses (human diseases caused by fungi) as a result of attempting to make spawn. At first, such problems may sound unlikely. However, if in attempting to grow mushroom mycelium, the grain or agar becomes contaminated with a human pathogen, the amateur may propagate it and give himself or those around him a massive inoculation of disease causing organisms. We are naturally equipped to resist infections, but we are not able to resist any disease if we receive massive amount of the causal organism.

Some of the need for absolute cleanliness can be reduced if “laminar hoods” with certified High Efficiency Particulate Air (HEPA) filters are used for all of the work, **Fig. 50**. If a hood is used, it must be absolutely clean, workers must be clean, and everything that goes into the hood must also be absolutely clean. In the use of hoods, it is difficult to be certain that everything that goes in is absolutely clean. In the directions that follow, precautions for things placed in the hood will be discussed.

OBTAINING MUSHROOM CULTURES

The best and usually easiest way to get a culture is to obtain one from an established source such, as a culture collection, a research laboratory, or possibly another spawn producer. Some sources will charge for their cultures. However, the cost is not usually high from the most reliable sources, compared to the risk of other sources.

There are several reasons that one might want to make a totally new culture. One is that there may be great problems with getting a culture from an established source. Another is the desire to establish a new line because one observes some desirable characteristics in wild mushrooms or in only



Fig. 50. A laminar hood, suitable for preparing spawn. However, clutter is not permissible.

one or two mushrooms that they are being grown. There are two ways to approach making the new culture: from a spore print, or from mushroom tissue. With most mushrooms, the spore print method is very difficult, since one must start with a number of separate, single spore cultures and then breed them, by growing two together until they fuse. Some mushrooms behave

76 OYSTER MUSHROOM CULTIVATION

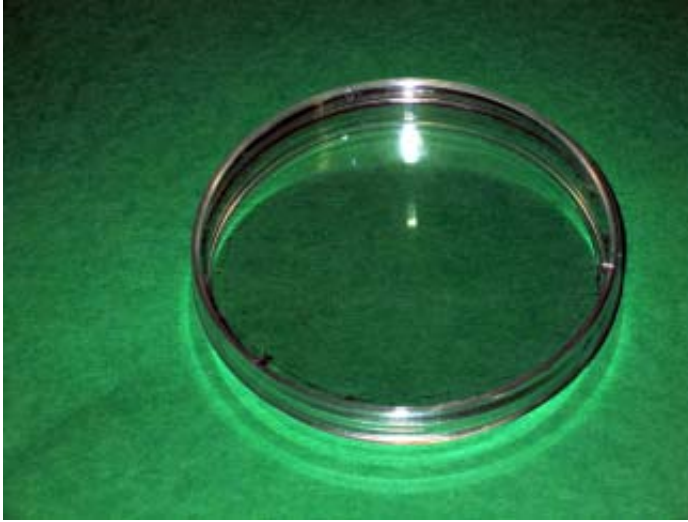


Fig. 51. A petri dish. Used for sterile cutting and for testing for contamination.

almost as if there were four sexes, and successful breeding will only occur with the correct two. We have not learned how to distinguish the “sexes” except by trial and error breeding. Thus, on average, breeding fails in three out of four attempts. *Agaricus bisporus* is an exception and single spores from it will normally produce a mycelium which can produce mushrooms, with no problems or benefit of breeding.

Spore Cultures: For culture purposes, spore prints are made by placing a piece of paper in the bottom of a jar and attaching a hook to the lid. The bottle is then sterilized. When the bottle has cooled, the mushroom is attached to the hook, with the gills down. The bottle is closed and allowed to stand for 8 to 24 hours, or less time if spores can be seen. A needle is used to scrape spores off the paper, one at a time. Each spore is placed on an agar “slant” in a test tube. In general, it will be wise to make at least ten tubes from the print of each mushroom. The tubes are then incubated at about 15 to 20°C (59 to 68°F) until substantial mycelium is seen.

Tissue Cultures: Tissue cultures are generally the best way to obtain a new culture. One can expect that the new culture will produce mushrooms with the same characteristics as the mushroom from which the tissue was



Fig. 52. A small laminar hood, suitable for work with small containers. For example starting new cultures and inoculating agar slants.

taken. While tissue cultures, done properly, are likely to succeed, some will be contaminated and some will fail to grow. For that reason, one will want to make several tubes from each mushroom.

To make a tissue culture, first take liquid chlorine bleach and dilute it with 10 parts of water to 1 part bleach in a small, clean container. Cut a piece of fresh mushroom about 1/2-1 cm (1/4-1/3 in) square. Drop the piece in the bleach and leave it for one minute. Open a sterilized petri dish, remove the mushroom piece from the bleach and put it in the plate, **Fig. 51**. A pre-sterilized plastic dish is preferred, glass works, but use care when cutting. Trim off the outside of the piece with a sterilized knife. Push the trimmings

78 OYSTER MUSHROOM CULTIVATION



Fig. 53. An autoclave. The steam-pressure vessel, used for sterilization.

aside and cut the remaining piece into cubes of about 2-3 mm. With a sterilized wire loop or with the knife, place the pieces on agar in tubes. Incubate at about 15 to 20°C (59 to 68°F) until substantial mycelium is seen.

Both new culture procedures are best done in a small laminar hood or a glove box, **Fig. 52**. It will not be surprising if your agar shows round glistening spots after it is incubated, if that should happen, discard the tube. You may also see mycelium that is not associated with the tissue cube or the spore, those tubes should also be discarded.

WHAT IS STERILIZATION?

It is very important not to confuse sterilization and pasteurization. Pasteurization is never used in spawn making, but it is generally used for cultivating mushrooms. Sterilization requires a pressure vessel called a retort or autoclave, **Fig. 53**. Sterilization requires a minimum of 121°C (250°F) steam (2 atmospheres pressure) for 20 minutes. That short time is only adequate if nothing contains more than 20 ml of liquid and all glass vessels or other equipment is thin and easily heated. Longer times are necessary for greater volumes of liquid, heavier equipment, and especially for anything solid that occupies a substantial volume. Containers with 200 ml of liquid will require 30 minutes. Grain used as substrate for spawn will require many hours. The exact time will depend on the size and shape of the containers.

Another means of sterilizing is to heat things in a flame. Wires, needles and many other things are heated until they are cherry red. Usually that method is only practical with small metal items and then only to about 3 cm above the area that is actually used. Although they have been sterilized, the openings of tubes and bottles are normally put in a flame as soon as they are opened, kept open for only a few seconds and put in a flame again, just before they are closed. Knives and some other utensils, are generally first dipped in 70% alcohol and then put in a flame, just to ignite the alcohol, and used as soon as the alcohol flame goes out.

AGAR SUBSTRATES

Two formula are often used for agar substrates. One is called **Potato Dextrose Agar** or **PDA**. Cook 200g of peeled potatoes in 1 L of water. Drain and save the water. You are finished with the potatoes, but they are good, so eat them. Make the water back up to 1 L with fresh water add 20 gm dextrose (= glucose – not common sugar), and 18 gm agar. Heat until the agar dissolves. Put into containers, plug and sterilize. Many people will tell you to use only distilled water and laboratory grade agar. Water that is safe to drink after it is

80 OYSTER MUSHROOM CULTIVATION

boiled and food grade agar are generally quite adequate for maintaining cultures. When the containers come out of the autoclave, they are generally laid down (“slanted”) in a manner that allows a maximum surface area for the mycelium.

I favor dilute **Malt Extract Agar**. Unfortunately, commercial dehydrated agar with that label may not even contain malt extract and will be very poor substrate for all fungi. Malt extract is available in places that supply amateur beer makers, as a syrup or occasionally a powder. Add 20 gm of the extract to 1 L of water and 18 gm of agar, heat until the agar is dissolved. Put into containers, plug and sterilize. As with PDA, the containers should be “slanted.”

KEEPING STERILIZED THINGS STERILE

If sterilization is done in a room that is washed daily, especially if chlorine bleach is used to wash it, it is only important to keep the items covered. However, if the room where sterilization is done is not very clean, things should be over-wrapped before they are sterilized and kept wrapped until they are in the laminar hood or other very clean place.

Although the table or bottom of the hood has been kept extremely clean, everything that touches it must be considered contaminated. Of course, that level of contamination is not a problem for the bottom of containers, workers hands or the handles of instruments. However, it is a problem for container covers, the working ends of needles, and other surfaces that will touch mycelium, agar, sterile grain, other substrate, or the inside of sterile vessels. Needles, knives and similar things will be re-sterilized with the flame every time they are used, so they may be laid down between uses, then flamed again.

Even with great care and careful cleaning, so that everything looks very clean, problems may remain. To be certain that everything has been done properly, and that filters are working properly, open agar plates should be placed in the laminar hood or other area, while culture and spawn containers



Fig. 54a. Glass ampules used to store mycelium in a liquid nitrogen refrigerator.



Fig. 54b. Plastic ampules also used to store mycelium in a liquid nitrogen refrigerator.

are open. When the work is completed, the plates should be closed and incubated with the cultures or spawn. Agar plates for this purpose will be petri dishes with a layer of the culture agar (PDA or malt). Any substantial number of colonies on the plates will indicate problems with cleanliness in the work area. Dishes must be labelled so that you will know what they are several days later.

KEEPING THE CULTURES

It will be necessary to have a stock of mycelium to keep a spawn making operation going. The usual method is to grow mycelium in tubes, then to take small samples and put them in a number of sterile ampules (small glass or plastic containers)(**Fig. 54**) with glycerol, seal them by melting the glass at the opening, then putting them into a liquid nitrogen refrigerator, **Figs. 55**. Liquid nitrogen must be added to the refrigerator at regular intervals so that the mycelium remains frozen in the liquid nitrogen. When it is time to prepare for a new batch of spawn, one ampule is removed, it is taken to the laminar hood or other sterile area, the top is broken off, and the contents put on the agar in a fresh, sterile tube. Then that tube is incubated at about 15 to 20°C

82 OYSTER MUSHROOM CULTIVATION



Fig. 55. A liquid nitrogen refrigerator.

(59 to 68°F) until the mycelium is well grown.

Liquid nitrogen refrigeration is now considered the only adequate method to preserve mushroom cultures. Continued re-culturing eventually results in reduced production and other losses in quality of mushrooms. More ordinary refrigeration of cultures at -10°C ($+14^{\circ}\text{F}$) and preferably lower, will allow storage of mycelium for a year or two with little loss in quality.

SPAWN INOCULUM

The mycelium from one tube is used to inoculate several large tubes of sterile agar. The inoculation must be done under the most careful and clean conditions. A wire loop or hook that is flamed to a cherry red and cooled will be used to remove the mycelium from the one tube and put a piece in each of the others. The mouths of the tubes will be flamed just after they are opened and just before they are closed.

There are several possible things that may be done after the large tubes are grown and there are different dangers of contamination associated with each.

1. Mycelium from the large tubes may be placed directly into containers of sterilized grain that will become the final spawn. Since only a small amount of mycelium is used compared to the total grain, growth will be slow and contamination will have time to become established, or even to leak in through the area provided for air.
2. Mycelium from the large tubes can be placed into similar tubes filled with grain. When that grain is thoroughly grown, it will be used to inoculate the containers of grain that will become the final spawn. The final spawn will grow more quickly because of the greater, more similar inoculum and it is easier to be certain that the tubes are adequately sterilized than to be sure that a large container is adequately sterilized. However, it is more difficult to detect contamination on grain, compared to agar and it will go through two growth cycles on grain.
3. Some other material may be used for an intermediate step. One large spawn producer uses a stick coated with a substrate as the inoculum for the final spawn.

STERILIZATION OF GRAIN FOR SPAWN

Rye, milo (grain sorghum), and millet are all commonly used for making spawn. One preparation method: 10 Kg grain, in 15 L water, boil 15 minutes allowed to cool 15 minutes, drain well and stir. 120 gm of gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) and 30 gm. ground limestone (CaCO_3) are mixed in, then the grain is placed in containers and sterilized at 121°C (250°F). The length of time required for sterilization will vary with the size of the containers, and how tightly they are packed. To some degree, you will need to determine the time by trial. One liter bottles containing 350 to 400 gm of the prepared grain will require at least three hours at 121°C (250°F). That is, no time is counted until the vessel is up to pressure and its exhaust thermometer shows 121°C

84 OYSTER MUSHROOM CULTIVATION



Fig. 56. A v-blendor, used by a major spawn maker to sterilize the grain for spawn. The blender rotates while high-pressure steam is put into the blender. Since the grain is kept loose, heat penetrates quickly to sterilize.



Fig. 57. Filling bags with sterile grain from the v-blender (**Fig. 56**).

(250°F). Cooling requires time as well, so one should expect that a minimum of five hours will be required from the time the autoclave is loaded until it is ready to be unloaded. If cooling is too rapid, the plugs or filters, that provide for air to enter the containers, will be blown out or damaged.

86 OYSTER MUSHROOM CULTIVATION

When the material has had a short time to cool, each container should be shaken to loosen the grain. They should be allowed to cool to 20 to 25°C (68 to 77°F) before they are inoculated.

Large spawn producers are able to use expensive and novel sterilizing system. One system is a high pressure steam V-blender, **Fig. 56** It is filled with the grain, then filled with steam. As it rotates every grain is in direct contact with steam, so sterilization is very rapid. The grain leaves the blender by sterile exit tube and goes directly into large, sterile, plastic bags with air fillers sealed in their sides. Inoculum of mushroom mycelium is added and the tops of the bags are heat-sealed.

INCUBATION

Once inoculated the containers should be placed on clean shelves at 15 to 20°C (59 to 68°F). After about one week, they should all be shaken to spread the mycelium evenly through the container. Shaking will speed growth and make the spawn a more even product. It may be wise to shake several times before the spawn is fully grown. If possible, the spawn should be used within a week after all of the grain has turned white with mycelium. If that is not possible, the spawn should be refrigerated at 4 to 5°C (39 to 41°F). Even in a refrigerator, the spawn will be old and weak after 30 days. If the spawn feels light in weight, it is old or it was not properly prepared.

SUMMARY

Spawn making facilities must be completely separate from mushroom growing facilities. Preferably the facilities should be separated by at least three kilometers (2 miles). Spawn making requires expensive equipment and a very high degree of careful work. Many operations required for production of quality spawn can be done at a lower cost per unit of spawn by large spawn producers. Spawn making requires full time work and management. The most important characteristic of an adequate spawn production facility is cleanliness.