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MUSHROOM SPAWN PRODUCTION

MATERIALS

1. **Wide Mouthed Jars** (1 litre)
2. **Pressure Cooker** with **pressure gauge** installed (22 litre – to fit approximately 8 Jars)
3. **Seed** or **Grain** (birdseed or millet seemed to work well)
4. **Cotton Wool** (to filter out contaminants)
5. **Bowls** (to soak grain)
6. **Sieve** and **Ladle** (to rinse and drain grain)
7. **Spoon**, **Knife** and **Fork** (for working with spawn)
8. **Aluminium Foil** (for wrapping jar lids etc in pressure cooker)
9. **For Multiplication: Mother spawn** (Pleurotus ostreatus
   For Agar Tissue Culture: **Scalpel**, **Alcohol Burner**, **Petri Dishes**, **Nutrient Agar** and **Young Mushrooms**
10. **Clean Room or inoculation box**

Step 1: Clean the room or inoculation box

To perform the grain inoculations, you require a sterile environment. Air is full of impurities and so it is important to reduce the level of containments where possible. This can be achieved by constructing a simple clean room or inoculation box

Step 2: clean jars

Preserving jars are ideal for preparing mushroom spawn. You should be able to find 1-2 litre jars at your local preserves outlet (I found using 1 litre jars better for fitting into the pressure cooker/autoclave). If you are unable to buy suitable jars, then recycling old jars is even better (we used 1 litre olive jars) Make sure you clean your jars thoroughly, inside and out.

Step 3; Boiling grains

Boil grains until they become soft (test by biting with teeth) for about 30 minutes help to prevent growth of unwanted moulds. Litre jars should be 3/4 filled with grain and lids (with breathing hole) fitted and capped with aluminium foil.







Step 4: Sterilization




Place the prepared grain jars into the modified pressure cooker, with an aluminum parcel of utensils (spoon, knife & fork) to use during the inoculation steps. Add approximately 3 litres of water, pouring it carefully around the jars. Pressure cook the jars at 15 psi for 60 minutes (if pressure cooker has pressure gauge). If the pressure cooker doesn't retain a vacuum, cover the valve with an alcohol or bleach soaked cloth as it cools. Wash your hands with antibacterial hand-wash and in the clean room remove jars (while still warm), giving them a shake to allow the grain to flow freely. Allow the jars of sterilized grain to cool completely. Place the parcel of sterilized utensils on your work bench for later.

Step 5: Inoculation

5.1: Using Mother spawn

There are a number of methods to inoculate the sterilised grain. One of the most straight forward and successful methods, is grain spawn transfer. You can purchase your initial grain from a known supplier. Spray down the clean room walls with a 1:20 ratio (5%) of bleach to water (It is suggested that a HEPA filter be employed to clean the air, however, this instructable is low tech). Make sure you have showered and are wearing clean clothes. Clean your hands with antibacterial soap or wear sterile gloves. A face mask and hair cap will also help reduce contamination (we are very dirty creatures). Open your spawn bag (or jar) and taking your sterile utensil of preference, break up the grains ready to transfer. Remove the aluminium foil and lid of your jar. Transfer 1-2 desert spoons of the spawn into your 1 litre jar of sterile grain. Quickly, push a small amount of cotton wool through the lid's breathing hole and attach to the jar. Finally, shake the jar vigorously to disperse the grain spawn throughout the jar. Place on a shaded shelf within the clean room to incubate. For pleurotus ostreatus incubate at 24°C (75°F) and for pleurotus pulmonarius (summer) 24°C to 30°C (75°F to 85°F).

5.2; Agar tissue culture transfer

Another method to inoculate your grain, is by first propagating the mushroom tissue on Agar (or cloning). Measure out 5.75 grams of nutrient agar powder to 1 cup of clean water (ample for 5 or more Petri dishes). Begin to heat and stir until the agar is completely dissolved. As it begins to boil, continue to stir for a minute and then remove from the heat. Pour a thin layer into your Petri dishes and cover with lids. Wrap in aluminium foil and pressure cook with your grain (or for at least 30 minutes at 15 psi). Move your Petri dishes, mushroom tissue and other equipment to the clean room. Allow the Petri dishes to cool completely. Spray the clean room walls, benches and floors with 5% bleach solution (as before wear clean clothes, wash your hands etc). A laminar flow bench and hepa filter would reduce

contamination during this stage, but it is possible (with a higher contamination rate) to succeed without one. Taking the mushroom by its base, carefully spit it in two. Place the mushroom (outside down) on to a clean surface, making sure you keep the inside tissue from touching anything. Sterilise the scalpel blade by holding it within the alcohol burner's flame. Lift the lid of the Petri dish and cool the scalpel blade by placing it centrally into your agar. With the scalpel, carefully cut a small square from the newly exposed mushroom tissue. Place the square of tissue centrally into the agar and cover with the Petri dish lid. You may wish to tape the lid (with a clean breathable tape) to reduce the chance of contamination. Repeat the process, making sure to sterilise the scalpel before each transfer. Leave the Petri dishes to incubate.

Incubate for pleurotus ostreatus at 24°C (75°F) and for pleurotus pulmonarius (summer) 24°C to 30°C (75°F to 85During this time, remove any Petri dishes that appears to be contaminated with other moulds. Once fully colonised (mycelium nearing the edges), it is time to transfer the agar to the sterilised grain. Choose only the most healthy cultures for the inoculations. Sterilise the scalpel blade, remove the Petri dish lid and cut 2 wedges from the centre of the dish. Remove the aluminium foil and lid of the grain jar. Using the scalpel, transfer the wedges to the sterile grain. Quickly, push a small amount of cotton wool through the lid's breathing hole and attach to the jar. Finally, shake the jar vigorously to disperse the mycelium from the agar throughout the grain. Place on a shaded shelf within the clean room to incubate (temperatures as above). Repeat for each jar of sterilised grain.

Note: *Cloning repeatedly (without introducing new strains) may lead to replicate fading, with subsequent cultures eventually losing vitality and therefore producing less mushrooms. In contrast to cloning, spores when germinated (see Step 8b), create many different strains that compete with each other. The resulting mushroom characteristics may therefore vary from culture to culture.*°F). Colonisation should take approximately 8 to 10 days.










Step 7: Inspect jars


With mother culture transfer, you should notice popcorn sized colonisation evenly spread throughout the grain after 1-2 days. If needed, give the jars a shake to evenly distribute the mycelium to aid colonisation. Once the mycelium is relatively uniform throughout the jar, leave it to incubate. The grain should be fully colonised with mycelium in seven to ten days.

With agar tissue culture transfer you should notice an initial fine mycelial network, jars may need to be shaken after 4-5 days. Colonisation should be complete by day ten.

Remove any jars showing signs of contamination (such as green mould) from the clean room. Once the jar is fully colonised, you can use it to inoculate more jars, or store it at low temperatures until needed and of course you can use it to inoculate straw substrate to grow mushrooms