Preparation of media and spawn for mushroom cultivation

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Introduction

About 2,000 kinds of edible mushrooms exist worldwide. However, only about 80 of them can be artificially cultured. Mushroom growers have been cultivating several kinds of mushrooms for more than a hundred years in Taiwan, such as white button mushroom, straw mushroom, shiitake, enokitake, and Jew's ear. In recent decades, more newly developed mushrooms such as king oyster mushroom, oyster mushroom, brown swordbelt mushroom, and maitake have been added to the production lists. These cultivable mushrooms can be divided into two types based on their difference of cultured material. One of them is cultured on fermented substrates and the other uses sawdust or natural logs. No matter which type of cultivable mushrooms, the good quality of spawn is important warrant for the success of cultivation.

The spawn preparation procedure

Spawn is similar to seed for a crop. However, spawn is not the spores of a mushroom. Spawn is the mycelia of a mushroom growing on grain or sawdust. Spawn quality is the key factor for a successful mushroom

business. Producing spawns for mushroom production requires isolating the mycelium from the mushroom fruitbody on a potato dextrose agar (PDA) medium in petri dishes or tested tubes. After multiplying the culture by subculture, the strain is incubated among a spawn substrate such as grain or sawdust. The spawn preparation consists of four common stages. Stage 1 is the mother culture isolated from spores, the fruitbody (mushrooms), or the wood log. Stage 2 is strain cultured on PDA medium. Stage 3 is mycelial culture transferred to the spawn substrate. Stage 4 is to multiply the spawn for mushroom production.

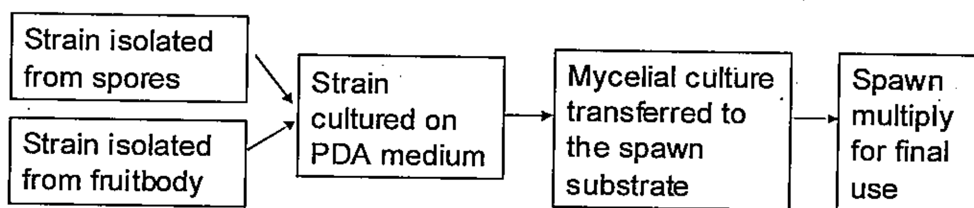


Fig 1. Four stages for making spawn.

Preparation of media suitable for most mushroom species

Preparation of agar slants

Most mushroom species grow on potato dextrose agar (PDA). The ingredients of PDA consist of 200 g diced potato, 20 g agar powder, 20 g dextrose or sugar, and 1 liter of water.

1. Wash and weigh the potatoes.
2. Dice potatoes into small pieces.
3. Boil for about 15 to 20 minutes until potatoes are soft.
4. Remove the potatoes through two layers of cheesecloth and add water to the broth to make exactly 1 liter.
5. Add the dextrose and the agar.
6. Stir occasionally and heat gently until the agar has melted.
7. Pour out the medium into test tubes when the agar is still hot and fill about one fourth of the test tubes (about 10 cc) or bottles.
8. Seal the tubes or bottles with cotton or silica plugs.

9. Sterilize the medium under pressure for 15 min.
10. Place the test tubes or bottles in an inclined position when the agar is still fluid.

Preparation of spawn

Grain spawn

Ingredients: 100% grains (wheat, sorghum, millet), 1% $CaCO_3$

1. Weight and wash the grain.
2. Boil the grain for about 15 to 20 minutes.
3. Cool down and add 1% $CaCO_3$.
4. Fill in bottles and seal with cotton plugs.
5. Sterilize at 121℃ for 40–60 min.
6. Mycelial culture is transferred to sterilized grain and incubate at 24 ℃ until mycelia grown through the substrate.

Sawdust spawn

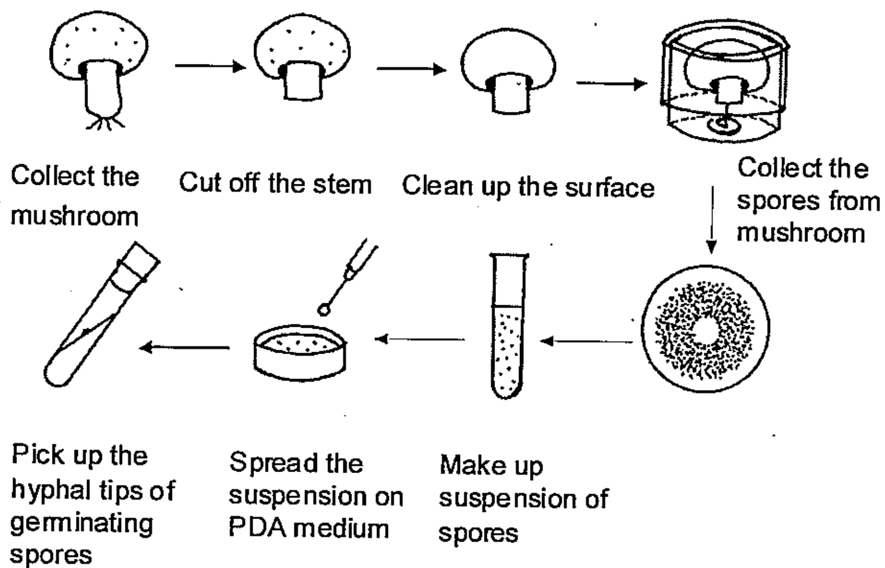
Ingredients: 80% sawdust, 20% rice brain, 1% $CaCO_3$

1. Weight and mix the sawdust, rice brain, and $CaCO_3$.
2. Add water and adjust the moisture content of the substrate to around 60~65%.
3. Fill bottles and seal with cotton plugs.
4. Sterilize at 120℃ for 40–60 min.
5. Mycelial culture is transferred to sterilized sawdust and incubate at 24℃ until mycelia grown through the bottle.

Isolation of mother culture from mushrooms

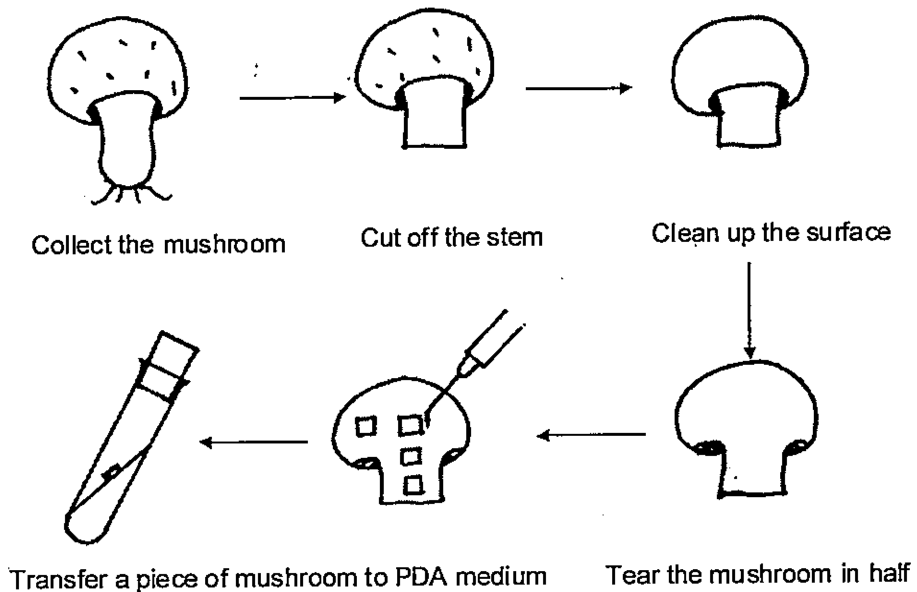
Isolation from spores

1. Collect the mushroom.
2. Cut off the stem and clean up the surface of mushroom.
3. Collect the spores from mushroom.
4. Make up suspension of spores.
5. Spread the suspension on PDA medium.
6. Pick up the hyphal tips of germinating spores.
7. Crossbreed monokaryotic mycelium to obtain a dikaryotic stain (hybrid).



Isolation from fruit body

1. Collect the mushrooms.
2. Cut off the stem and clean up the surface of mushroom.
3. Flame a scalpel until red-hot, then dip it in 95% alcohol for cooling, and flame it again to remove the alcohol.
4. Break the mushroom in half by hand and do not touch the inner surface.
5. Use the heated scalpel to take out a small piece of the inner tissue.
6. Heat the mouth of the tube in the flame and put the tissue in the middle of agar.



Preservation of mother cultures of mushroom

Spawn variations always affect the yield and quality of mushroom production. To prevent the effect of spawn variation, it is important to preserve mother cultures. Several methods have been developed for this purpose, such as subculture, refrigerating in 4℃, -20℃or -80℃, storage in liquid nitrogen, mineral oil, and sterilized water. This practicum includes three simple methods as following.

Preservation with mineral oil on agar slant

1. Subculture on PDA medium and incubate at 25℃ for 7~14 days.
2. Sterilize the mineral oil with autoclave.
3. Put sterilized mineral oil into oven overnight.
4. Add the sterilized mineral oil to agar slant until the cultured is flooded.
5. Store at 16℃.

Preservation with sterilized water

1. Subculture on PDA medium and incubate 25℃ for 7~14 days.
2. Mycelial disks are punched out with a 5-mm cork border form the margin of the colony.
3. Five mycelial disks are put into a vial containing sterilized distilled water.
4. Store at 16℃.

Preservation of stock cultures by deep-freezing

1. Subculture on PDA medium and incubate at 25℃ for 7~14 days.
2. Cut the mycelial colony by 3 X 3 X 3 mm for the agar blocks or with a 5-mm cork border.
3. Place agar blocks onto vial containing 10% sterilized glycerol.
4. Incubate at 25℃ for 2 days.
5. Vials are stored at -20℃, -80℃ or liquid nitrogen.
6. Thaw the frozen culture for 1-3 hr at room temperature before using.