

ISOLATION AND PURIFICATION OF MUSHROOM FOR DEVELOPMENT OF MOTHER CULTURE






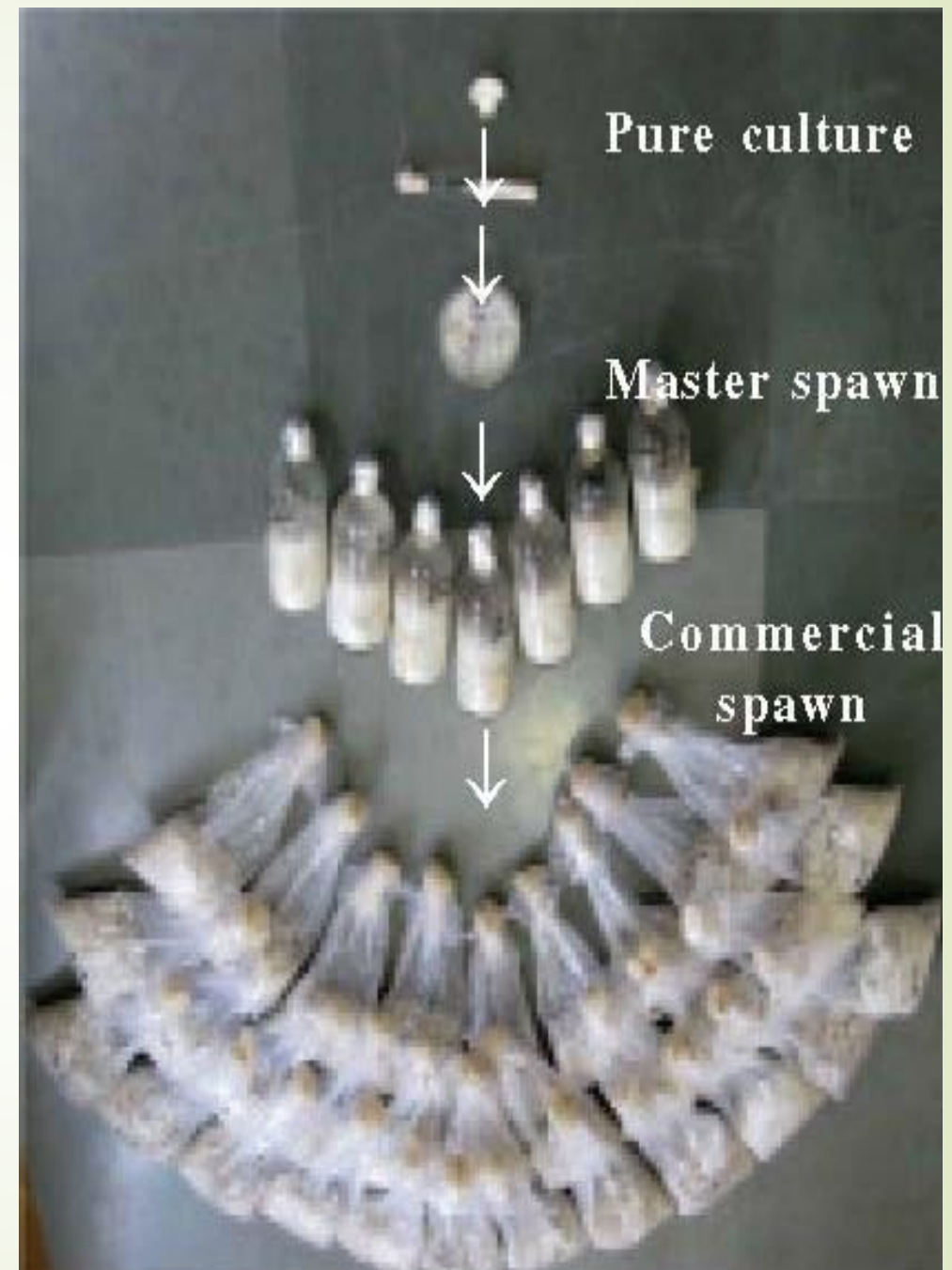
Base spawn / Nucleus culture:

Tissue culture technique is used to bring the edible mushroom to pure culture so that the mushroom fungus can further be used to prepare spawn, which is an essential material for mushroom cultivation. This nucleus culture is grown on Potato Dextrose Agar medium in test tubes. A small tissue from a well-grown mushroom is aseptically transferred to agar medium in a test tube in a culture room. The test tubes are incubated under room temperature for 10 days for full white growth of fungal culture. This is further used for preparation of Mother spawn.

Procedure:

- Select well grown, disease free mushroom early in the morning and keep it on a clean paper for 2-3 hr to get certain amount of moisture present in the mushroom to get evaporated.
- Clean the culture room/ laminar flow chamber with antiseptic solution.
- Keep the sterilized PDA slants, razor blades, forceps etc. inside the chamber and put on the UV light.
- After 20 minutes put off the UV light and start working after 5 minutes.
- Sterilize all the instruments to be used by exposing to Bunsen burner.

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- ❑ Take in the mushroom and split open the mushroom longitudinally into two halves.
 - ❑ Using a new, sterilized blade cut a small piece of tissue from the centre of the split mushroom at the junction of pileus and stipe.
 - ❑ Remove the cotton plug of the agar slant and the tissue is aseptically placed inside the slant by using a sterilized forceps and closes it immediately.
 - ❑ After transferring tissues from the mushroom, the tube are arranged in a wire basket and kept in a clean room at room temperature for the growth of the fungus.
 - ❑ Observe the tube at periodical intervals and remove the contaminated ones. The tubes will be ready for further use within another ten days. The base spawn is used for preparation of mother spawns.





Precautions to be observed:

1. Wash the hands with antiseptic lotion before start working inside the chamber. If possible, it is better to use hand gloves while operation.
2. It is better that the maximum of two persons may work inside the room at a time. Avoid unnecessary talking while working inside the room.
3. While separating the tissue from the centre of the mushroom it should not touch the bottom or sides of the mushroom.




Mother spawns:

Mother spawn is nothing but the mushroom fungus grown on a grain based medium. Well-filled disease- free grains are used as substrate for growing the spawn materials. The various steps involving in preparation of mother spawn are listed below here under.

- Wash the grains in water thoroughly to remove chaffy and damaged grains.
- Cook the grains in an autoclave / vessel for 30 minutes just to soften them.
- Take out the cooked grains and spread evenly over a Hessian cloth on a platform to remove the excess water.
- Mix Calcium carbonate (CaCO_3) thoroughly with the cooked, dried grains @ 20 g / Kg.
- Fill the grains in polypropylene bags up to 3/4th height (approximately 300-330 g / bag), insert a PVC ring , bold the edges of the bag down and plug the mouth tightly with non-absorbent cotton wool.

- ❑ Cover the cotton plug with a piece of waste paper and tie tightly around the neck with a jute thread.
- ❑ Arrange the bags inside an autoclave and sterilize under 20-lbs. pressure for 2 hours.
- ❑ Take out the bags after cooling and keep them inside the culture room and put on the UV light.
- ❑ After 20 minutes put off the UV light and start working in the culture room. Cut the fungal culture into two equal halves using a inoculation needle and transfer one half portion to a bag. Similarly, transfer another half portion of the culture to another bag.
- ❑ Incubate the inoculated bags in a clean room under room temperature for 10 days for further use to prepare bed spawn.



Note: The purpose of mixing Calcium carbonate is i) to remove the excess moisture present in the cooked grains, ii) to neutralize the pH of the grains and iii) to avoid caking of grains after sterilization.

Precaution to be observed:

1. Avoid over cooking of sorghum grains, as over cooking lead to splitting of grains.
2. Don't dry the cooked grains on the floor. Always dry over hessian cloth spread on a raised platform
3. Use only recommended dose of CaCo_3 for mixing with the cooked grains. Mixing over dose reduces the fungal growth in the inoculated bags.



Thank you..