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MUSHROOM CULTURE: MEDIA, PREPARATION AND MAINTENANCE

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1. **A. Media for cultivation of mushroom fungi**

   The pure cultures are raised on a convenient culture medium which is generally in solidified state due to the addition of Agar-agar. In laboratory, the edible mushroom strains may be cultured on different media. The composition of media and the methods of preparation are as given under:

2. **Potato - dextrose Agar medium (PDA)**
   a. Peeled and sliced potato: 250g
   b. Dextrose: 20g
   c. Agar –agar powder: 20g
   d. Water: 1000 ml

   About 250gpotatoes are peeled, cut into small pieces, boiled in water for 25-30 minutes and filtered through a muslin cloth. The volume of the extract is raised to 1000 ml with water and boiled along with dextrose and agar-agar powder so as to get a thoroughly mixed solution. Before pouring in the test tubes or flasks, the pH is adjusted to 7.0 and then after plugging with non-absorbent cotton and sterilization at 15 p.s.i. for 15 – 20 minutes in an autoclave.

3. **Potato -dextrose Yeast Agar Medium (PDYA)**

   Just like preparation of PDA, PDYA can be prepared by adding 2g Yeast extract in the solution for selected fungi.

4. **Malt Extract Agar medium (MEA)**
   a. Malt extract ---- 25g
   b. Agar- agar powder ---- 20g
   c. Distilled water ---- 1000ml

   Malt extract and agar are mixed in 1 litre water and boiled by continuously stirring with a glass rod so as to avoid formation of clumps followed by sterilization at 15 p.s.i. for 15 – 20 minutes in an autoclave

5. **Compost Extract Agar medium (CEA)**
   a. Pasteurized compost ---- 150g
   b. Agar –agar powder ---- 20g
   c. Water ---- 1000ml

   Compost is boiled in 1.5 to 2.0 litre water for few minutes till volume of the water is reduced to half and after filtering through muslin cloth, the volume is again made to 1 litre and autoclaved after mixing agar powder in it and filling in the test tubes.

6. **Malt Peptone Grain Agar Medium (MPGA)**
   a. Malt extract ---- 20g
   b. Rye or Wheat grains ---- 5g
   c. Yeast (Optional) ---- 2g
   d. Agar-agar powder ---- 20g
   e. Peptone ---- 5g (pH -7.0)

   Wheat or rye grains are boiled in water for 1-1.5 hours; the filtrate is mixed with other ingredients and continuously stirred while heating before filling and autoclaving.

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7. Oat meal agar:

a. Oat meal flakes 30g
b. Agar-agar: 20g
c. Distilled water: 1000ml

Cook oatmeal in water for 15 – 30 minutes. Filter through three or four layers of cheesecloth and bring filtrate back to volume with water. Add agar and autoclave it at 15 p.s.i. or 121°C for 15 minutes.

8. Wheat extract agar

a. Wheat grain: 32g
b. Agar-agar powder: 20g
c. Distilled water: 1000ml

Boil 32g wheat grains with 1 litre of distilled water for about 2 hrs and filter after 24 hrs. Bring filtrate back to volume with water. Add agar and autoclave it at 15 p.s.i. or 121°C for 15 minutes.

9. Rice bran decoction medium

a. Rice bran: 200g
b. Agar-agar: 20 g
c. Distilled water: 1000ml

Boil 200g rice bran with 1 litre of distilled water for about 2 hrs and filter it. Bring filtrate back to volume with water. Add agar and autoclave it at 15 p.s.i. or 121°C for 20 minutes.

The pH of the medium adjusted by adding N/10 NaOH or N/10 HCl drop by drop to raise it to 7 or brought down to be adjusted to 7.0, respectively before sterilization. Wheat grain and compost extract are most suitable culture media for *A. bisporus* and *A. bitorquis* cultures. Cultures of *Volvariella spp.* and *Pleurotus spp.* can be maintained on PDA or Malt extract agar medium. It is desirable that cultures are not maintained on the same type of culture medium in each sub-culturing.

B. MUSHROOM CULTURE PREPARATION

Culture isolation: Fresh and healthy mushroom fruit body (basidiocarp) showing all the desirable attributes of that species/strain or their spores are used to raise mycelial cultures by following methods:

1. Vegetative mycelium culture (tissue culture)

Step 1: Cleaning of young basidiocarp with sterilized distilled water and dipping in 2.5% sodium hypochlorite solution for 1 min under aseptic conditions using laminar flow.

Step 2: In case of button mushrooms, the basidiocarp is air dried and split open longitudinally from centre and vegetative mycelial bits are cut from the collar region (junction of pileus and stipe). Whereas, in black ear mushrooms, the ear is cut along the edge with a sterilized scissor and inner tissues are scraped and small bits of tissues are removed.

Step 3: These bits are then washed in sterilized water to remove sodium hypochlorite and placed in oven sterilize Petriplates having culture media.

Step 4: Incubation of inoculated plates at 25°C ± 2°C in a BOD incubator.

Step 5: New mycelium growth over the tissue is observed within 4-5 days.

Step 6: Purification of cultures by carefully transferring young mycelium from growing edge of the colony from Petriplate to test tubes.

Step 7: Incubation of inoculated tubes at 25°C± 2°C for 10-14 days (35°C for *Volvariella volvacea*).

2. Multispore culture

Step 1: Under aseptic conditions, spore mass is scraped from a fresh spore print or basidiocarp and suspended in 100 ml of sterilized distilled water in flasks and shake to obtain uniform spore suspension.

Step 2:A few drops of this suspension is added to lukewarm culture medium and poured into oven sterilize Petriplates. Petriplates are rotated to homogenize the spore suspension into culture medium. The culture medium is allowed to solidify and then Petriplates are inoculated at 25°C± 2°C for 3-4 days (35°C for *Volvariella volvacea*).

Step 3: The spore germination is observed under microscope and germinating spores along with a

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piece of agar are transferred carefully to culture tubes.

Step 4: Incubation of culture tubes of*Agaricus bisporus and A. bitorquis*at 25°C for 10-14 days and *Volvariella volvacea*at 32°C for 7 to 10 days.

3. Single spore culture: *Agaricus bitorquis* and *Pleurotus spp.* are heterothallic with tetraspored basidia, therefore single spore is self-sterile but this technique can be successfully used for breeding new strains. *Agaricusbisporus* being secondary homothallic with bispored basidia and majority of its spores being self-fertile, can be used to raise fertile cultures. Single spore cultures are procured in the same way as that in multispore cultures except serial dilution of spore suspension for single spore culture isolation. Its methodology is given below.

Step 1: Single spore culture isolation: serial dilution of spore suspension to obtain 10-12 spores/petriplate so that individual germinating spore is marked and could be lifted under aseptic conditions.

Step 2: Transfer of above individual germinating spore into culture tubes and its incubation at 25°C for 10-14 days in BOD incubator.

Step 3: Procurement ofpure mycelial cultures followed by their preservation for a particular need.

C. MUSHROOM CULTURE MAINTENANCE AND STORAGE:

There are following methods used for preservation of mushroom cultures.

1. Regular sub-culturing

For storage purposes cultures are prepared on agar slants in culture bottlesor test tubes. These cultures can be stored in racks at room temperatures for one to few weeks. The periods between sub-culturing can be extended up to 46 months by storage at cooler temperatures, i.e., at 4-7°C in a refrigerator or cold room. However, all mushroom species cannot be stored at low temperature. For example,*Ganoderma* and *Volvariella* sp. should be kept at temperature less than 15°C or so. *Volvariella volvacea* is incubated at 32°C for 7 to 10 days. The other mushroom strains are incubated at 25°C for 2-3 weeks until the slants are fully covered with mycelium. *V. volvacea* should be sub-cultured every 2 months. Species of *Lentinula, Pleurotus* and *Agaricus* strains can be kept in a refrigerator at 4°C, and they should be sub-cultured every 6 months.

2. Storage under mineral oil

In this, actively growing mycelial cultures are covered up to 1 cm above the slant level, with the help of mineral oil (liquid Paraffin) sterilized in an autoclave at 121°C for 15 minutes for two consecutive days. Alternatively, 0.5 cm mycelial discs are suspended in 1-2 ml of sterilized liquid paraffin. Covering cultures on agar slants with mineral oil prevents dehydration, slows down metabolic activity and growth through reduced oxygen tension. In conjunction with maintenance of the culture in a refrigerator at 4°C, this is an effective method of preserving fungal cultures. The culture stored in this way remained viable for 8 years.

3. Water storage

The cultures are grown on a suitable culture medium and after full growth, 4-5 bits of 0.5 mm diameter are transferred aseptically to precooled and sterilized McCartney bottles containing demineralized water and the lids tightly screwed down and are stored at room temperature. All mushroom cultures except V. volvacea can be stored by this method. Revival of culture is by removal of a block and placing the mycelium on a suitable growth medium. Survivals of fungal cultures stored this way are reported for 2 to 5 years' period satisfactorily.

4. Lyophilisation (freeze-drying)

It is a method for long-term preservation of spore-bearing fungi. Mycelial mushroom cultures are not well preserved in this way. However, spore collected from a young and

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healthy mushroom aseptically can be stored for several years by this method. In freeze-drying, spore is frozen and water is removed by sublimation. The drying of the spores is accomplished by freezing under reduced pressure in vacuum. Primary drying is achieved at -40°C for 4 hours. Vacuum is released and glass ampoules are stored at - 20°C (or - 70°C). Secondary drying is done in freeze-dryer under vacuum at 20°C for 2 hours. The ampoules are then stored at 4°C to 6°C for longer shelf life inside a refrigerator.

5. Preservation at -70°C

Glycerol (10%) in aqueous solution is sterilized by autoclaving at 121°C for 15 minutes. Alternatively, Dimethyl sulfoxide (DMSO) is sterilized by filtration using 0.22 micron Teflon filter. Usually 10% glycerol suspensions of cultures are made (0.5 ml to 1 ml) and the aliquots are distributed in small vials or tubes. The vials/tubes are placed at -70°C.

6. Cryopreservation in liquid nitrogen

Glycerol (10%) suspension of young mushroom mycelium is prepared and distributed in aliquots of 0.5 ml to 1 ml in plastic screw cap cryo-vials, which can withstand ultra-cold temperature. Programmed cooling at 1°C to 10°C per minute is ideal. In case where programmable freezer is not available, vials are first placed in a mechanical freezer (-70°C) for an hour and then to check viability of a culture before and after freezing. In cryopreservation, the prepared suspension of mushroom mycelium is stored at ultra-low temperatures (-196°C in liquid nitrogen).

7. Granular structure medium

The mycelial viability and the economic properties of mushroom strains can be retained for at least five years, if the mycelium is preserved at 2-4°C on granular structure medium. The ingredients of granular structure culture medium are saw dust or mixed straw powder (72%), wheat powder (20%), soybean powder (5.5%), complex additives (2%) and adhesive (0.5%).

8. Cryopreservation in mechanical freezers

In this method, the cultures are prepared in the same way as for liquid nitrogen preservation and placed first at -20°C and then at -70°C and finally in freezers maintained below -130°C (-140°C or -150°C).

Table 1. Comparison of mushroom culture preservation methods

Method of preservation	Cost		Longevity	Genetic stability
	Material	Labour		
Storage at room temperature	Low	High	4-6 weeks	Variable
Storage in refrigerator	Medium	High	4-6 months	Variable
Storage under oil	Low	Low/medium	4-5 years	Moderate
Storage in water	Low	Low/medium	2-3 years	Moderate
Storage in deep freezer (-70°C)	Medium	Low/medium	4-5 years	Moderate
Freeze-drying of[1]Basidiospore	High	Initially high	20 years	Good/medium
Liquid nitrogen	High	Low	Indefinite	Good[1]
Ultra-low mechanical freezers (-150°C)	High	Low	(-)	(-)

(-) = Ultra low temperature freezers are new. In this longevity & genetic stability of culture yet to be proved that it is superior to other methods of preservation.

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The choice of methods will depend on the requirements of the collection, the equipment and facilities available. Table 1 compares different methods of preservation with regard to costs of materials, labour, longevity and genetic stability. It is recommended that each mushroom strain/isolate should be maintained by at least two different methods. In general, storage in liquid nitrogen and mineral oil preservation technique are best suited for preservation of edible mushrooms. The handling techniques, freezing protocols, cryopreservation and thawing rates can be optimized for a particular strain to obtain maximum survival. Once the mushroom has been successfully frozen and stored in liquid nitrogen, the storage period appears to be indefinite, because no chemical and or physical changes can occur at such low temperatures. The flow chart of mushroom culture preparation and conservation is given below:

<table>
<tr><td colspan="4">Mix 20g malt extract and 20g Agar-agar in 1 lit water</td></tr>
<tr><td colspan="4">▼</td></tr>
<tr><td colspan="4">Boil for 10-15 minutes till agar is dissolved</td></tr>
<tr><td colspan="4">▼</td></tr>
<tr><td colspan="4">Transfer 10ml medium in culture tubes (150x18mm)</td></tr>
<tr><td colspan="4">▼</td></tr>
<tr><td colspan="4">Plug culture tubes with non-absorbent cotton</td></tr>
<tr><td colspan="4">▼</td></tr>
<tr><td colspan="4">Autoclave at 15 p.s.i. for 20 minutes</td></tr>
<tr><td colspan="4">▼</td></tr>
<tr><td colspan="4">Keep autoclaved tubes in inclined position supported by a metallic rod for 1 hr</td></tr>
<tr><td colspan="2">▼</td><td colspan="2">▼</td></tr>
<tr><td colspan="2">Transfer 5mm tissue bit in culture tubes</td><td colspan="2">Transfer 1ml spore suspension (50-100 spores/ml)</td></tr>
<tr><td colspan="4">▼</td></tr>
<tr><td colspan="4">Incubate for 8-10 days at 25 ^{0}C for A. bisporus, Pleurotus spp., L. edodes, Auriculariaspp and 32-35 ^{0}C for G. lucidum, C. indica and V. volvacea</td></tr>
<tr><td colspan="4">▼</td></tr>
<tr><td colspan="4">Culture preservation</td></tr>
<tr><td>▼</td><td>▼</td><td>▼</td><td>▼</td></tr>
<tr><td>±4 ^{0}C in refrigerator — Sub-culturing after 75-90 days</td><td>Liquid paraffin (room temperature) —Sub-culturing after 12 months</td><td>Lyophilization (room temperature) — Long term storage</td><td>Liquid nitrogen (-19 ^{0}C) — Long term storage</td></tr>
</table>

Fig. Flow chart of mushroom culture preparation and conservation/ preservation

REFERENCES

1.Ahlawat, O.P. and Rai, R.D. (2000). *Mushroom Science XV*, 695-699.

2. Aneja, K.R. (2004). Mushroom production technology. In: Experiments in Microbiology Plant Pathology and Biotechnology (Fourth

Date of Acceptance: August 10, 2022 **Published:** October 15' 2022

Edition). *New Age International Limited*, Publishers. New Delhi. pp. 496-519.
3. Chadha, K.L. and Sharma, S.R. (1995). Mushroom research in India: History, Infrastructure and Achievements. *Advances in Horticulture*,**13:** 1-12.
4. Chang, S.T (1991). Cultivated Mushrooms. *In Handbook of Applied Mycology*. Vol.3: Foods and Fees, 221231. Edited by D. K. Arora, K.G. Mukerji and E. H. Marth. New York Marcel Dekker Ink.
5. https://nrcmushroom.org/html/technicalbulletin.html
6. https://www.agrimoon.com/
7. Singh, M., Vijay, B., Kamal, S., Wakchaure, G.C. (2011). Mushrooms: cultivation, marketing and consumption. Directorate of Mushroom Research. ICAR-Chambaghat, Solan –173213 (HP), 266pp
8. Stamets, P. (1994). Permaculture with a mycological twist. The Permaculture Activist. May. p. 8–10.
9. Upadhyay, R.C., Singh, S.K., and Tewari, R.P. (2004). Mushroom spawn production and infrastructure requirements. Technical Bulletin, DMR, Solan. pp. 38.