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Standard Operating Procedure (SOP)

Long-term Storage of Mushroom & Fungal Cultures

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Prepared by:	
Reviewed by:	Approved by:

1. Purpose

To describe a standardized, reproducible, and compliant method for long-term storage of mushroom cultures using multiple storage media, ensuring genetic stability and viability.

2. Scope

This SOP applies to all laboratory personnel involved in cultivation, drying, storage, and preservation of mushroom cultures in research, training, and commercial laboratories.

3. Responsibilities

- laboratory staff: Execute the procedure aseptically and record observations
- lab supervisor/QA: Ensure compliance with SOP and review deviations
- quality manager: Maintain document control and revision history

4. Safety & Biosafety Precautions

- perform all open handling steps inside a certified Laminar Airflow (LAF)
- wear laboratory coat, gloves, mask, and eye protection
- handle DMSO and glycerol with gloves; avoid skin contact
- follow autoclave and hot air oven safety protocols
- dispose of biological waste as per institutional biosafety guidelines

5. Preliminary Planning and Setup

The cultures are intended for **long-term preservation (5–25 years or longer)**. Therefore, **robust forward planning** is essential to ensure sustained viability, genetic stability, and reliable accessibility over time.

The recommended storage strategy is as follows:

5.1. Preparation of Mycelia Suspension

Dispense clean, actively growing mycelial biomass into:

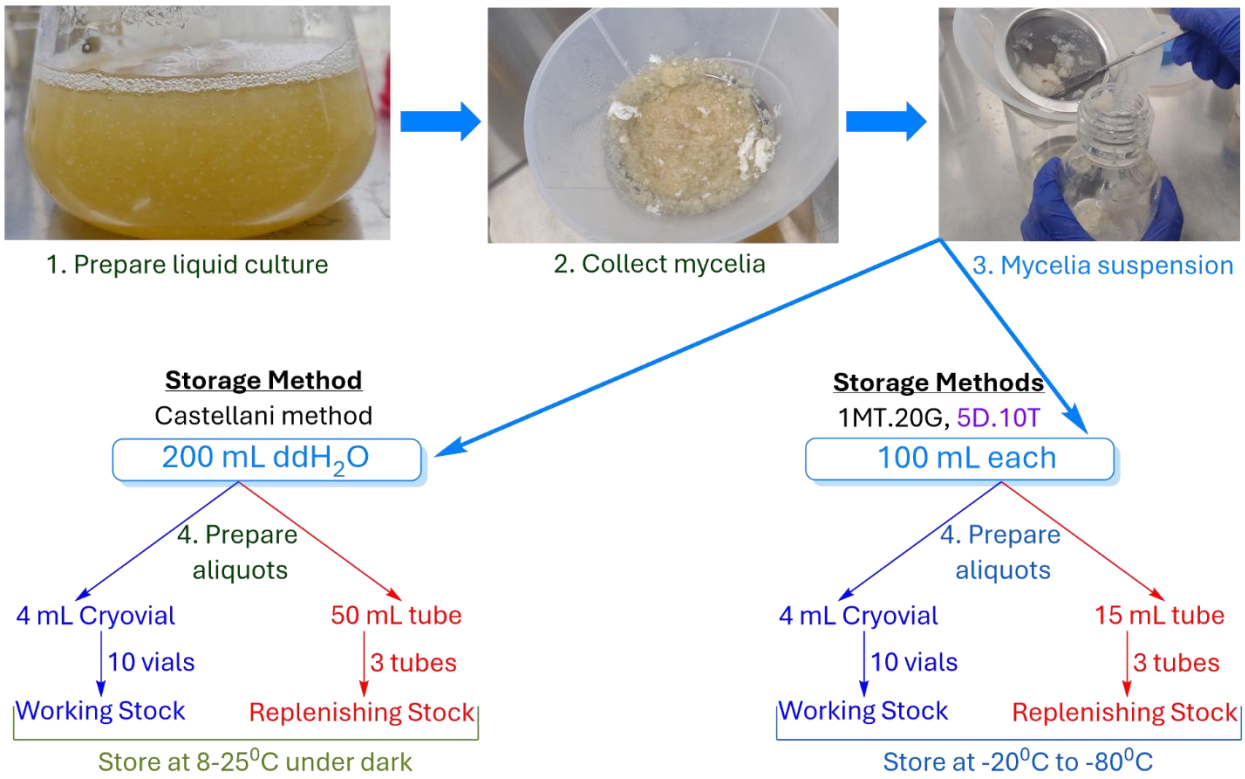
- 100 mL storage media for preservation at −20 °C or below.
- 200 mL sterile distilled water for storage at room temperature

5.2. Aliquoting Strategy

Prepare two categories of aliquots to balance routine use and long-term genetic stability:

- **small-volume aliquots (2–4 mL):**
  Intended for **frequent access and routine operations**, minimizing repeated disturbance of primary stocks.
- **large-volume aliquots:**
  Reserved for **replenishment of small aliquots**, ensuring continuity without reverting to master stocks:
  - 15 mL aliquots for −20 °C storage.
  - 50 mL aliquots for room temperature storage

A workflow is outlined below-



6. Long-term Storage Methods

Among the numerous long-term preservation methods available for mushroom and other fungal cultures, each laboratory should select the method(s) best suited to its available infrastructure, technical expertise, and financial resources. It is important to recognize that no single preservation method is completely fail-safe. Therefore, cultures should always be maintained in multiple preservation formats—preferably two to four independent methods—to minimize the risk of irreversible culture loss due to unforeseen failures associated with any one storage technique. This chapter summarizes four reliable and cost-effective preservation methods that can be readily implemented in most laboratories equipped with only basic facilities, such as a domestic refrigerator, while still providing excellent long-term culture security.

SM01: Filter Paper: The filter-paper storage method is well-established preservation technique by cultivating the fungal mycelia on a filter paper section (FPS) supported by an underneath agar medium. The myceliated FPS is controlled-dried to a final moisture content of 7-10% to minimize fungal metabolic activity. It requires minimal equipment, occupies little storage space, is inexpensive, and consistently provides excellent recovery after prolonged storage (typically 5–25 years) when maintained under appropriate conditions.

SM02: Castellani Method: One of the most widely accepted low-cost methods for long-term fungal preservation. Storage in sterile distilled water effectively suppresses metabolism while maintaining high culture viability for many fungal species for up to 25 years. The method is simple, reproducible, and requires no specialized cryopreservation equipment.

SM03: 1M Trehalose + 20% Glycerol: Trehalose stabilizes cellular membranes and proteins, while glycerol functions as a cryoprotectant during freezing. This combination provides good long-term preservation but requires careful preparation, freezing, and thawing to achieve consistent recovery. Performance is generally species-dependent and less robust than filter paper or Castellani preservation.

SM04: 5% DMSO + 10% Trehalose: DMSO acts as a penetrating cryoprotectant and trehalose provides membrane stabilization, together reducing freezing injury. Although effective for preserving viable cultures, DMSO must be handled carefully because of its cytotoxicity at room temperature and the method demands stricter preparation and handling procedures, making it less practical for routine long-term culture maintenance.

Name	Composition	Notated as	Storage period with good recovery
SM01	Filter paper	FP	10 – 20 years ★★★★★
SM02	Double-distilled water	Castellani method	20 – 25 years

			★★★★★
SM03	1M Trehalose + 20% Glycerol	1MT.20G	>5 years ★★★☆☆
SM04	5% DMSO + 10% Trehalose	5D.10T	>5 years ★★★☆☆

Ratings represent the relative suitability of each preservation method for long-term fungal culture storage, considering ease of implementation, expected culture viability, reliability, and supporting literature/experimental observations. They are not direct measurements of culture viability.

6.1. Chemicals: Double-distilled water, Trehalose, Glycerol, Dimethyl sulfoxide (DMSO).

6.2. Equipment: Autoclave, Laminar Air Flow hood, Weighing balance, Rotary shaker, Desiccator, hot air oven, etc.

6.3. Labware and Consumables:

SS304 Sieve	To filter and collect mycelia from liquid culture, pore size 1 mm
Funnel	To hold the SS304 sieve during filtration. Note that the funnel shall be sufficiently large to hold the SS304 sieve in it. Prefer 150 mm polypropylene funnel.
Tall-form Beaker Reagent bottle Conical flask	To collect the filtrate coming down through the sieve. A sterile tall-form beaker or conical flask or reagent bottle or similar containers work well. Ensure these containers are large enough to hold 2X volume of the liquid culture.
Other Glassware	Reagent bottles, standard volumetric flask, conical flask, Petri dish, etc.
Spatula	Long SS304 or polypropylene spatula or spoon to transfer mycelia from SS304 sieve to storage media bottles to prepare mycelia suspension. Prefer a spatula with spoon size of 5-15 mm. Also, prefer long-handle spatula for transfer to minimize risks of contamination while transferring mycelia.
Cryovials	To make 'Working Stock' aliquots. The size of cryovial may depend on choice and availability of the lab. 1.5 mL, 2 mL, and 4 mL cryovials are most common.
Centrifuge tubes	To prepare 'Replenishing Stock' aliquots. 15 mL polypropylene centrifuge tubes for–20^{0}C aliquots. 50 mL polypropylene centrifuge tubes for room temp. aliquots.
Micropipette	To dispense mycelia suspension from storage bottles to cryovials and centrifuge tubes. Prefer FULLY AUTOCLAVABLE, variable volume micropipettes of 10 mL capacity for faster aliquoting.

Microtips	Cut the first 3-5 mm of the tip of the micropipettes
Filter paper	Cellulose filter paper, Whatman filter paper No. 1 Prepare Filter Paper Section (FPS) of size 15-25 mm. Matrix for growth for fungal mycelia
Forceps	Sterile forceps to transfer filter papers through Petri dish(es)
Borosilicate Petri dish	To sterilize filter papers
Silica gel desiccant	For drying myceliated filter papers

6.4. Advantages

- Scalable & efficient **–** Easy to prepare and scale; one 500 mL liquid culture media in 1 liter conical flask equals dozens of agar plates.
- Agar-free pure mycelia – Harvests clean mycelial biomass, unlike agar plates (mycelia + agar).
- Bulk availability – Enables easy aliquoting into multiple sterile storage vials.
- Faster biomass generation – Rapid mycelial growth compared to solid media.
- Better genetic stability – Reduced sectoring and senescence seen in aging agar plates.
- Lower contamination risk – Fewer transfers and less surface exposure.
- Ideal for cold & cryostorage – Compatible with glycerol and long-term storage at 4 °C to −80 °C.

7. Preparation of Storage Media

7.1. SM01: Filter Paper: Discussed below as a separate Section

7.2. SM02: Castellani's method (in double-distilled water)

It is the cheapest and easiest method of preservation of mushroom cultures at room temperature. Use double-distilled water for mycelia storage.

Autoclave sterilize double-distilled water. Cool it to room temperature before use.

7.3. SM03: 1 Molar Trehalose + 20% Glycerol

Calculation:

Required volume of solution = 100 mL

Required quantity of Trehalose = Molarity x Molar mass x Volume of soln.

= 1.0 moles L-1 x 342.30 g mol^{-1} x 0.100 L

= 34.23 g

Using $(C_1V_1)_{\text{stock solution}} = (C_2V_2)_{\text{Final solution}}$

Now, required quantity of Glycerol, $V_1 = (C_2V_2) / C_1$

Where, C_1 = [Glycerol] in the original solution, 100%
V_1 = Volume of the original glycerol solution
C_2 = [Glycerol] in the final solution, 20%
V_2 = Volume of the final glycerol solution, 100 mL
Or V_1 = (20% x 100 mL) / 100%
Hence, V_1 = 20 mL

Taking density of glycerol to be 1.261 g mL^{-1}, the weight of 20 mL glycerol equals to 25.22 g.

Preparation: To a small amount of water taken in a 100-mL standard volumetric flask, add 34.23 g of Trehalose, dissolve well. Add 20.0 mL glycerol (or weigh 25.22 g) and mix well. Make the final volume up to the mark with double-distilled water. Mix well to get a homogenous solution.

7.4. SM04: 5% DMSO + 10% Trehalose

Calculation:

Required volume of solution = 100 mL

Using $(C_1V_1)_{\text{stock solution}} = (C_2V_2)_{\text{Final solution}}$

Required quantity of DMSO, $V_1 = (C_2V_2) / C_1$

Or V_1 = (5% x 100 mL) / 100%
Hence, V^1 = 5 mL

Required quantity of Trehalose, $W_1 = (C_2W_2) / C_1$
Where, W = weight of the solute
Or W_1 = (10% x 100 g) / 100%
Hence, W_1 = 10 g

Preparation: To a small amount of water taken in a 100-mL standard volumetric flask, add 10.0 g of Trehalose, dissolve well. Add 5.0 mL DMSO and mix well. Make the final volume up to the mark with double-distilled water. Mix well to get a homogenous solution.

Note: *About 0.001% of DMSO undergoes thermal decomposition per day at 120 °C. For a 30-minute autoclave cycle, the estimated decomposition is (30/1440) × 0 001% = 0.00002%. This level of decomposition is negligible. Therefore, a 5% DMSO solution may be autoclave sterilized under standard conditions without any meaningful loss of cryoprotective performance. Autoclaving the 5D.10T formulation enables one-step preparation without requiring the slower and more labor-intensive sterile membrane filtration of DMSO and subsequent mixing with the sterile storage component.*

Ref: https://www.gchemglobal.com/resources/dmso-university/dmso-physical-properties/

8. Autoclave sterilization of Labware, Media and Other Necessities

Steam sterilization, i.e. autoclave sterilization, is effective only when sufficient steam is generated within the vessel during autoclaving. Unlike culture media, vials, tubes, caps, micropipettes, tips, etc. are not in liquid form; therefore, sufficient water must be present in the container holding them. This ensures enough steam is produced to fully saturate their surfaces, enabling efficient and uniform sterilization during the autoclave cycle.

8.1. Preparing Cryovials and Microcentrifuge tubes for Sterilization: Separate the body and cap of the cryovials and place them in separate containers (e.g. appropriately sized beakers). Add 20–50 mL of double-distilled water to each container. Cover the mouth of the containers using an inverted half of a Petri dish or a suitable polypropylene cover.

For microcentrifuge tubes, keep the caps open and place them in a suitable beaker with a small volume of double-distilled water. Cover the container using an inverted half of a Petri dish or a polypropylene cover.

8.2. Preparing Centrifuge tubes for Sterilization: Separate the body and caps of the centrifuge tubes.

- Place the tube bodies in a suitable centrifuge tube rack (if available) or in an appropriately sized beaker.
- Place the caps in a separate beaker.

If using a rack, add 5 mL (in 15 mL tube) and 10 mL (in 50 mL tube) of double-distilled water to each tube. Loosely place the caps on tube after adding water. If stacking tubes in a beaker, add ~50 mL of double-distilled water to the beaker.

Close the rack lid (if applicable) and cover the containers using an inverted half of a Petri dish or a polypropylene cover.

8.3. Preparing Micropipette for Sterilization: Refer to the manufacturer's specifications and user manual before sterilization. Only fully autoclavable micropipettes should be autoclaved. **Do NOT autoclave a non-autoclavable micropipette.**

Place micropipettes and tips in separate beakers or suitable containers. Add 20–50 mL of double-distilled water to each container. Cover the containers using an inverted half of a Petri dish or polypropylene film.

8.4. Preparing storage media and water for sterilization:

- dispense 100 mL of storage media (1MT.20G and 5D.10T) into separate 250 mL borosilicate (3.3) reagent bottles or any other suitable vessel as available.
- dispense 200 mL of double-distilled water into 250 mL reagent bottles.

- prepare few extra bottles with double-distilled water for rinsing collected mycelia on the SS304 sieves.

Loosely fit the caps of all reagent bottles to allow pressure equilibration during sterilization.

8.5. Sterilization Process: Place all prepared items in the autoclave, ensuring sufficient space for proper steam circulation.

Sterilize at 121°C for 15 minutes under standard autoclaving conditions.

9. Procedure

9.1: Cultivating Mycelia

I. Prepare 1 Liter of liquid culture medium using the following formulation optimized for *Cordyceps militaris*: 20 g dextrose, 0.70 g Soyatone, 1 g yeast extract, 0.57 g $MgSO_4.7H_2O$ and 0.57 g Celtic salt.
II. Dispense the media in conical flasks up to half-the capacity of the flask. Insert an SS 304 mesh roll in it. Sterilize at 121 °C for 15 minutes**,** then allow to cool to room temperature (RT).
III. Inoculate aseptically under a laminar airflow (LAF) cabinet.
IV. Incubate at 25 °C at 150 rpm to obtain luxurious mycelia density.

9.2. Collecting Mycelia: *Carry out this whole process aseptically under a pre-cleaned LAF. Everything contacting the mycelia in all forms must be sterilized and cooled before use.*

I. Harvest the mycelial biomass by filtering the liquid culture through a sterile SS 304 strainer. *Note: Cover the SS304 sieve with a sterile, half-Petri dish all the time except during handling to minimize direct exposure of LAF airflow and possibilities of contamination.*
II. Wash the mycelia on the strainer 2–3 times with sterile and cold double-distilled water at an interval of 10-15 minutes.
III. After the final wash, let the excess water drip off due to gravity to obtain mycelia with minimal quantity of water in it.
IV. Though water does not exert any adverse effect, its minimal quantity helps obtaining higher mycelia yield per unit volume of collected mycelia.

NOTE: Since all storage media are prepared in water, presence of little water in harvested mycelia is acceptable without seeking a dry media yield.

9.3. Preparing mycelia suspension: *Carry out this whole process aseptically under a pre-cleaned LAF. Everything contacting the mycelia in all forms must be sterilized and cooled before use.*

Mycelia can be suspended in 10-100X volume of storage media depending on its availability.

I. Roughly divide the collected mycelia into four roughly equal volumes. No need to place in four different containers, just make four parts in the sieve itself using spatula.

I. Transfer two parts to 200 mL distilled water, one part to 100 mL 1MT.20G and one part to 100 mL 5D.10T. [*Note: Labs can proceed with one or more long-term storage formats as required.*]
II. Tightly close the cap of the storage media bottles. Gently shake and swirl to mix well.
III. Allow the mycelial suspension to stand for 1–2 days. Swirl the suspension intermittently during this period to facilitate diffusion of the cryoprotectants into the mycelial biomass.

At this stage, the mycelia suspension is ready to be dispensed in aliquots.

9.4. Preparing Working and Replenishing Aliquots: *Carry out this whole process aseptically under a pre-cleaned LAF. Everything contacting the mycelia in all forms must be sterilized and cooled before use.*

Start with a single mycelia suspension at a time to keep things tidy and prevent mixing up tubes. Prepare both working and replenishing stocks before proceeding to another one. Adopt aseptic parameters like using gloves, opening the Laminar Air Flow (LAF) cabinet door only at minimum height (6-8 inches from base), etc.

I. Aseptically decant water from the beaker with cryovials, if any.
II. Mix the mycelia suspension by gentle reverse pipetting using sterile micropipette and cut tips. Remember to mix well before dispensing into every aliquot.
III. Dispense mycelia suspension to fill 3/4th volume of the cryovial. Secure its mouth with cap.
IV. Dispense mycelia in the remaining vials, a total of 10 vials is sufficient. (It is totally the choice of the lab to determine these numbers and proceed so).

The process remains the same for working aliquots of long-term storage formats (Castellani method, 1MT.20G and 5D.10T). The remaining media will be used to prepare replenishing aliquots.

V. Dispense 10 mL mycelia suspension to respective 15-mL centrifuge tubes. Secure the tubes with sterile caps. It shall make around 6-7 tubes each bottle.
VI. Dispense 40 mL mycelia suspension from sterile water bottle to 50-mL centrifuge tubes. Secure the tubes with sterile caps. It shall give around 3-4 tubes each bottle.

9.5. Labelling:

Since these cryovials and tubes are intended for long-term storage over several years, it is essential to use high-quality labels–preferably printed–that are resistant to ethanol and other solvents, and capable of withstanding freezing conditions without degradation.

I. Ensure the cap is tightly secured. Clean the tubes with a clean wipe or tissue paper to remove any residue of water and storage media.
II. Fix the label bearing *Genus-species*, culture *ID.*, storage media and date. The label can also be upgraded with QR or bar code and other details as per lab need.
III. Also label the container or boxes containing these cryovials and tubes for easy identification without opening the container.

9.6. Storage:

I. Store 1MT.20G and 5D.10T vials and tubes at–20^{0}C to–80^{0}C as available.

II. Store Castellani method vials and tubes at 8-25^{0}C under dark.

10. Culture Preservation on Filter Paper

Long-term culture preservation of fungal mycelia on filter papers has been in use for decades. Affordable yet efficient, this method can also be extended to mushrooms including *Cordyceps militaris*.

10.1. Preparation of agar plate with filter paper

I. Put the required number of cellulose FPS of size 15-25 mm (circle or square as suited) in a borosilicate glass Petri dish. *Note: At this stage, the FPS can be stacked over each other for bulk sterilization.*

II. Carefully wrap the Petri dish in a porous cellulose paper.

III. Sterilize in hot air oven at 160^{0}C for 1 hour. Let it cool to RT. *Note: sterilization at higher temperature may lead to browning of FPS.*

V. Aseptically transfer 4-8 sterile FPS to a new agar plate using sterile forceps under laminar air flow cabinet. *Note: The FPSs in an agar plate shall not overlap.*

10.2. Growing fungal mycelia on filter paper

I. Place a small inoculum at the center of each FPS. A 100-200 mL of liquid culture or 5–8 mm agar culture disk is sufficient as inoculum.

Note: A 100-200 mL of liquid culture as inoculum yields a faster and uniform mycelia growth because of spontaneous spreading of the inoculum over FPS at the time of inoculation. Fungal mycelia almost evenly appear on the FPS during incubation period and thickens over time.

The agar disk inoculum grows outwardly toward the periphery. This localized growth yields a relatively slower growth rate compared to the liquid culture inoculum. Moreover, the

inoculum often appears as a mycelium bump compared to a uniform flattened growth with liquid inoculum.

II. Seal the Petri dish with parafilm by stretching it well. Stretching a parafilm creates some micropores (like larger inter-weaving pores when a sheet of cloth is stretched). These micropores facilitate gaseous exchange while preventing loss of moisture and contamination.

III. Incubate at 25^{0}C under dark for 2-3 weeks till the FPS is fully covered with fungal mycelia.

10.3. Preparing the desiccator

I. Borosilicate Desiccator with or without vacuum facility: A 5-lilter vacuum desiccator costing around ₹ 5, 000 ($ 50) is usually sufficient for small batches. Clean the borosilicate desiccator with or without a vacuum facility with soap water, potable water and air dry it. Place some silica gel desiccator granules at its bottom. Sterilize the whole setup at 160^{0}C for 1 hour in hot air oven. Let it cool to RT. This setup is ready for drying myceliated FPS.

Note: Vacuum desiccator speeds up drying of myceliated filter paper but requires an additional investment in the vacuum pump, preferably oil-free vacuum pump costing around ₹ 40, 000 ($ 400) or above. Uncontrolled vacuum drying may cause rapid drying of the mycelia and may subsequently cause loss of viability. Moreover, high vacuums may also cause cracks and breakage of the glass desiccator.

A normal desiccator without vacuum facility also works perfectly fine but may take additional 1-2 days to complete the drying process while minimizing the possibility of rapid drying of mycelia. Moreover, it also saves around ₹ 40, 000 ($ 400) by eliminating the need for a vacuum pump.

II. Polycarbonate Desiccator with or without Vacuum facility: Clean a polycarbonate vacuum desiccator (with or without a vacuum facility) with soap water, potable water and air dry it. Wipe the whole surface with 70% isopropyl alcohol under LAF, let it air dry for few minutes. Place the inner surface of the two halves of the desiccator exposed to the UV light of a Laminar Air Flow (LAF) cabinet for 30 minutes.

Aseptically place some sterile silica gel (sterilized at 160^{0}C for 1 hour) at its bottom. This setup is ready for drying myceliated FPS.

10.4. Final moisture content in the dried, myceliated FPS

The final moisture content of 7-10% in the dried, myceliated FPS is crucial for its prolonged viability during storage at non-freezing (4 - 25^{0}C) and freezing temperatures (-20 to –80^{0}C). A lower moisture content, say 2%, may exhibit rapid loss of viability. A higher moisture

content, say 15%, is inefficient in inactivating metabolic activities of the fungal mycelia. The metabolically active fungal mycelia often exhibit gradual viability loss during prolonged storage at non-freezing temperatures after 1 year. Formation of intracellular ice-crystals in metabolically active mycelia (with moisture content > 12%) at sub-zero temperatures also reduces its viability in a few months.

10.5. Water adsorption capacity of Silica gel desiccant

At ambient 50-70% relatively humidity (RH), silica gel adsorbs around 30-35% of water by its weight. That is, a 100 grams sample of dried silica gel granules adsorbs around 30-35 grams of water at ambient RH of 50-70%. The amount of water adsorbed is directly proportional to ambient RH in the desiccator. For example, a 100-gram sample adsorbs around 15% water and 40% water of its dry weight at RH 20% and 80%, respectively.

10.6. Controlled drying of the myceliated FPS

Following sections 10.4 and 10.5, the amount of dry silica gel becomes crucial to dry myceliated FPS to a final moisture content of 7-10%. The controlled drying process is recommended for prolonged viability of the myceliated FPS.

For simplicity of calculations, we proceed with a 5-liter desiccator with 10 FPS, weighing 2 grams each.

10.6.1. Calculating water content of 5 Liter air: Saturated air holds around 23.0 grams water per cubic meter volume at 25^0C. Assuming a 60% ambient RH condition, the amount of water in a 5-liter desiccator is equal to – 60% of (23.0 g / 1000 L) x 5 L = 0.069 g = 69 mg

This water content of 69 mg already present in the desiccator appears significantly small compared to the weight of myceliated FPS. However, the humidity content of air may become significantly high while using larger volume desiccator.

10.6.2. Calculating water content of FPS: The weight of a dry filter paper section may vary depending on the quality and manufacturer. A cellulose paper may absorb 2–3 times water of its weight. A 1” x 1” dry FPS weighs around 65 mg with soaked weight of 143 mg.

The weight of mycelia per unit area is technically difficult to predict as the colony characteristics exhibit huge variations among species, culture media and incubation conditions.

For the simplicity of calculations, let the assumed weight of a myceliated FPS is 2.0 grams, with fresh mycelia weight accounting up to around 85-90% weight of the whole FPS. For a five-set sample of FPS, the total wet weight of the FPS sums up to 10 grams.

10.6.3. The required quantity of Silica gel Desiccant: Calculating the exact weight of dry silica gel desiccant to obtain a final moisture content of 7-10 in the dried FPS is practically infeasible because of inherited variation in weight of moisture in air, dried FPS and mycelia growing over it. However, any closer approximation becomes crucial to prevent excess and under drying of mycelia to preserve its prolonged viability.

Following section 10.5, silica gel adsorbs around 30-35% of water by its weight. The required amount of silica gel to dry 10 grams of fresh FPS is around 28 – 33 grams. Start so with smaller quantity of silica gel to minimize the chances of excess drying.

10.6.4. Drying the myceliated FPS

I. Aseptically transfer the fully colonized FPS from the agar plate to a sterile Petri dish using sterile forceps under Laminar Air Flow (LAF) cabinet.

II. Aseptically transfer the FPS loaded Petri dish to a sterile vacuum desiccator with around 28 – 33 grams silica gel per 10 grams fresh FPS.

Note: Weighing fresh FPS under sterile conditions is practically difficult. To keep it simple, use 28-33 grams dry silica gel per 5-7 fresh FPS.

III. Seal the gap between the two halves of the desiccator with cellophane tape.

Note: Cellophane tape is usually sterile because of its manufacturing process. Since it does never directly contact the fungal mycelia, it's assumed to be sterile for the purpose. Sealing the desiccator with cellophane tape ensures prevention of entry of environmental air as well as accidental dislodging of the lid.

IV. Let the myceliated FPS dry for 3-4 days. The FPS may gradually curl over the drying period, a natural process not to worry about.

V. A dried FPS sample can also be dried at 105^0C to test its residual moisture content. If needed, a small quantity of silica gel can be placed in the desiccator and dry it for another 1-2 days.

The finally dried FPS with moisture content of 7-10% is ready for storage.

10.6.5. Storage of dried mycelia on FPS:

I. Aseptically transfer few (3-5 or as feasible) myceliated FPS to a dry sterile cryovial, glass vial or centrifuge tube.
II. Tightly secure the cap. Seal the cap with Petri-seal (preferred) or parafilm (minimal stretching).

III. Affix identity label with species name, strain, batch number, date, QR code, etc. as feasible.
IV. The dried mycelia on FPS can safely be stored at room temperature, 4-8^{0}C, –20^{0}C and -80^{0}C for years.
However, periodic viability test is crucial to assess the storage period at specific temperature for the specific fungal species.

11. Culture Revival

I. In a pre-cleaned LAF hood, clean the outer surface of culture vial with 70% ethanol.
II. Thaw the vial by briefly dipping in a beaker with sterile water at 25-30^{0}C. - (Note: For frozen stocks SM03 and SM04, do not fully thaw the vial; clean the surface and scrape the required µL directly from the semi-solid/viscous block to prevent freeze-thaw degradation).
III. Revive the cultures as follow-

a. aseptically scratch a bit of mycelia from FPS (SM01) using sterile forceps or needle and transfer to a suitable culture plate.

b. aseptically transfer 100-200 mL of liquid inoculum (SM02- SM04) to a suitable culture media.

IV. Seal the Petri dish with a stretched parafilm strip.
V. Incubate under dark at 25^{0}C for 2-4 weeks.

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