Experiment-3

SUBCULTURE TECHNIQUES FOR PURIFICATION OF FUNGI

Primary fungal cultures often result in **mixed growth**. To isolate and purify a specific fungus, **subculturing techniques** are applied. These methods allow transfer of selected fungal parts (e.g., spores or hyphae) to fresh medium for **pure culture development**. The techniques used are:

- **A. Spotting**
- **B. Plug Inoculation**
- **C. Streaking**

Material

Common Materials for All Techniques

1. **Sabouraud Dextrose Agar (SDA) plates**
2. **Primary culture plate or old growth**
3. **Incubator (25±3°C for molds, 37°C for yeasts)**
4. **Parafilm or tape for sealing plates**
5. **Marker for labeling plates**
6. **Gloves, lab coat, and sterile workspace**

A. Spotting

7. Inoculating needle (platinum, aluminum, silver/iron types)
8. 70% ethanol for sterilization
9. Spirit lamp or Bunsen burner

B. Plug Inoculation

10. Sterile scalpel (optional)
11. Well borer (cork borer)
12. Forceps (optional)

C. Streaking

13. Inoculating loop
14. Loop sterilizer or spirit lamp

A. Spotting Technique

Used for filamentous fungi (molds)

Two variations:

- **Single Spot** – One central inoculation point
- **Multi-Spot** – Three points in triangular form

Single Spot Technique – Procedure

1. Sterilize the inoculating needle:
    - If **platinum**: heat until red-hot.
    - If **aluminum** or **silver/iron**: dip in ethanol, then briefly flame.
2. Touch the sterile needle to a **colony from primary/old culture**.
3. Gently place it in the **center of a fresh SDA plate**.
4. Incubate upright at **22–28°C for 3–5 days**.

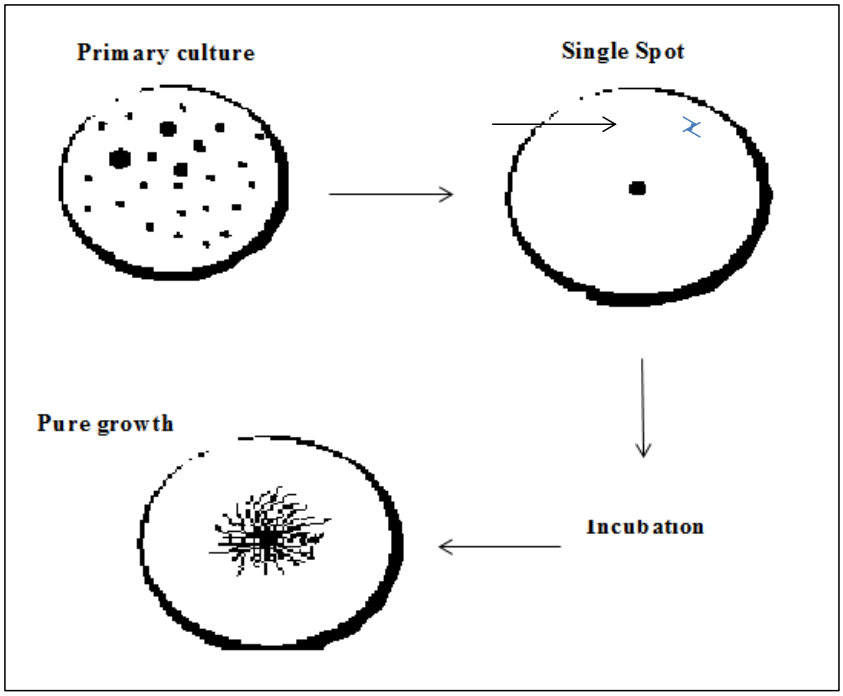

Figure 3.1: Subculture by single spot technique

Multi-Spot Technique – Procedure

1. Sterilize needle as above.
2. Touch a colony from the primary culture.
3. Gently spot the spores onto **three points** on a fresh SDA plate in **triangle formation**, rotating the needle slightly at each point.
4. Incubate at **22–28°C for 3–5 days**.

💡 *Multi-spot technique provides better distribution of nutrients and reduces contamination risk compared to single spotting.*

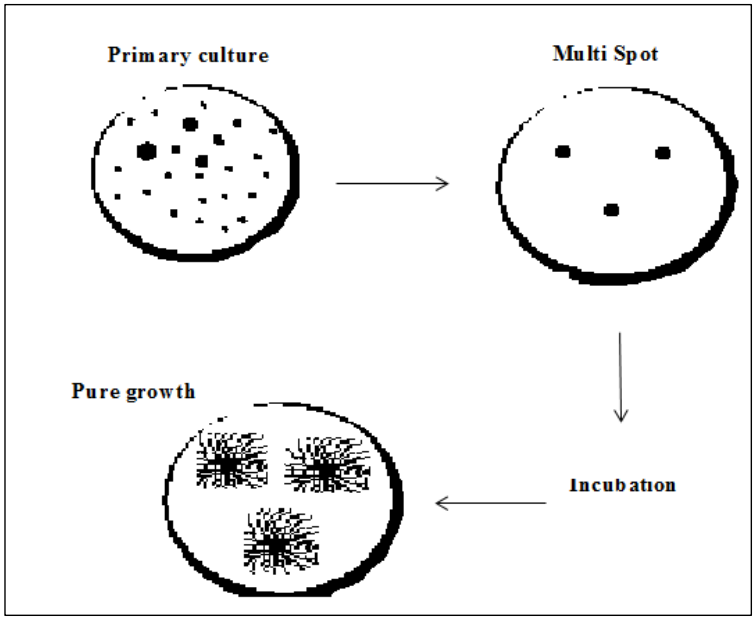

Figure 3.2: Subculture by multi spot technique

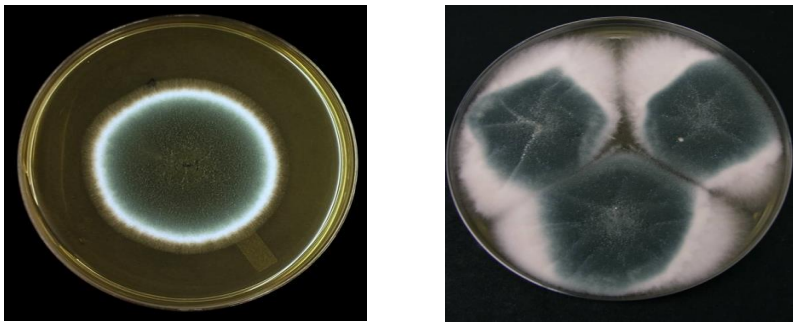

Figure 3.3: Single spot of mold of left side and multispot of mold on right side

B. Plug Inoculation

Used for transferring hyphal/mycelial plugs of molds

Procedure

1. Sterilize the **well borer** by dipping in 70% ethanol, then flame to evaporate.
2. Press the sterile borer into a **primary culture plate** to extract a **mycelial plug**.
3. Transfer the plug to the **center of a fresh SDA plate**.
4. Gently press the plug to ensure contact with the medium.
5. Incubate at **25±3°C for 3–5 days**.

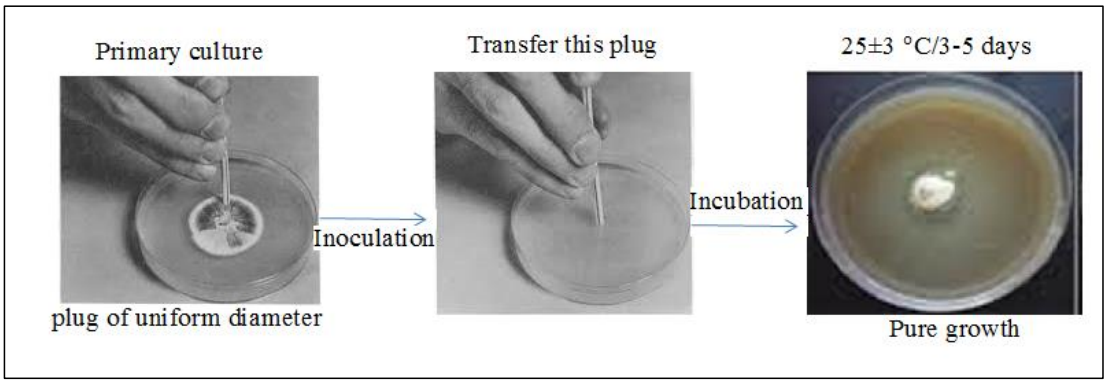

Figure 3.4: Agar plug inoculation for mold

C. Streaking Technique

Used for yeast purification

Types of streaking:

- One-way
- Two-way
- Three-way
- **Four-way (recommended)**

Procedure

1. Sterilize the **inoculating loop** and allow it to cool.
2. Touch a colony of yeast from primary/old culture.
3. Perform **four-way streaking** on an SDA plate for isolation.
4. Incubate **inverted at 37°C for 24–48 hours**.

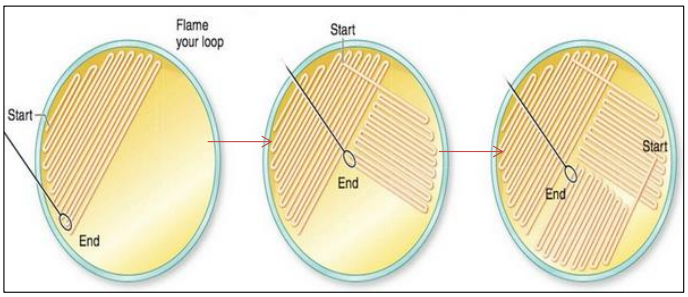

Figure 3.5: Streaking for sub-culture of yeast

Figure 3.6: Yeast growth on SDA

PRESERVATION AND REVIVAL OF FUNGI

To avoid repeating the lengthy process of isolation and purification, fungal cultures should be **preserved** in a way that maintains their viability and characteristics over time.

1. Sub-culturing

Short-term method; involves transferring fungi onto **PDA or SDA slants**.

Material

1. SDA/PDA slants
2. Inoculation tools
3. Refrigerator (4°C)

Procedure

- Inoculate slants and incubate:
  - **Molds**: 25°C for 7 days
  - **Yeasts**: 37°C for 48 hrs
- Store at **4°C**
- **Revival**: Subculture onto fresh slants every 15 days

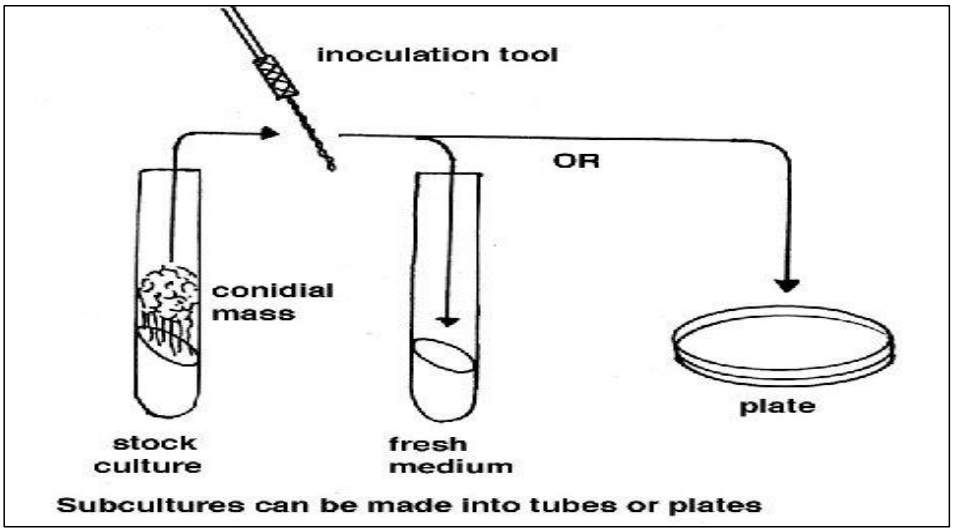

Figure 16.1: Subculturing for revival of cultures

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2. Lyophilization *(For Yeast)*

Procedure

- Place yeast culture in a **lyophilizer** to dry and powder
- **Revival**: Inoculate powder into broth and incubate under suitable conditions

(Demonstration only)

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3. Cryopreservation

Materials

1. Overnight broth culture
2. Sterile **glycerol (15%)**
3. Cryovials
4. -20°C or -80°C freezer

Procedure

- Mix 1 mL broth culture with 150 µL sterile glycerol
- Freeze at **-20°C or -80°C**

Revival

- Thaw at room temp
- Centrifuge at 6000 rpm (10 min)
- Wash pellet with sterile saline, re-suspend, and inoculate on SDA

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4. Soil Preservation (for molds)

Materials

1. Sterile soil in a bottle
2. Autoclave
3. Spore suspension (in normal saline)

Procedure

- Autoclave soil **twice** on consecutive days
- Add fungal spore suspension
- Incubate at **25°C for 10 days**
- Store at **room temp or 4°C**

Revival

- **Sprinkle soil** on SDA or
- Suspend in saline, settle, and spread supernatant on SDA

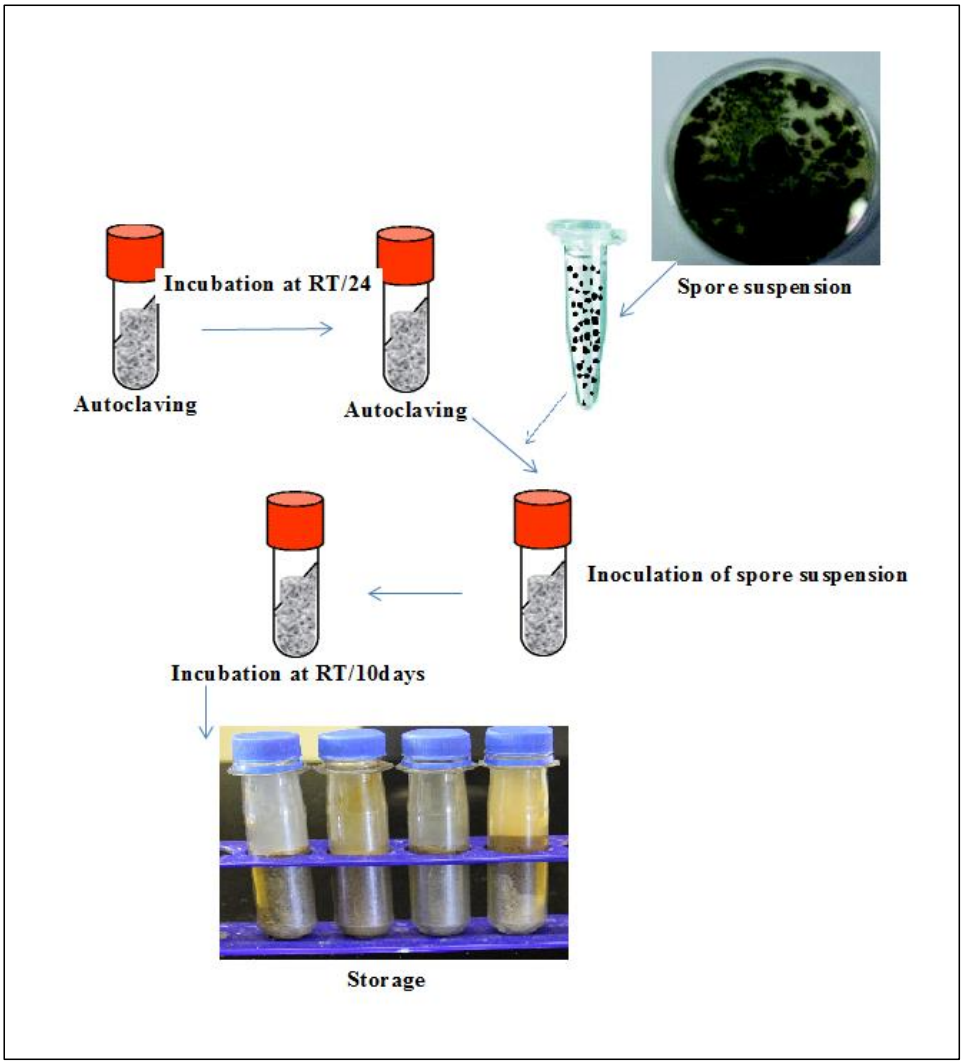

Figure 16.2: Preservation of filamentous fungi in soil

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5. Microbank (Commercial Bead System)

Materials

1. Cryovials with **polystyrene microbeads**
2. Buffer

Procedure

- Transfer pure culture into vial
- Wait 2 min, discard buffer
- Microbes adhere to beads
- Store at **-20°C or -80°C**

Revival

- Remove one bead
- Streak onto agar plate
- Incubate at recommended temperature

(Demonstration only)

Figure 16.3: Cryovial with microbeads (left side) and microbank (Rightside)