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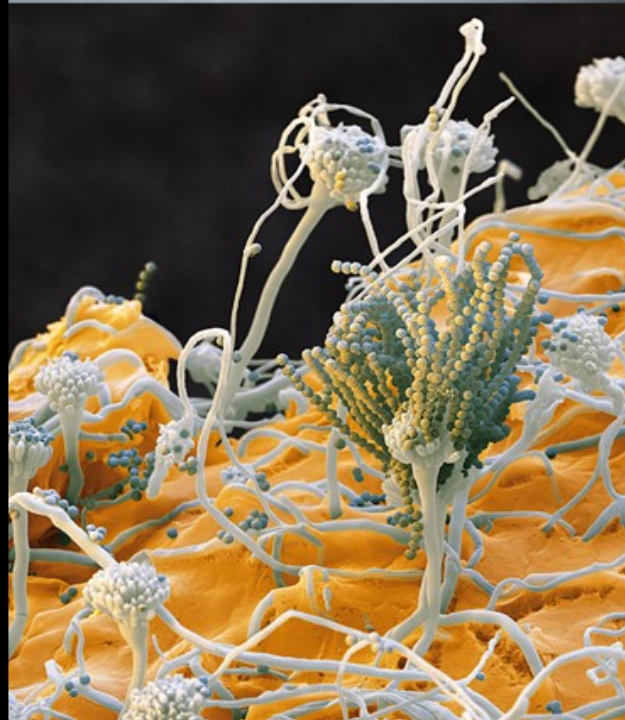
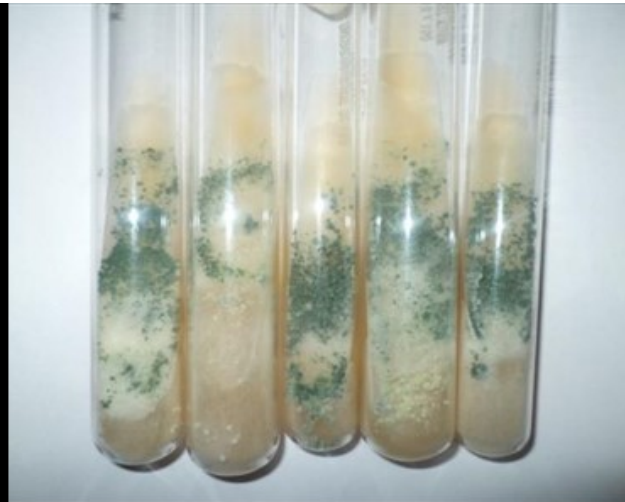
POST GRADUATE (P.G.),
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VMC 609: TECHNIQUES IN
MICROBIOLOGY AND
IMMUNOLOGY

**TOPIC: METHODS OF
FUNGAL PRESERVATION**

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Primary
methods of
culture
preservation

Continuous growth

Drying

Freezing

1. Continuous growth

Cultures are grown on agar

typically are used for short-term storage

Normally, stored at temperatures of from 5°-20°C

May be frozen to increase the interval between subcultures

Simple and inexpensive

Specialized equipment is not required

2. Drying

Most useful method of preservation for cultures ,
which produce spores or other resting structures

Silica gel, glass beads, and soil are substrate for drying

Technically simple and do not require expensive
equipment

Freezing methods include:

Cryopreservation

Freeze drying or lyophilization

Maintenance and preservation of cultures

Short-term preservation

Long-term preservation

Short-term preservation

- Involves maintenance of cultures for up to 1 year
- Most fungal cultures can be maintained for that period by serial transfer
- Time consuming and labor intensive
- Small collections with cultures in constant use for short periods (less than 1 year) are preserved by this method.

- Periodic transfer of inoculum is done from an actively growing fungus culture to test tubes (screw cap or plugged with cotton or foam) or Petri dishes (wrapped with Parafilm to reduce drying) containing an agar medium of choice.
- Alternating nutrient-rich with nutrient-poor media at serial transfer helps to maintain healthy cultures.

Contd...

- After a culture is established, it is kept at room temperature or at 4°C.
- Cultures are checked periodically for contamination and desiccation.
- Vigorous, sporulating cultures are sealed tightly and stored in freezer at -20°C or stored at -70°C to enhance survival and increase the interval between required transfers

Fungi are preserved using an array of different preservation protocols

SUBCULTURING

potato dextrose agar
(PDA)

Potato carrot agar
(PCA)

Czapek-Dox agar (CDA)



Subculturing for 30-90 days

Depending upon fungal species

Storage under mineral oil

Covering fungal culture with sterile mineral oil

reduces the penetration of available environmental oxygen



Reduces metabolic activity and increases the longevity of the strains



low-cost method, for those fungi not withstanding cryopreservation & lyophilisation

Cultures can be revived by inoculating on fresh medium

incubating them on appropriate temperature

Storage under distilled water

Cultures are grown in optimum conditions and cut using a 3 mm corkborer

5–6 discs are inserted in the tube with sterilized distilled water and tube kept at 4 °C



low-cost method and best suited for non spore forming or low sporulating fungi

Preservation in Silica Gel

The silica gel method is used to preserve sporulating fungi as alternate to freeze-drying or for storage in liquid nitrogen.


Viability after storage on silica gel depends on the strain of fungus and the medium on which it was grown before storage.


Advantage - silica gel , prevents all fungal growth and metabolism.


Revival of cultures from silica gel is easy.


Protocol


- Cotton-plugged test tubes (13-mm diameter × 100 mm high) partially filled (to 65 mm) with 12- to 20-mesh silica gel without indicator dye are heat sterilized at 180°C for 2 hours and stored at room temperature in tightly sealed containers until needed.
- Fungal cultures are grown for a week on suitable agar slants.

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- For sporulating strains, about 0.5-ml sterile water is pipetted gently into the tube of a sporulating culture.
 - Spores are suspended using a vortex-type mixer.

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- For nonsporulating strains, aerial hyphae and mycelia are scraped from the agar surface with a sterile blade and transferred to a test tube (10-mm diameter × 75 mm tall) containing 0.5 ml water.
 - The mixture is ground with a pipette or glass rod against the tube wall to obtain a creamy homogenate.
 - About 0.5 ml sterile skim milk is added and stirred gently.

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- The cotton plug covering the silica is removed, and the suspension is pipetted over it, drop by drop.
 - The tube is agitated briefly with a mechanical mixer to distribute inoculum over as many particles of silica gel as possible and then is placed in an ice-water bath for 15 minutes.

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- After 1 day at room temperature, the particles appear dry, and the tube is sealed against moisture with Parafilm.
 - Tubes are stored at 5°C or -20°C in a moisture- proof box.
 - Storage at low temperatures can increase the survival period twofold to threefold over storage at room temperature.

Cryopreservation

Most reliable methods for long-term fungal preservation

strains are stored at ultralow temperature in deep freezers (-80 °C)

or in liquid nitrogen—LN2 (-196 °C) with cryoprotectants

Lyophilisation

Lyophilization is one of the best methods for long term storage

not appropriate for all fungi.



EQUIPMENT AND ACCESORIES REQUIRED.

- High-quality mechanical vacuum pump; vacuum gauge; vacuum manifold; cold trap; hoses to connect pump, trap, and manifold; insulated bath; support stand for manifold;
- Oxygen-gas torch; oxygen supply;
- 10-cm lengths of 6-mm soft glass tubing with one end heat sealed, or lyophilization ampoules;
- Cotton for plugging tubes;
- Pasteur pipettes;
- Mechanical or electrical pipetting aid;
- Sterile menstruum;
- permanent ink suitable for writing on glass.

To be continued...





Thank You