Post Graduate (P.G.), Monsoon semester

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VMC 609: TECHNIQUES IN MICROBIOLOGY AND IMMUNOLOGY

TOPIC:

"Maintenance and preservation of bacteria and fungi"

PART-II

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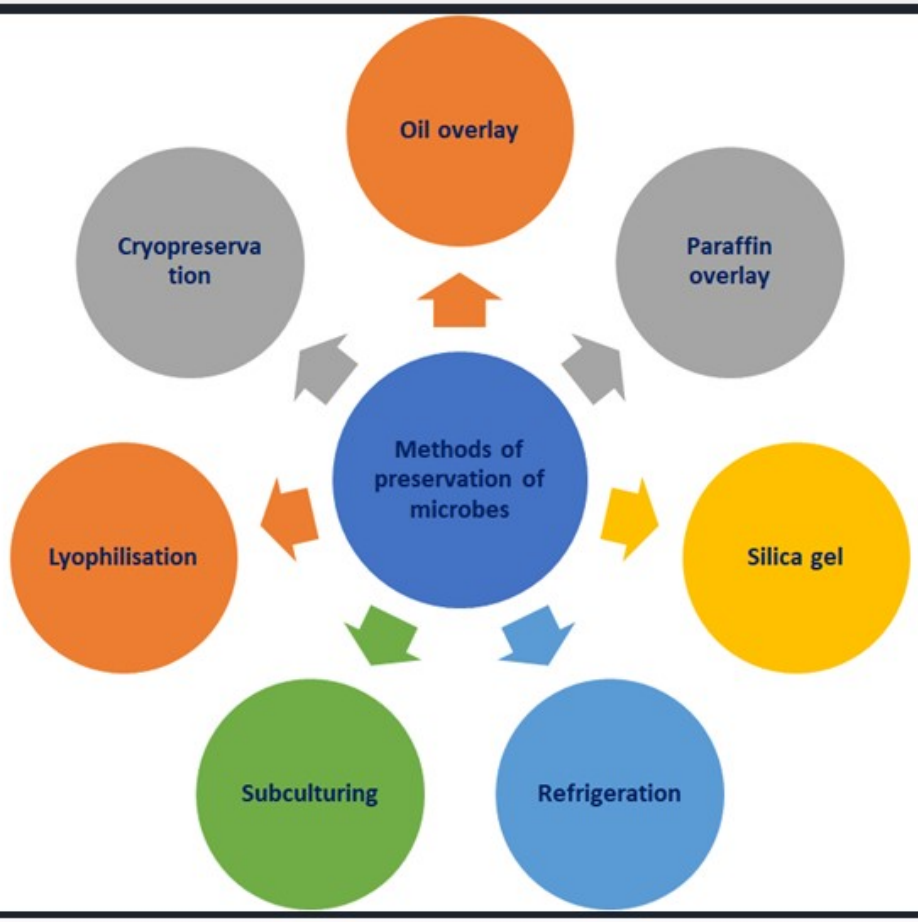
Preservation methods



Short term preservation

Agar Slant culture

- **Cultures are preserved on Agar stabs or slants**
- **Microbes are inoculated with individual culture and incubated overnight.**
- **stored at 5 to -20°C.**
  - sub-culturing is done after an interval of six months
- **cultures are covered with mineral oil to a depth of 1 cm above the slant.**
  - sub-cultured after the storage of about 1 year.

Short term preservation

- At high concentration salts act as bacteriostatic (temporary growth inhibitor).
- bacterial culture are suspended in 1% salt concentration (NaCl) & stored in screw-capped tubes to prevent evaporation.
- The tubes are stored at room temperature.

Regular Subculture

- Periodic transfer on fresh, sterile media can maintain microbial culture.
- To keep the viability of cultures appropriate growth medium and a proper storage temperature should be maintained.
- culture preserved by alternate cycles of active growth and storage periods obtained by series of subcultures.

Contd...

Considerable factors for maintaining a microbial culture by using subculture method

Nature of media:

- Solid media should be chosen in preference to liquid as there is a higher chance of contamination in liquid media
- Slope cultures are often used for preservation but oxygen sensitive bacteria may benefit from stab culture.
- After subculturing the tubes should be sealed properly.
- Cotton wool plugged tubes are not adequate - media will quickly dry out and cultures will be lost.

Disadvantages

I. Change of characteristics-

- characteristics may be lost, reduced, or intensified.
- Changes may occur among strains where intervals between transfers are short.

II. Contamination-

- occurs frequently, especially when large numbers of cultures are involved

III. Mislabelling-

- labeled with the wrong name or number, may become distorted and unrecognizable

IV. Loss of cultures-

- Temperature fluctuations in incubation or refrigeration of equipment may contribute to loss.

Paraffin Method

- Very simple and cost effective method of preserving cultures of bacteria and fungi for longer time at room temperature
- In this method sterile liquid paraffin is poured over the slant culture of microbes and stored upright at room temperature.
- Paraffin layer prevents dehydration of the medium and ensures anaerobic conditions.
- It slows the metabolic activity by reduced
- Growth through reduced oxygen tension.

Mineral oil method

- Cultures can also be maintained by covering the agar slants with a layer of sterile mineral oil about half inch above the surface of the slant.
- The oil must be above the tip of the slanted surface.
- Mineral oil covered cultures are stored at room temperature or preferably at 0-5°C.

Mineral oil method

While preserving the cultures in oil following points should be considered:

- oil should be above the uppermost level of the medium, for preventing dry out
- Oil should be separate from the wall of the tube and float to the surface of the wall.
- oil free of as any rancidity or toxic substance.
- preferably the oil should be to sterilized in hot air oven at 150ºC to 170ºC for one hour;
- If autoclaved moisture becomes mixed with the oil, giving it a milky appearance.

Silica Gel Method

- **The basic principle in this technique is quick desiccation at low temperature -** allows the cell to remain viable for a long period.
- **bacteria and yeast can be stored in silica gel powder at low temperature for a period of 1-2 years**
- **Finely powdered, heat sterilized and cooled silica powder is mixed with a thick suspension of cells and stored at low temperature.**

Storage At Refrigerator Or Cold Room Storage

- Live cultures on a culture medium can be successfully stored in refrigerators or cold rooms (4ºC).
- metabolic activities of microbes slows down greatly at this temperature but do not altogether stop.
- bacterial metabolism slows and only less quantity of nutrients will be utilized.
- cannot be used for a very long time because toxic products get accumulated which can kill the microbes.

Cryopreservation and Lyophilization

- *Cells are subjected to cryogenic temperatures - promote ice crystal formation in the suspension medium and within the cell interior.*

- *Resulting osmotic imbalance induces biophysical and biochemical changes (e.g. Disruption of organelles and loss of membrane integrity) and causes cryo-injuries and cell death.*

- *Cryoprotectants protect the cells from cryo-injuries during cryopreservation.*

Cryoprotectants

–Broadly classified as penetrating or non-penetrating

–Cell penetrating cryoprotectants are generally considered ideal.

–They protect the cell by

- lowering the freezing point of water
- promoting hydrogen bond formation
- vitrification of solvents
- preventing ice crystal formation inside the cells .

–Glycerol (10–15%) and dimethyl sulfoxide (5%) are frequently used in cryopreservation of microorganisms, and both have cell-penetrating capacity.

Need for cryoprotectant

As water is removed during freezing as ice, electrolytes become increasingly concentrated in unfrozen water → **may be harmful**

Electrolyte concentrations outside cells become very different from inside those cells → **leading to osmotic stress**.

Cultures can be preserved very effectively if frozen in the presence of a cryoprotectant → **reduces damage from ice crystals**.

PROCESS OF FREEZING

About 2 ml of glycerol solution is added on to the agar slant culture

Shaking is performed to emulsify the culture

Emulsion is then transferred to ampoules, with each ampoule having 5 ml of the culture

Ampoules are placed in a mixture of industrial methylated spirit and carbon dioxide and frozen rapidly to -70°C

Ampoules are then removed and placed directly in a deep freeze at -40°C for utilization of the stock cultures

Ideal cryoprotectant

An ideal cryoprotectant should meet all of the following criteria:

- *be highly water soluble*
- *penetrate inside the cell*
- *have a low toxicity*
- *be non-reactive*
- *not precipitate at high concentrations.*

Cryopreservation

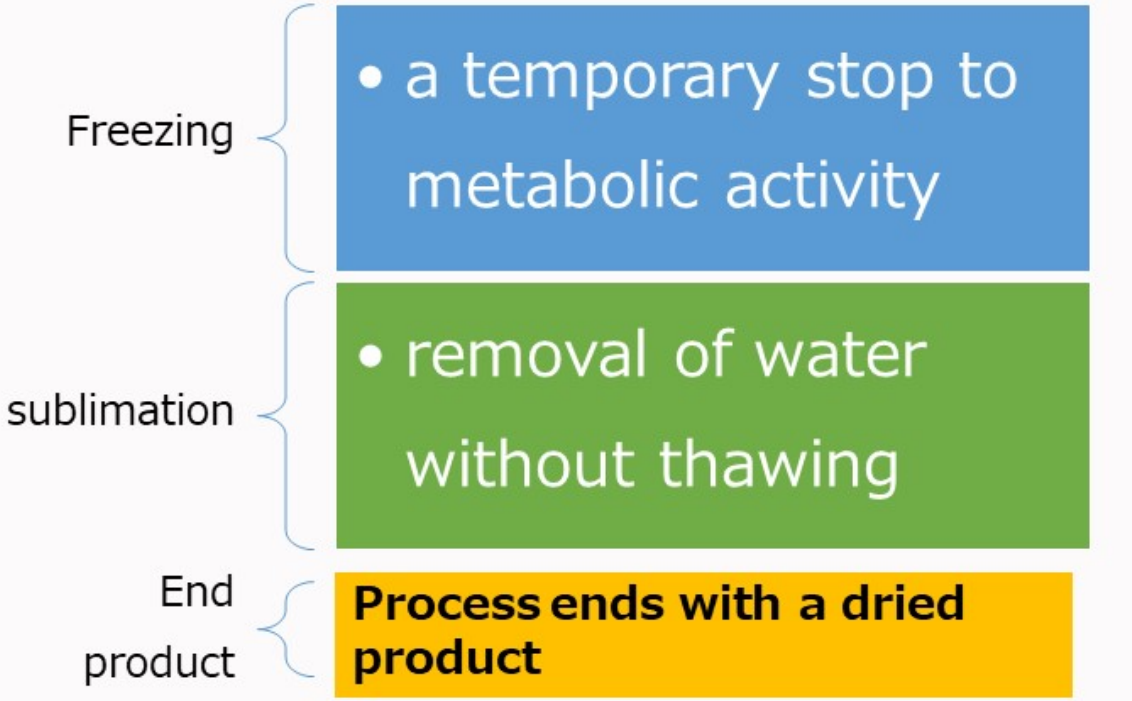
"cryopreservation refers to the preservation of biological materials at cryogenic temperatures, generally - 80 °C, (dry ice) or -196 °C, (liquid nitrogen)."

- Low temperature protects proteins and DNA from denaturation and damage
- slows the movement of cellular water.
- biochemical and physiological activities of the cells are essentially halted and cells are protected for long periods of time.
- Preservation at - 80 °C is adequate
- **Preservation at -196 °C is considered ideal** because the chances of DNA mutations are almost zero at this temperature.
- Preservation of cells at -20 °C is not recommended for long-term preservation.

Freeze-drying or lyophilization

- *Preferred long-term preservation method due to the low cost of maintenance and ease of transportation of lyophilized cultures.*
- *Lyophilization gives satisfactory results for the preservation of many bacteria, yeast and sporulating fungi*
- *Does not adequately preserve non-sporulating fungi (vegetative hyphae), some species of yeast*

Stages of freeze-drying process



The dried product is sealed either under vacuum or under an inert gas, can be stored at room temperature with no further metabolic activity until water and nutrients are restored


THANKS