Preservation of Microorganism :-

All practising microbiologists have felt the need to preserve the viability of m/o with further work.

While preservation all the cultural characteristic of culture was conserved.

There are two criteria for selecting a method of preservation for a given culture

(i) The period of preservation desired, and

(ii) The nature of culture preserved.

With the increasing importance of m/o to industry (eg. biochemical & antibiotics production, bioassay) human, animal & plant pathologists, geneticists, taxonomists and teachers have felt the need of culture collections.

There are several large public service collections.

C.B.S – Central Bureau Voor Schimmelculture – 1906

ATCC – American Type Culture Collection – 1925

C.M.I – Commonwealth mycological Institute – 1947.

MTCC – Microbial Type Culture Collection.

There are 3 basic aims in maintaining and preserving the M/o

(i) to keep culture alive

(ii) Uncontaminated

(iii) as healthy as possible (both physically & physiologically)

(iv) To have adequate stocks and appropriate systems for replenishing stocks.

1. Serial Subculture method:-

≠ Simplest and most Common method.

≠ microbes are grown in agar slants and transferred to fresh media before they exhaust all the nutrient or dry out.

≠ Solid media should be preferred compared to liquid media, as growth of Contaminants can be more readily observed.

eg:

Bacteria	Medium	Transfer time	Incubation Temp. °c.	Storage temp °c
1. Bacillus spp.	- N.A. slant	12 months or longer	28°c	4°
2. pseudomonas spp.	- N.A. slant	3 months	28°c.	4°
3. Clostridium spp.	:- Robertson Cooked meat medium	6 months or longer	28°c.	Room temp.

≠ The time period appropriate for subculture may range from a week to even some years.

2. Preservation by overlaying cultures with mineral oil:-

≠ It is a modified technique of Serial Subculture technique

≠ It was first extensively used by Buell & Weston.

≠ Even store more than 10 years.

≠ This method was cheap & easy.

The steps involved in this method are,

1. Inoculation of the agar slant contained in a screw-cap tube with a given culture is practised.

2. Inoculated agar slant is subjected to incubation until good growth appears.

3. Using sterile technique, a healthy agar slant culture is covered with sterile mineral oil to a depth of about 1cm above the top of the agar slant.

4. Oiled culture from step(3) can be stored at room temp. For better viability it is stored low temp (15°C)

∴ The oil is used should be good quality & free from m/o.
eg. Liq. paraffin oil - Specific gravity 0.865 to 0.890.

3. Lyophilization or Freeze-drying :-

≠ Lyophilization is the most satisfactory method of long-term preservation of m/o.

≠ It is universally used for the preservation of bacteria, viruses, fungi, sera, toxins, enzymes and other biological materials.

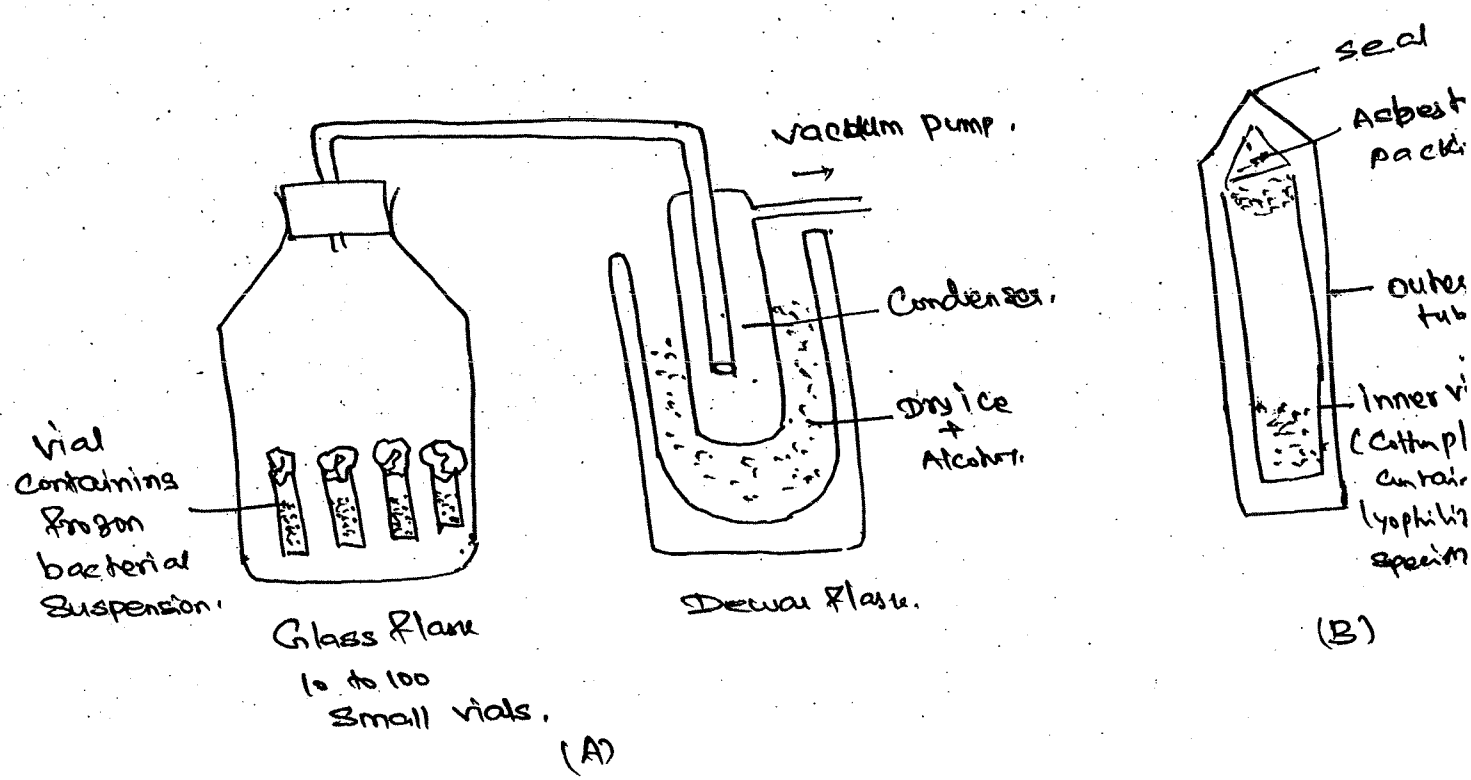
Major steps involved in this technique are.

(I) A cell or spore suspension is prepared in suitable protective medium

(II) Using a sterile technique the suspension from (I) distributed in small quantity into glass ampoules.

(iii) The ampoules are connected with a high vaccum system usually incorporating a desiccant (eg. phosphorous pentoxide, silicagel or a freeze trap) and immersed in to a freezing mixture of dry ice and alcohol (−78°c)

(iv) The vaccum pump is turned on and the ampoules are evacuated till drying is complete, after which they may be sealed.



(A) Small cotton-plugged vials containing frozen suspension of the m/o are placed in the glass-flask. W/c is attached to a condenser.

The condenser is connected with high vaccum pump and this system brings about desiccation of the cultures.

(B) After desiccation of culture (A), the vials are removed, placed individually in a large tube, covered c asbestos packing & sealed under vaccum.

(4) Storage at very low temperatures (or) Nitrogen storage:-

≠ It is also called Cryogenic storage

≠ It is a satisfactory method for long-term preservation of m/o.

≠ It is used as successful with many specimens w/c cannot be preserved for Lyophilization.

Major steps involved in the performance of this method are,

(i) ≠ The culture is suspended as a cell or a spore suspension

≠ as finely broken up particles of mycelium or as a piece of fungus mycelium in a suitable suspending medium (10% glycerol (or) Dimethyl sulfoxide).

(ii) The suspension prepared in step (i) is distributed into ampoules (it must be resistant to cold shocks).

(iii) The ampoules filled with a culture suspension are frozen and are hermetically sealed.

(iv) The frozen ampoules are kept in aluminium canes. and kept in the liquid nitrogen..

Advantages:

1. It is an effective method for preservation
2. No Sub culture is required
3. The cultural characteristics remain unchanged.
4. Contamination is not possible due to the ampoules are sealed.

Disadvantage:-

1. It is very expensive, costly apparatus are required.
2. reliable supply of nitrogen is needed
3. Needed more alert due to usage of liq. Nitrogen (explosive gas)

5. Soil Culture:

This method is particularly used for the preservation of spore producing microbes.

eg. Bacillus, Penicillium, Aspergillus & Strephmyces.

Steps involved in this method are,

(I) A spore suspension is first prepared

(II) A mixture of soil (20%), Sand (78% & $CaCO_3$ (2% is prepared and distributed (1gm) in tubes.

(III) Sterlize the tubes 8 to 15 hr at 130°C. & cooled.

(IV) A spore suspension is added to the sterilised tube and allowed to grow for 10 days.

(V) The inoculated tube (step IV) kept in desicator under vacuum (to evporate excess water).

(VI) Then tubes are sealed & stored at 5 to 8°C.