Maintenance and Preservation of Lab cultures and Pure Cultures

Once a microorganism has been isolated and grown in pure culture, it becomes necessary to maintain the viability and purity of the microorganism by keeping the pure culture free from contamination. Normally in laboratories, the pure cultures are transferred periodically onto or into a fresh medium (subculturing) to allow continuous growth and viability of microorganisms. The transfer is always subject to aseptic conditions to avoid contamination. To maintain pure culture for extended periods in a viable conditions, without any genetic change (Mutations) as well as avoiding its contamination is referred as Preservation. During preservation most important factor is to stop microbial growth or at least lower the growth rate. Due to this toxic chemicals are not accumulated and hence viability of microorganism is not affected.

Application of Preservation

1. Academic Use
2. Research Purpose
3. Fermentation Industry
4. Biotechnological Field

Techniques for the Preservation of microbes broadly divided into two

1. Methods where organisms are in Continuous metabolic active state
2. Methods where organisms are in Suspended metabolic state

1. **Continuous metabolic active state preservation technique**: In this technique, organisms preserved on nutrient medium by repeated sub-culturing. In this technique, any organisms are stored by using general nutrient medium. Here repeated subculturing is required due to depletion or drying of nutrient medium. This technique includes preservation by following methods.

a. **Periodic transfer to fresh media:** Organisms grown in general media on slant, incubated for particular period at particular temperature depending on the characteristics of the selected organisms, then it is stored in refrigerator. These cultures can be stored for certain interval of time depending on the organism and its growth conditions. After that time interval, again these organisms transferred to new fresh medium and stored in refrigerator

b. **Overlaying culture with mineral oil**: Organisms are grown on agar slant then they are covered with sterile mineral oil to a depth of 1 cm. above the tip of the surface. This method is simple; one

can remove some organisms in aseptic condition with the help of sterile wire loop and still preserving the initial culture. Some species preserved satisfactorily for 15 – 20 years by this method.

c. **Storage in sterile soil:** This method is widely used for preserving spore forming bacteria and fungi. In this method, organisms will remain in dormant stage in sterile soil. Soil sterilized then spore suspension added to it aseptically, this mixture dried at room temperature and stored in refrigerator. Viability of organisms found around 70 – 80 years.

d. **Saline suspension:** Normal Saline used to provide proper osmotic pressure to organism's otherwise high salt concentration is inhibitory for organisms. Organisms kept in screw cap bottles in normal saline, stored at room temperature, wherever required transfer made on agar slats, and incubated.

2. **Methods where organisms are in Suspended metabolic state:** Organisms preserved in suspended metabolic state by either drying or storing at low temperature. Microbes are dried or kept at low temperature carefully so that their revival is possible.

a. **Drying in vacuum:** In this technique, organisms dried over chemical instead of air dry. Cells passed over $CaCl_2$ in a vacuum and then stored in refrigerator. Organisms survive for longer period.

b. **Lyophilization**: Lyophilization is vacuum sublimation technique. Cells grown in nutritive media and then this culture distributed in small vials. These vials culture then immersed in a mixture of dry ice and alcohol at -78 °C. These vials immediately connected to a highvacuum line, and when they are completely dried, each vial sealed under vacuum. This is most effective and widely

Methods of Preservation of Lab cultures/Pure cultures

Since repeated subculturing is time consuming, it becomes difficult to maintain a large number of pure cultures successfully for a long time. In addition, there is a risk of genetic changes as well as contamination. Therefore, it is now being replaced by some modern methods that do not need frequent subculturing. These methods include

1. Periodic transfer to fresh media (Subculturing)
2. Oil overlay (Paraffin method)
3. Storage at low temperature

a. Refrigeration

b. Cryopreservation

4. Freez dying (Lyophilization)

5. Storage in sterile soil

6. Saline suspension

A. **Periodic transfer to fresh media (Subculturing) -**Strains can be maintained by periodically preparing a fresh culture from the previous stock culture. The culture medium, the storage temperature, and the time interval at which the transfers are made vary with the species and must be ascertained beforehand. The temperature and the type of medium chosen should support a slow rather than a rapid rate of growth so that the time interval between transfers can be as long as possible. Many of the more common heterotrophs remain viable for several weeks or months on a medium like **Nutrient Agar**.

Advantages

I. It is a simple method, any special apparatus are not required.

II. Easy to recover the culture

Disadvantages:

I. Since repeated subculturing is time consuming, it becomes difficult to maintain a large number of pure cultures successfully for a long time.

II. Risk of contamination is more

III. Possibility of genetic changes due to the development of variants and mutants and changes in biochemical characteristics Therefore, it is now being replaced by some modern methods that do not need frequent subculturing.

Example: All microbiology laboratories preserve microorganisms on **agar slant.** The slants are incubated for 24hr or more and are then stored in a refrigerator. These cultures are periodically transferred to fresh media. Time intervals at which the transfers are made which varies with the origin and condition of growth.

B. **Oil overlay (Paraffin method)-** The agar slants are inoculated and incubated until good growth appears. Many bacterial species can be preserved by covering the growth on the agar slants with sterile mineral oil. They are then covered with sterile mineral oil to a depth of 1 cm above the tip of slant surface. Oil must cover the slants completely. Oil reduces the loss of water and also slow down the exchange of gas within organism and surrounding. This is a simple and most economical method of preserving bacteria and fungi where they remain viable for several years at room temperature. The layer of paraffin prevents dehydration of the medium and by ensuring an aerobic condition,the microorganism remain in dormant state.

C. **Refrigeration-** Pure cultures can be successfully stored at 0-4°C either in refrigerators or in cold-rooms. This method is applied for short duration (2-3 weeks for bacteria and 3-4 months for fungi) because the metabolic activities of the microorganisms are greatly slowed down but not stopped. Thus their growth continues slowly, nutrients are utilized and waste products released in medium. This results in, finally, the death of the microbes after sometime.

D. **Cryopreservation-** Cryopreservation (i.e., freezing in liquid nitrogen at -196°C or in the gas phase above the liquid nitrogen at -150°C) helps survival of pure cultures for long storage times. In this method,the microorganisms of culture are rapidly frozen in liquid nitrogen at -196°C in the presence of stabilizing agents such as glycerol or Dimethyl Sulfoxide (DMSO) that prevent the cell damage due to formation of ice crystals and promote cell survival. This liquid nitrogen method has been successful with many species that cannot be preserved by lyophilization and most species can remain viable under these conditions for 10 to 30 years without undergoing change in their characteristics, however this method is expensive.

E. **Freez dying (Lyophilization)-** Freeze-drying is a process where water and other solvents are removed from a frozen product **via sublimation.** Sublimation occurs when a frozen liquid goes directly to a gaseous state without entering a liquid phase. It is recommended using slow rates of cooling, as this will result in the formation of vertical ice crystal structures, thus allowing for more efficient water sublimation from the frozen product. Freeze-dried products are hygroscopic and must be protected from moisture during storage. Under these conditions, the microbial cells are dehydrated and their metabolic activities are stopped; as a result, the microbes go into dormant state and retain viability for years. Lyophilized or freeze-dried pure cultures and then sealed and stored in the dark at 4°C in refrigerators. Freeze-drying method is the most frequently used technique by culture collection centers. Many species of bacteria preserved by this method have remained viable and unchanged in their characteristics for more than 30 years.

Advantage of Lyophilization

I. Only minimal storage space is required; hundreds of lyophilized cultures can be stored in a small area.

II. Small vials can be sent conveniently through the mail to other microbiology laboratories when packaged in a special sealed mailing containers.

III. Lyophilized cultures can be revived by opening the vials, adding liquid medium, and transferring the rehydrated culture to a suitable growth medium.

F. **Storage in sterile soil-** This method is widely used for preserving spore forming bacteria and fungi. In this method, organisms will remain in dormant stage in sterile soil. Soil sterilized then spore suspension added to it aseptically, this mixture dried at room temperature and stored in refrigerator. Viability of organisms found around 70 – 80 years.

G. **Saline suspension-** Sodium chloride in high concentration is frequently an inhibitor of bacterial growth.Bacteria are suspended in 1% salt solution (sublethal concentration in screw cap tubes to prevent evaporation). The tubes are stored at room temperature. Whenever needed the transfer is made on agar slant.