Module – 07: The Preservation of Industrially Important Microbes

Introduction

Microbes are required for the production of fermentation products. They are very valuable for specific product. Not all the microbes will give one product produced efficiently by specific microbe.

The isolation of a desired organism for a fermentation process may be time consuming and very expensive procedure and it is therefore essential that it retain the desirable characteristics that led to its selection. In addition, the culture used for the fermentation process should remain viable and free from contamination. Thus, industrial cultures must be preserved and maintained in such way as to eliminate genetic change, protect against contamination, and retain viability.

Different techniques are used for maintenance and preservation of different organisms based on their properties. Selected method should also conserve the properties of the organisms.

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 sub-culturing is required due to depletion or drying of nutrient medium. This technique includes preservation by following methods.

1.1 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.

1.2 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.

1.3 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.

1.4 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.

2.1 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.

2.2 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^{o}C. These vials immediately connected to a high-vacuum line, and when they are completely dried, each vial sealed under vacuum. This is most effective and widely

used technique due to long time survival, less opportunity for changes in characteristics of organisms and small storage area. Organisms can survive for period of 20 years or more.

2.3 Use of Liquid nitrogen

Microorganisms grown in nutritive media and then this culture frozen with Cryoprotective agents like Glycerol and Dimethyl Sulfoxide. Frozen culture kept in liquid Nitrogen refrigerator. Organisms can remain alive for longer period.

2.4 Storage in silica gel

Both bacteria and yeast stored by this method. By this technique, organisms can survive for 1 – 2 years. Finely Powdered Heat sterilized Silica powder mixed with thick suspension of cell at low temperature.

Note:

- Cells should be harvested when actively growing (mid logarithmic phase)
- One method used for few organisms or specific organism; not all the organisms preserved by any one technique mentioned above.

Quality control of the preserved stock culture

Whichever technique used for the preservation and maintenance of industrially important organisms it is essential to check the quality of the preserved organisms stocks. Each batch of newly preserved cultures routinely checked to ensure their quality. A single colony transferred into a shake-flask to ensure growth of particular kind of microorganism; further shake-flask subculture used for the preparation of huge quantity of vials. For the assessment of purity, viability, and productivity of cultures, few vials are tested. If samples fail any one of these tests, the entire batch destroyed. Thus, by the use of such a quality-control system stock cultures retain and used with confidence.

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