

Preservation of Industrially Important Microbes

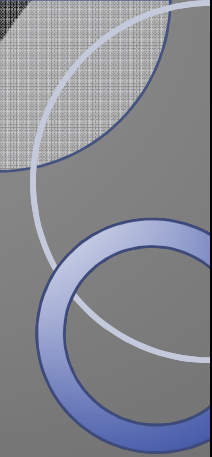
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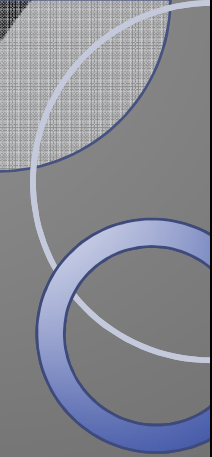
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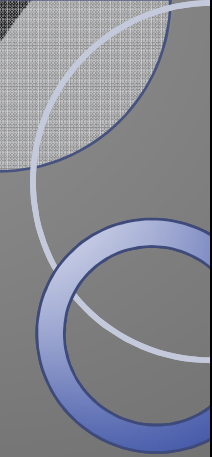
Introduction

- Microbes are required for the production of fermentation products. They are very valuable for specific product.
- One product produced efficiently by specific microbe will not be given by all the microbes.



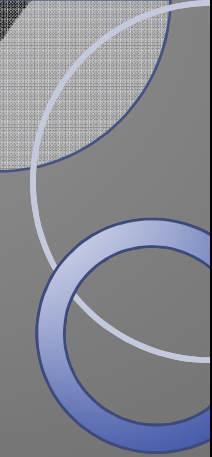
Need of Preservation

- The isolation of a desired organism for a fermentation process may be a time consuming and very expensive procedure.
- Also, 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.



Techniques of Microbial Preservation

- Different techniques are used for maintenance and preservation of different organisms based on their properties.
- Techniques for the Preservation of microbes are broadly divided into two -
 - 1) Methods where organisms are in Continuous metabolic active state
 - 2) Methods where organisms are in Suspended metabolic state



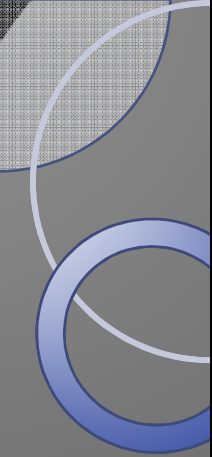
Methods where organisms are in Continuous metabolic active state –

- In this technique organisms are 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. Periodic transfer to fresh media –
 2. Overlaying culture with mineral oil –

Periodic transfer to fresh media

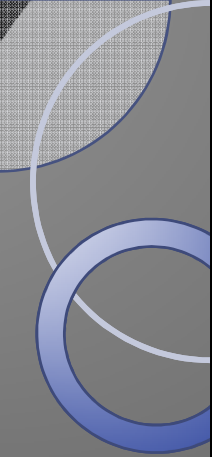
- Organisms are grown in general media on slant, incubated for particular period of time at particular temperature depending on the characteristics of the selected organisms, then it is stored in refrigerator.
- These cultures can be stored for certain interval of time depending on the organism and its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





Overlaying culture with mineral oil

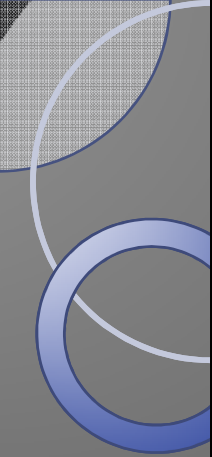
- Organisms are grown on agar slant then they are covered with sterile mineral oil to a depth of 1 cm. above the tip of the surface.
- This method is simple; one can remove some organisms in aseptic condition with the help of sterile wire loop and still preserving the initial culture.
- Some species have been preserved satisfactorily for 15 – 20 years by this method.



Methods where organisms are in Suspended metabolic state

Preservation by Drying in Vacuum

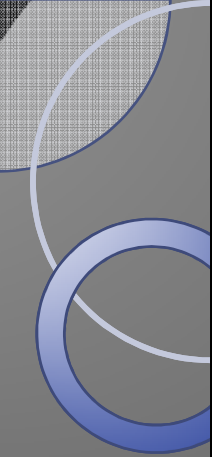
- The cultures are dried over CaCl_2 in a vacuum, then stored in a refrigerator. At such conditions, the organisms survive for longer period than the air dried cultures.



Cryo-preservation

- The cultures are grown in culture tubes. Then 10-30% of a cryo-protective agent (e.g. glycerol or dimethyl sulfoxide) is added into it so that it could protect the culture from cold shock.
- The culture is dispensed into ampoules. The ampoules are properly sealed and kept in liquid nitrogen at -176 to -196°C .



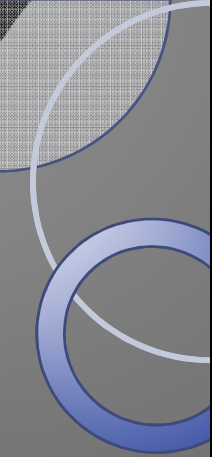


Lyophilisation or Freeze-drying

- In this method the microbial suspension is put in small ampoules and frozen through drying under vacuum. Vacuum drying.
- Then the ampoules are sealed off in vacuum. The lyophilised cultures are stored at 4°C.
- The cultures remain viable for about 10 years. By this method a large number of cultures are maintained without variation in their characteristics.

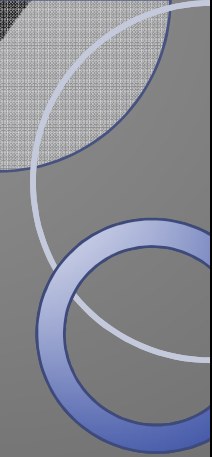


Manifold tree is placed on lyophilizer and ampules (with culture) placed tightly in the slots



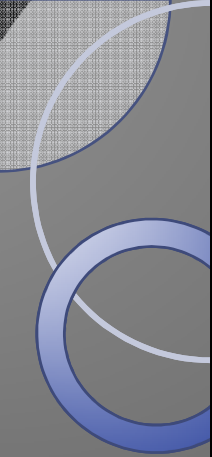
Instruments require for microbial preservation

- Refrigerator (4°C)
- Deep Freezer (-20°C)
- Deep Freezer (-80°C)
- Crayo can
- Lyophilizer
- Cold Room



Microbial Conservation Centers

- Approximately, every country in the world have one or more microbial culture collection centers. Some microbial collection and conservation centers in the world are enlisted –
 1. American Type Culture Collection (ATCC), USA
 2. Bioresources Collection and Research Center (BCRC), Taiwan
 3. Collection of Aquatic Important Microorganisms (CAIM), Mexico
 4. Culture Collection of Algae and Protozoa (CCAP), UK
 5. Marine Biotechnology Institute Culture Collection (MBIC), Japan



Microbial Conservation Centers

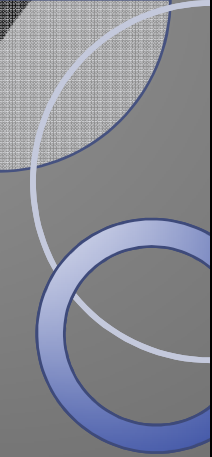
Microbial Type Culture Collection and Gene Bank
(MTCC), India

National Bank for Industrial Microorganisms and Cell
Cultures (NBIMCC), Bulgaria

National Collections of Industrial, Food and Marine
Bacteria (NCIMB), UK

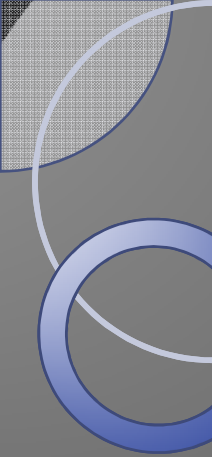
National Collection of Yeast Cultures (NCYC), UK

Agricultural Research Service Culture Collection (NRRL),
USA



Laws of Microbial Conservation

- The basic functions of existing culture collection centers in isolation, identification, maintenance; promotion and development of new collection banks or encouragement for new initiatives of collecting cultures and provision of standard scientific services are governed by WFCC (World Federation of Culture Collections) of IUMS (International Union of Microbiological Societies) and IUBS (International Union of Biological Sciences) commission.
- They recommend good culture collection practices; provide guidance in the form of guidelines to maintain authenticity of each culture and also to maintain microbiologically high standards of operation of all types of culture collection centers in the world.



Thank you