

PURE CULTURE PRESERVATION AND MAINTENANCE



Submitted By:

Meghali Kalita

***Guided By-* Dr. Diganta Narzary**

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❑ WHAT IS PURE CULTURE?

Pure culture is a culture obtain from a single spore/cell.



❖ **PRESERVATION**

- To maintain pure culture for extended periods in a viable conditions, without any genetic change is referred as Preservation.
- The aim of preservation is to stop the cell division at a particular stage i.e. to stop microbial growth or at least lower the growth rate.
- Due to this toxic chemicals are not accumulated and hence viability of microorganisms is not affected.

❖ **OBJECTIVES OF PRESERVATION**

- To maintain isolated pure cultures for extended periods in a viable conditions.
- To avoid the contamination.
- To restrict genetic change (Mutation)

METHODS OF PRESERVATION AND MAINTENANCE OF MICROBIAL CULTURE:

The method of preservation is mainly of two types-

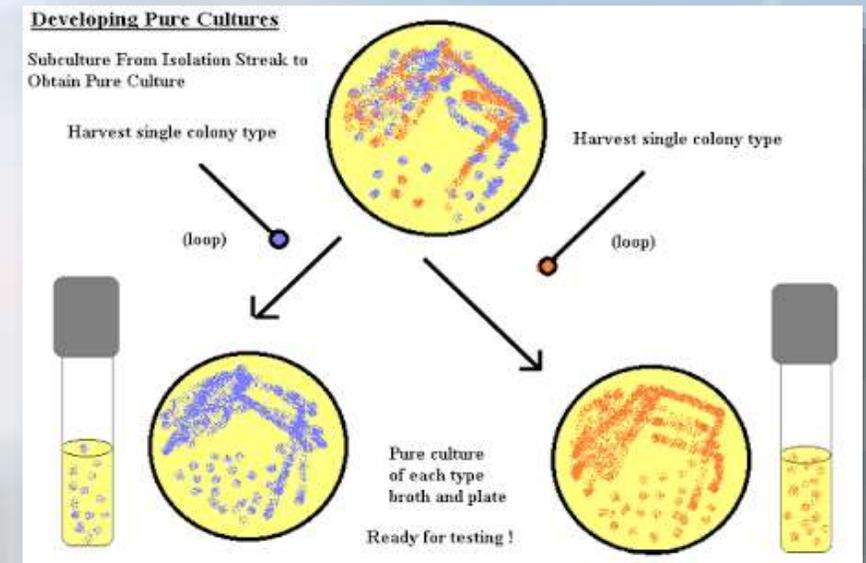
❑ SHORT TERM METHODS

❑ LONG TERM METHODS

✧ **SHORT TERM METHODS**

■ Periodic transfer to fresh media-

- Culture can be maintained by periodically preparing a fresh culture from the previous stock culture.
- Many of the more common microbes remain viable for several weeks or months on a medium like Nutrient agar.
- It is an advantageous as it is a simple method and any special apparatus are not required. However it is easy to recover the culture.
- The transfer has the disadvantage of failing to prevent changes in the characteristics of a strain due to development of variants and mutants and risk of contamination is also more in this process.



❑ PRESERVATION OF BACTERIA USING GLYCEROL

- Bacteria can be frozen using 15% glycerol.
- The glycerol is diluted to 30% and an equal amount of glycerol and culture broth are mixed, dispensed into tubes, and then frozen at -10°C .
- The viability of organisms varied such as *Escherichia coli*, *Diplococcus pneumoniae* etc. viable for 5 months, *Haemophilus influenzae* viable for 4 months, *Neisseria meningitidis* for 6 weeks and *Neisseria gonorrhoeae* for 3 weeks

○ STORAGE BY DRYING METHOD

- Spores of some microbes which are sensitive to freeze-drying, can be preserved by drying from the liquid state rather than the frozen state.
- Different procedures of drying methods are as follows:
 - Paper disc: A thick suspension of bacteria is placed on sterile discs of thick absorbent paper, which are then dried over phosphorus pentoxide in a desiccation under vacuum.
 - Gelatin disc: Drops of bacterial suspension in gelatin are placed on sterile plastic petriplates and then dried off over P₂O₅ under vacuum.
 - L-drying: Bacteria in small ampoules are dried from the liquid state using a vacuum pump and desiccant and a water bath to control the temperature. In this suspension of the organisms are dried under vacuum from the liquid state without freezing taking place.

- Apart from the mentioned methods the organisms are also dried over Calcium Chloride in vacuum and are stored in the refrigerator.
- At such conditions the organisms survive for longer period than the air dried cultures.



● STORAGE BY REFRIGERATION

- Culture medium can be successfully stored in refrigerators or cold rooms, when the temperature is maintained at 4°C.
- At this temperature range the metabolic activities of microbes slows down greatly and only small quantity of nutrients will be utilized.
- This method cannot be used for a very long time because toxic products get accumulated which can kill the microbes.



✧ LONG TERM METHODS

❑ MINERAL OIL OR LIQUID PARAFFIN STORAGE

- In this method sterile liquid paraffin is poured over the slant culture of microbes and stored upright at room temperature.
- Where as cultures can also be maintained by covering agar slants by sterile mineral oil which is stored at room temperature or preferably at 0-5°C.
- It limit the oxygen access that reduces the microorganism's metabolism and growth, as well as to cell drying during preservation.
- The preservation period for bacteria from the genera *Azotobacter* and *Mycobacterium* is from 7-10 years, for *Bacillus* it is 8-12 years.



❑ STORAGE IN SALINE SUSPENSION:

- Bacterial culture is preserved in 1% salt concentration in screw capped tubes to prevent evaporation.
- The tubes are stored in room temperature.
- Whenever needed the transfer is made on Agar Slant.



❑ IMMERSION IN DISTILLED WATER:

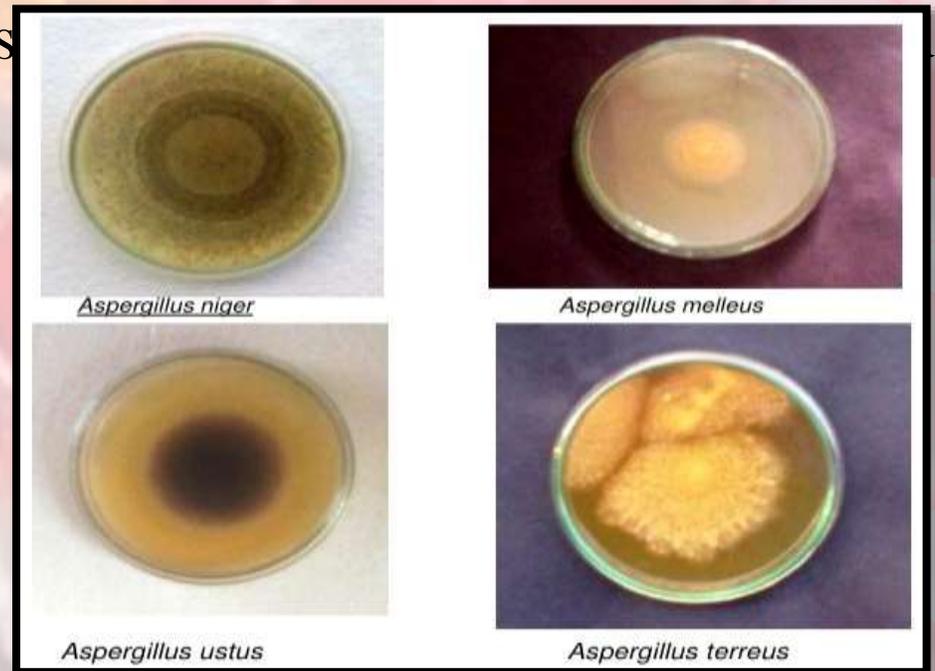
- Another inexpensive and low-maintenance method for storing fungal culture is to immerse them in distilled water.
- Fungi can be stored in this method at 20°C, survived up to 2-10 years depending upon the species.

❖ FOR SPORULATING FUNGI:

- It involves inoculating agar slants of preferred media with fungal cultures and then incubating them at 25°C for several weeks to induce sporulation.
- Sterile distilled water(6-7 ml) is added aseptically to the culture, and the surface of the culture is scraped gently with a pipette to produce a spore and mycelial slurry.
- This is kept in sterile glass vial at 25°C and to retrieve a culture, 200-300µl of the suspension is removed from the vial and placed on fresh medium.

❑ STORAGE IN STERILE SOIL

- It is mainly applied for the preservation of sporulating microorganisms. *Fusarium*, *Penicillium*, *Alternaria*, *Rhizopus* etc. proved successful for store in sterile soil.
- Soil storage involves inoculation of 1ml of spore suspension into soil (autoclaved twice) and incubating at room temperature for 5-10 days.
- The initial growth period allows the fungus gradually to become dormant.
- The bottles are then stored at refrigerator.
- Viability of organisms found around 70-80 years.



■ Lyophilization (Freeze-drying)

- It is a vacuum sublimation technique.
- Freeze drying products are hygroscopic and must be protected from moisture during storage.
- By freezing the cells in a medium that contain a lyoprotectant (usually sucrose) and then pulling the water out using vacuum(sublimation), cells can be effectively preserved.
- Freezing must be very rapid, with the temperature lowered to well below 0°C (as such -20°C).
- Lyophilized cultures are stored in the dark 4°C in refrigerators.
- Many microbes preserved by this method have remained viable and unchanged in their characteristic more than 20 years.
- It is very advantageous as only minimal storage space is required to preserve.



■ **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 time.
- 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.
- By this method species can remain viable for 10-30 years without undergoing change in their characteristics.



● STORED IN SILICA GEL

- Microbes can be stored in silica gel powder at low temperature for a period 1-2 years.
- The basic principle in this technique is quick desiccation at low temperature, which allows the cell to remain viable for a long period of time.
- Some of the species which are preserved on anhydrous silica gel are such as- *Saccharomyces cerevisiae*, *Aspergillus nidulans*, *Pseudomonas denitrificans*, *Escherichia coli* etc.

CONCLUSION:

Whichever technique is 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 should be routinely checked to ensure their quality.

However preservation is essential as it reveals great importance in the field of science. Preservation helps in research purposes, industry as well as in biotechnological field.



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