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Techniques Involve in Preservation of Pure Culture

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Abstract
Pure culture is usually obtained from a mixed culture by transferring a small sample into new one. The maintenance and preservation of cultures of microbes requires very and careful attention. In this article, important techniques involved in preservation of pure culture have been outlined.

Introduction
A culture which is obtained from the solid nutrient medium that contains mixed population of microbes is known as pure culture. Pure culture may be defined as the process involving isolation and culturing of the microbial colonies. This method is used to isolate an individual or single colony from the mixed microbial population. It includes the transfer of a single cell to the fresh nutrient agar medium under aseptic conditions. The isolation techniques of the pure culture were developed by Robert Koch during the mid- 19th century.

Types of Pure Culture

Why Pure Culture is done?
- It maintains the characteristics of microbial cell.
- It helps to preserves the viability of culture.
- The culture which is preserved can be used for various taxonomic studies or research purposes.
- Helps in assessing tools for designing vaccine and various industrial productions.

Techniques Involve in Preservation of Pure Culture

1.	REFRIGERATION
2.	PARAFFIN COATING
3.	CRYOPRESERVATION
4.	LYOPHILIZATION

1. **Refrigeration:** Temperature ranges between 0-4°C under refrigeration, pure culture can be preserved. Through streaking the discrete colony of microbes from the solid nutrient medium, slant cultures are prepared. Through refrigeration, cultures of bacteria can be preserved up to 2-3 weeks and a culture of fungal for 3-4 months. The metabolic activity of the microorganisms can be slowed down by temperature of refrigeration but do not

stop it completely. Thus, the microbes slowly utilize the nutrient medium, increase in number and finally die due to the nutrient scarcity and accumulation of toxic wastes.

2. **Paraffin Coating:** This method involves the covering of microbial culture with paraffin or mineral oil. For full covering the growth of microbes, mineral oil should be added up to the slant height. It preserves the culture of cell for 1 to 2 years. It is very simple and in-expensive technique. Slant cultures should be store at room temperature with sterile liquid paraffin as it maintains the moisture content of the nutrient medium.

3. **Cryopreservation:** This method preserves the suspension of culture of microbes by applying various cryopreservative agents *ie*., dimethyl sulfoxide and glycerol. In this method, small vials are used to preserve the culture of microbes at low temperature. There are two types of cryo-freezers:
    a. **LIQUID NITROGEN FREEZERS** (preserves cell culture in liquid or vapour phase at a freezing temperature of -196°C).
    b. **MECHANICAL CRYOGENIC FREEZERS** (preserves cell culture viability at -150°C**).**

    The viability of cell under cryopreservation ranges between 10 to 30 years. It is a costly method.

4. **Lyophilization:** This process also maintains or preserves a dense suspension of cell of microbial culture. This method involves the use of lyoprotectant (sucrose) to stabilize the culture of microbial cell. In this method, the culture of cell is freeze at a temperature range between -60 to -78 °C. After that, sublimation of cell culture is done that helps in dehydrating the moisture content of the microbial sample by vacuuming. It helps in maintaining the viability of cell for more than 30 years. The advantage of this method is that it covers less space for storage and their transportation to other labs becomes easy. But this method is cost-effective and requires special reagents and equipments.

Conclusion

Lastly, we can conclude that in maintaining integrity of cell, characteristics, and functioning, bio preservation plays an important role. Thus, preservation of culture efficiently maintains the cells when present in dormant state by evading the growth of variants and contaminants.