ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 11| Issue: 7| July 2025|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2025: 8.691|| ISI Value: 1.188

SUB-CULTURING AND CRYOPRESERVATION OF MICROBIAL CULTURES: TECHNIQUES AND APPLICATIONS

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ABSTRACT

Microorganisms are fundamental tools in biological research and biotechnology and it's Applications to maintain their viability and genetic stability,More efficient preservation techniques are essential for this sub culture and cryopreservation of microbial Culture. This paper reviews two fundamental methods – (a)sub-culturing (b)cryopreservation – focusing on their methodologies, advantages, limitations, and applications. Sub-culturing is a widely practiced short-term method for routine laboratory use, whereas cryopreservation serves as a long-term strategy to minimize genetic drift and maintain microbial stocks. Understanding the principles and protocols of both methods is crucial for microbiologists, biotechnologists, and researchers handling microbial resources.The preservation of this biological material has achieved the safeguarding of industrial potential and the possibility of researching new functions and the use for the benefit of humanity. Various conservation and preservation methods have been developed over the years, such as: sterile distilled water, cryopreservation, freeze-drying, sub-culture, and sterile mineral oil, the use depending on the microorganism and the desired viability and preservation time for long periods.The most used conservation methods are cryopreservation and freeze-drying, guaranteeing the stability of the characteristics of the strain (their viability, their vitality, and their biological potential) over time; however, the need to continue evaluating different methods.

IMPORTANT WORDS:- *Sub-Culturing, Cryopreservation,Microbial Strain,Phenotypic and Genotypicstrain, Microorganisms , Fungus,Biotechnology.*

1. INTRODUCTION

Preservation of microbial cultures is a vital aspect of microbiology and biotechnology. Microbial strains must be maintained in a viable and genetically stable state to ensure consistent results in research, diagnostics, and industrial applications. Two commonly used strategies for culture maintenance are sub-culturing and cryopreservation. While sub-culturing is simple and suitable for short-term use, cryopreservation is preferred for long-term storage due to its ability to retain phenotypic and genotypic traits over extended periods.In order to ensure controlled conditions and for a specific time (long or short), it is necessary not only to preserve the strain but also the viability, characteristics, and safeguarding of all properties such as morphology, growth rate, and stability of the preserved strains.

2. SUB-CULTURING

Definition and Principle:-

Sub-culturing (also known as passaging or subcultivation) is the process of transferring microorganisms or cells from one culture medium to another to maintain their growth and viability.

2.1 Purpose in Cryopreservation during sub culture

Ensure viability and Actively growing cells survive freezing better to maintain purity of Sub-culturing helps remove contaminants before storage.Standardize growth phase Cultures are preserved during the log phase for optimal recovery to prepare for cryoprotectant addition (e.g., glycerol, DMSO).

Important Steps Involved in sub culture processing:-

1.Select a healthy, pure culture.
2. Sub-culture to fresh medium to rejuvenate cells.
3. Incubate until log phase (actively growing).
4. Harvest cells and mix with cryoprotectant.

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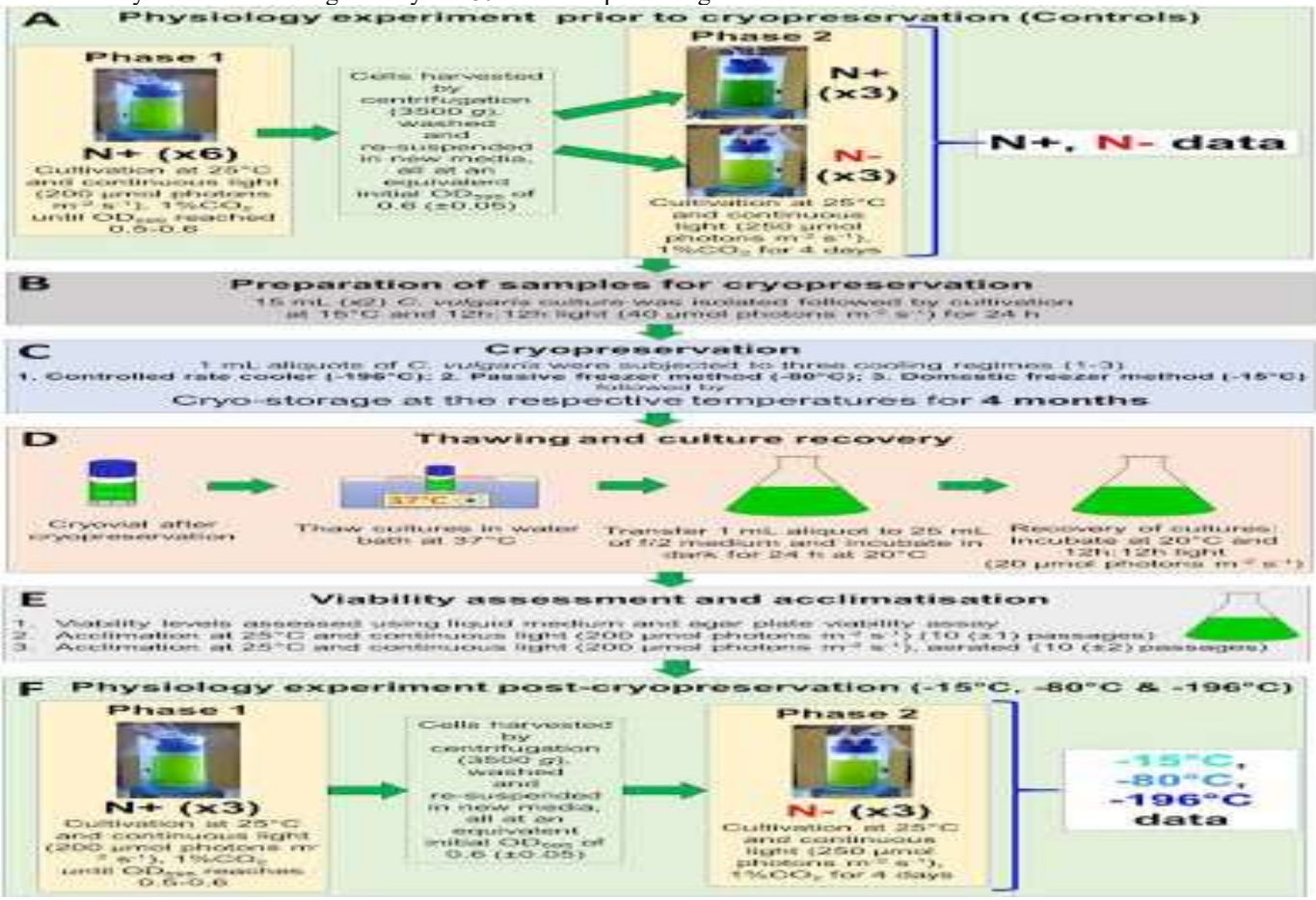
5. Transfer to cryovials and freeze gradually to –80°C or in liquid nitrogen.



2.2 METHODOLOGY

•Use sterile techniques to transfer a small portion of the culture.
•Freshly prepared media is inoculated using a sterile loop or pipette.
•Incubate the culture under suitable conditions (temperature, oxygen, etc.).
Monitor growth and repeat sub-culturing at appropriate intervals .
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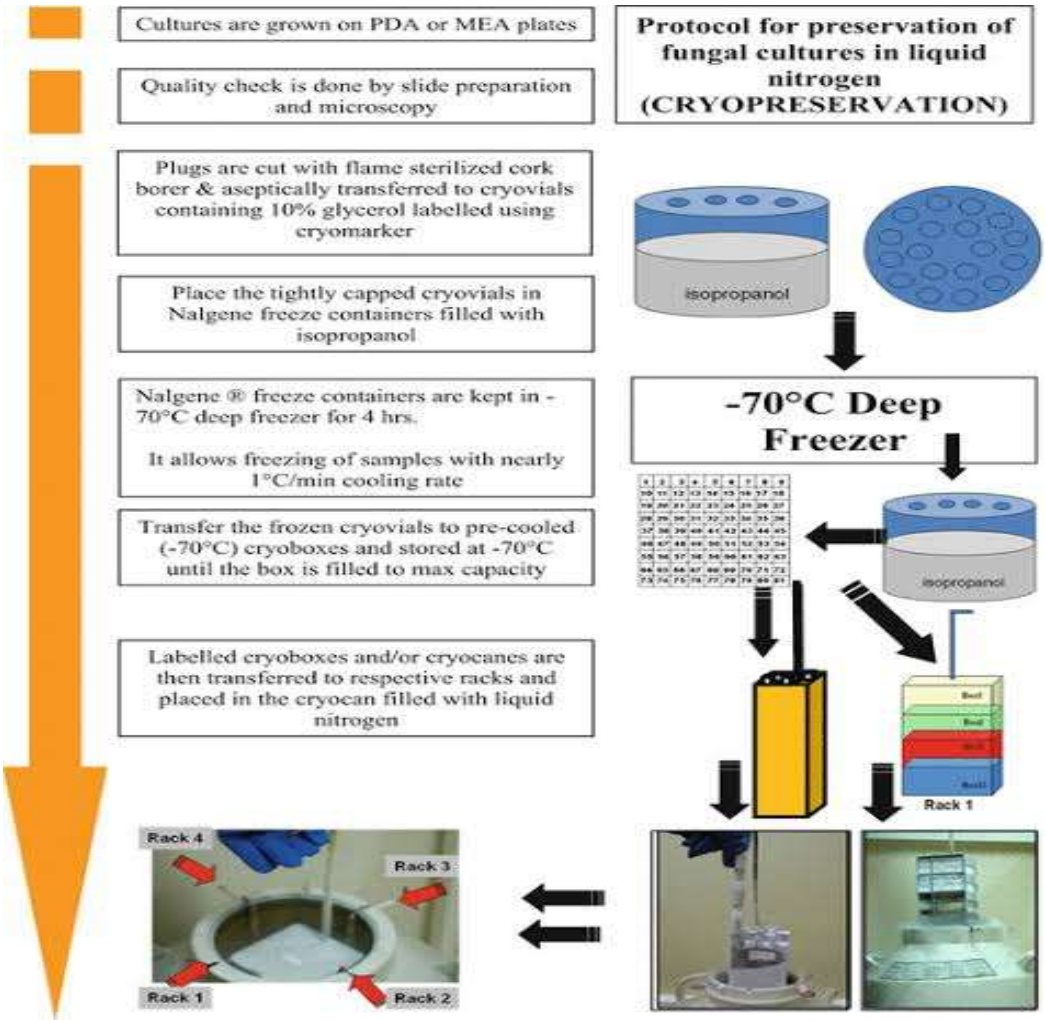

2.3 APPLICATION

Routine maintenance of bacterial, fungal, and cell cultures.Preparation of cultures for experimental or diagnostic use.Isolation and purification of microbial strains.

2.4 LIMITATIONS

Risk of contamination.

Genetic drift or mutation due to repeated culturing.Labor-intensive and time-bound

3. CRYOPRESERVATION

3.1 Definition and Principle:-

Cryopreservation involves storing biological materials at ultra-low temperatures to halt metabolic activity, preserving the structural and functional integrity of cells.

3.2 CRYOPROTECTANTS

Glycerol (10–20%): Prevents ice crystal formation; widely used for bacteria.

DMSO (5–10%): Effective for mammalian and some yeast cells.

Sugars (sucrose, trehalose): Stabilize membranes during freezing.

3.3 CRYOPRESERVATION PROTOCOL

1. Grow culture to exponential phase.
2. Mix with cryoprotectant in sterile cryovials.
3. Cool gradually (e.g., −1°C/min).
4. Store in:−80°C for short to medium-term.Liquid nitrogen (−196°C) for long-term storage.

3.4 Revival

Rapid thawing (37°C water bath).Transfer to appropriate growth medium.

Incubate and monitor for recovery.

3.5 Advantages:-Long-term storage with minimal genetic changes.

Reduced labor and contamination risk.High viability upon revival.

3.6 More Challenges

Expensive equipment (e.g., liquid nitrogen tanks).

Risk of ice crystal damage if not cryoprotected properly.

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4. COMPARISON OF SUB-CULTURING AND CRYOPRESERVATION

Feature: -Sub-Culturing&Cryopreservation

Storage Duration:-Short-term (days to weeks),Long-term (months to years)
Labor Requirement :- High Low
Contamination Risk:- High Low
Genetic Stability:- Low (with repeated passaging) High
Cost and Equipment Low High

5. APPLICATIONS IN RESEARCH AND INDUSTRY

Microbiology Research: Maintaining rstrains.
Clinical Microbiology: Preserving pathogenic isolates.
Biotechnology: Stocking genetically modified strains.
Pharmaceuticals: Quality control of microbial production strains.

6. CONCLUSION

Both sub-culturing and cryopreservation are essential in microbiological and biotechnological workflows. While sub-culturing allows easy and quick access to cultures, it carries a risk of genetic variation over time. Cryopreservation offers a superior method for long-term maintenance with preserved genotypic and phenotypic characteristics. Laboratories should employ both methods in combination to balance accessibility and stability of microbial strains.

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