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Chapter 12

Paraffin preservation of microbial cultures: Techniques, mechanisms, and long-term storage applications

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1. Introduction:

Preservation of microbial cultures is essential in microbiology for maintaining genetic stability and ensuring reproducibility of experiments. The paraffin method is a simple and effective technique used for long-term preservation of microbial cultures (Tripathi et al., 2018; Tortora et al., 2020). This method involves embedding a microbial culture in a layer of molten paraffin wax, which provides physical protection and creates an anaerobic environment, thus preventing desiccation and microbial contamination (Smith et al., 2014). Here's a detailed overview of the preservation of microbial cultures using the paraffin method:

1. **Physical Encapsulation:** The microbial culture is encapsulated within a layer of molten paraffin wax, forming a protective barrier against physical damage and environmental stressors.
2. **Anaerobic Environment:** The paraffin wax layer creates an anaerobic environment, which inhibits the growth of aerobic microorganisms and reduces oxidative damage to the preserved culture.
3. **Moisture Retention:** Paraffin wax helps retain moisture within the preserved culture, preventing desiccation and maintaining the viability of microbial cells over extended periods.
4. **Temperature Stability:** Paraffin wax provides thermal insulation, helping to maintain a stable temperature within the preserved culture, which is crucial for long-term viability.

2. Materials Required:

1. Pure culture of the microorganism
2. Sterile nutrient agar plates
3. Sterile test tubes with nutrient broth
4. Sterile distilled water
5. Sterile mineral oil

6. Paraffin wax
7. Bunsen burner or other flame source
8. Incubator set to appropriate temperature for the microorganism

3. Procedure: (Maloy et al., 2007)

1. Prepare sterile nutrient agar plates according to standard microbiological techniques.
2. Inoculate a single colony of the pure culture onto the surface of a nutrient agar plate using a sterile inoculating loop. Streak the culture for isolation if necessary.
3. Until colonies are clearly separated and visible, incubate the plate at the proper temperature and conditions needed for the microorganism's growth.
4. Select a well-isolated colony and inoculate it into a sterile test tube containing nutrient broth. Incubate the broth culture until it reaches the desired growth phase (usually mid-log phase).
5. Prepare cryoprotective solution by mixing equal volumes of sterile distilled water and sterile mineral oil.
6. Add a few drops of cryoprotective solution to the broth culture containing the microorganism and mix gently to ensure even distribution.
7. In a sterile environment, transfer the cryoprotected culture into a sterile test tube.
8. Melt paraffin wax in a water bath or similar apparatus to ensure sterility. Maintain the temperature just above the melting point of the wax.
9. Once the paraffin wax is melted, pour a layer of the molten wax over the surface of the culture in the test tube. Ensure that the entire surface is covered with a layer of paraffin wax to prevent contamination.
10. Allow the paraffin wax to solidify at room temperature or in a refrigerator.
11. Put the name of the microbe, the date of preservation, and any other pertinent information on the test tube's label.
12. Store the sealed test tube in a refrigerator or other suitable storage conditions appropriate for the long-term preservation of the microorganism.

4. Precautions:

1. Ensure all equipment and materials are sterile to prevent contamination of the microbial culture.
2. Handle paraffin wax and embedding materials carefully to avoid burns and maintain cleanliness.
3. Use appropriate fixatives and methods for preserving microbial cultures prior to embedding in paraffin wax.
4. Embed microbial cultures gently and evenly in paraffin wax to ensure uniform preservation.

5. Control embedding temperature to prevent overheating or rapid cooling, which could damage microbial structures.
6. Store paraffin-embedded microbial cultures in a dry, cool environment to prevent mold growth or degradation.
7. Label embedded cultures clearly with relevant information such as date, organism type, and preservation method.
8. Monitor embedded cultures periodically for signs of deterioration or contamination.
9. Use proper disposal methods for unused paraffin wax and contaminated materials according to laboratory safety protocols.

5. References:

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