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Exercise 4
Isolation into Pure Culture of a Bacterial Species

Introduction

A **pure culture** is a culture of cells containing only a single species. A number of procedures are available for isolation of pure cultures from mixed populations of microbes. A pure culture may be isolated using special medium with specific chemical or physical agents that allow selection of one organism over another. Methods for isolation of a pure culture include: (i) **SPREAD PLATING** on solid agar medium with a glass spreader and (ii) **STREAK PLATING** with a loop and (ii) **POUR PLATING**. The purpose of these is to **isolate** individual bacterial cells (colony-forming units) on a nutrient medium.

Objective

To isolate a single, pure bacterial species from a mixed culture using the streak plate method.

Materials

- Cultures: from spread and pour plating cultures
- Nutrient agar plates
- Inoculating loop
- Alcohol lamp
- Sterile gloves
- Disinfectant (e.g., 70% ethanol)
- Marker or wax pencil
- Incubator (set at 37°C or appropriate temperature)

Safety Precautions

1. Wear gloves and a lab coat to prevent contamination.
2. Use a alcohol carefully to sterilize the loop.
3. Work near the flame to maintain sterility.

Procedure

Part 1: Preparatio
1. Label the bottom of the agar plate with your name, date, and type of sample.
2. Sterilize the workspace with disinfectant.

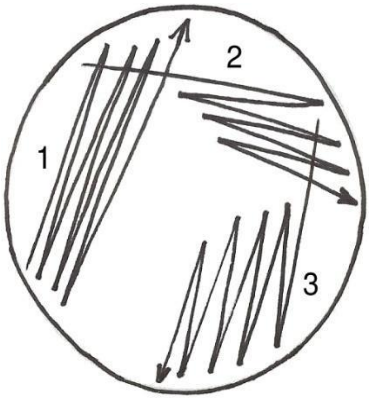
Part 2: Streak Plate Method
1. Sterilize the Loop:
   - Heat the inoculating loop in the Bunsen burner flame until red-hot. Allow it to cool for a few seconds.

2. Obtain the Inoculum:
   - Dip the cooled loop into the mixed culture tube or solution.

3. Streak the Plate:
   - Divide the plate into three quadrants visually or draw a guide on the underside.
   - Streak quadrant 1 by gently dragging the loop back and forth across the agar surface.
   - Re-sterilize the loop.

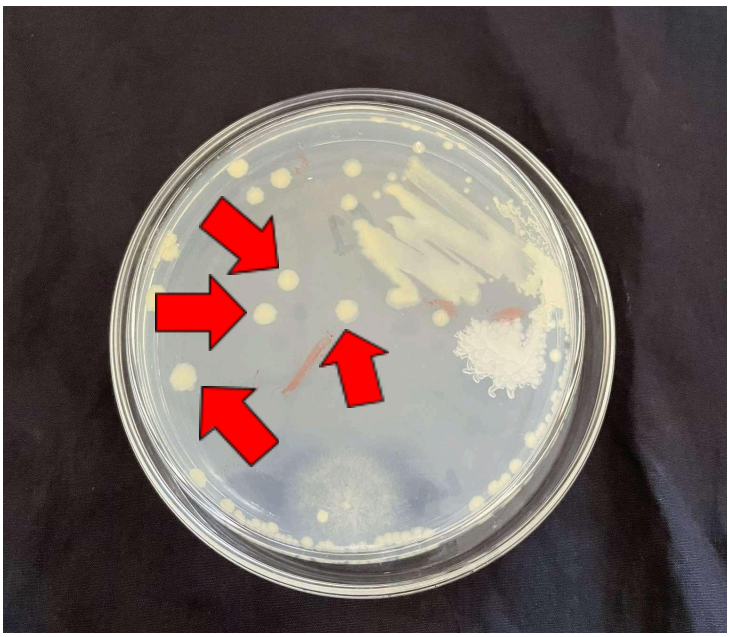
- Drag the loop from quadrant 1 into quadrant 2 and streak. Repeat for quadrant 3, sterilizing the loop each time.

4. Incubation:
   - Place the agar plate in the incubator upside down.
   - Incubate for 24–48 hours at the appropriate temperature for the bacterial species.



3-way Streak Technique

Part 3: Observations and Results
1. Observe the plate after incubation.
   - Look for well-isolated colonies in the final quadrants. (Provide pictures)



2. Select a discrete colony and describe it as follows:
a. Form: Circular

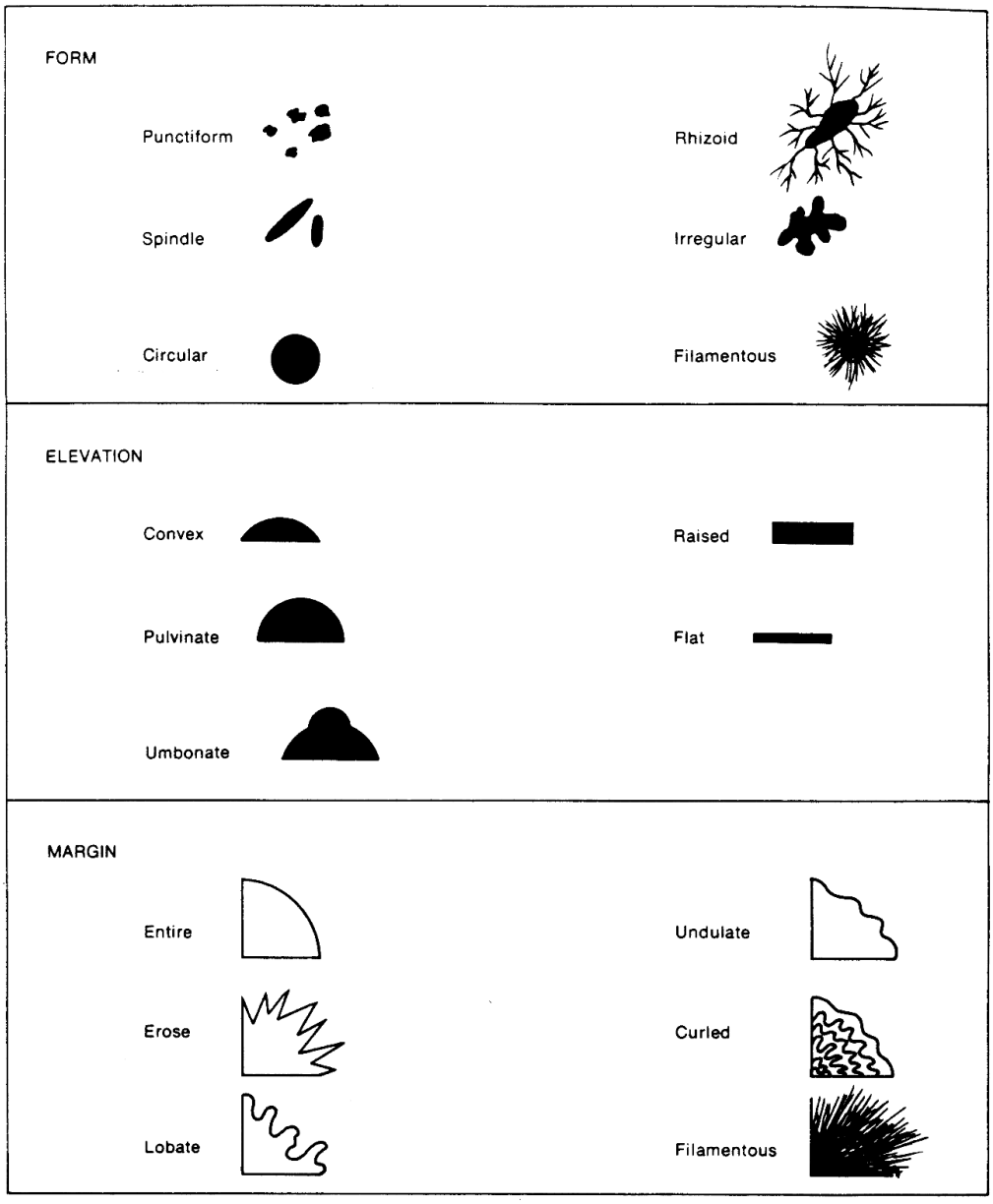
b. Elevation: Convex

c. Pigmentation: Pale yellow

d. Margin: Entire

COLONIAL MORPHOLOGY

Both colonial and cellular morphology are characteristic of each species of bacteria and are sometimes useful in the identification of an unknown microorganism. When a bacterium grows on a solid agar surface, the number of cells increases until a visible mass of cells, called a colony, appears. It is usually inferred that each colony arises from the division of a single cell. The most useful culture characteristics are morphology, size and pigmentation of the colony. The figures presented below illustrate some of the morphological characteristics of bacterial colonies and provide helpful terminology for the description of colony morphology.



Discussion Questions

1. What is a colony? Can you consider it a pure culture? How do you develop pure working cultures from a colony?

   A colony is a visible growth of microorganisms on a solid growth medium. It can be considered a pure culture if it originates from a single microbial cell called inoculum. To obtain pure working cultures from a colony, you can use the streak plate technique.

   Colonies formed by different bacteria can be very different from the bacterial species that formed them. Consequently, examining a characteristic known as colonial morphology—the way the colonies look on an agar plate or slant—is a helpful first step in identifying bacteria. Although it is ideal to make these conclusions by examining a single colony, some colonial traits, such the texture and color of the bacterial growth, can still be described if colonial growth is more prevalent and single colonies are not present.

2. What is the purpose of streaking in quadrants?

   The streak plate method involves inoculating the sample across a plate in overlapping quadrants. This dilutes the inoculum and isolates individual colonies, allowing you to select and further culture a single, pure colony.

   First, the petri plate is divided into three or four sections or zones, by then, creating sequential streaking zones on the petri plate and flaming the inoculation loop in between zones, the quadrant streak plate reduces or dilutes the quantity of cells. Only the first section or zone receives a full strength of bacteria directly from the inoculum. The rest are overlapping fragments of the previously streaked zone.

3. How does sterilizing the loop between streaks help in isolating pure colonies?

   Full-strength bacteria are deposited from the source in the first section. Then, it is followed by sterilization of the inoculating loop. No further cells are later added to the agar surface. The sterile loop is then used to spread or streak out the cells that have already been previously added by overlapping. Sterilizing the inoculation loop between quadrants prevents cross-contamination and ensures each section is inoculated with only the desired microorganism.

4. What are some potential sources of contamination during the process?

   Potential sources of contamination during the isolation process include improper aseptic technique, introduction of unwanted microorganisms, and the choice of isolated inoculum. Poor technique, such as not properly sterilizing the inoculation loop or working near open flames, can lead to the transfer of contaminants onto the agar plate. Also, airborne microbes in the surrounding environment can settle onto the plate and grow, interfering with the isolation of the target organism. Additionally, the choice of isolated inoculum could play a role with contamination as maybe it was not isolated enough to become a pure culture and the growth of bacteria in the plate interferes with their modes of living.

5. Why is it important to incubate the plate upside down?

   Incubating the plate upside-down is an important step to prevent condensation or moisture from forming on the agar surface. If moisture develops, it can drip down and spread the microorganisms, causing cross-contamination between the isolated colonies. By inverting the plate, the condensation forms on the lid rather than directly on the agar, minimizing the risk of colony disruption or unwanted mixing of the microbes. This helps ensure the integrity of the isolated colonies and facilitates the selection of a pure culture.