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Obtaining pure culture of microorganism

It involves the common plating techniques employed in microbiology

1. Streak Plate Method
2. Spread plate method
3. Pour Plate Method.

1. Streak Plate Method

- This method was developed by two bacteriologists, Loeffler and Gaffkey in the laboratory of Robert Koch.

- In this method a sterilized inoculating loop or transfer needle is dipped into a suitable diluted suspension of microorganisms which is then streaked on the surface of an already solidified agar plate to make a series of parallel, non-overlapping streaks. The process is known as streaking and the plate so prepared is called a streak plate.
- The main objective of the streak plate method is to produce well separated colonies of bacteria from concentrated suspensions of cells.

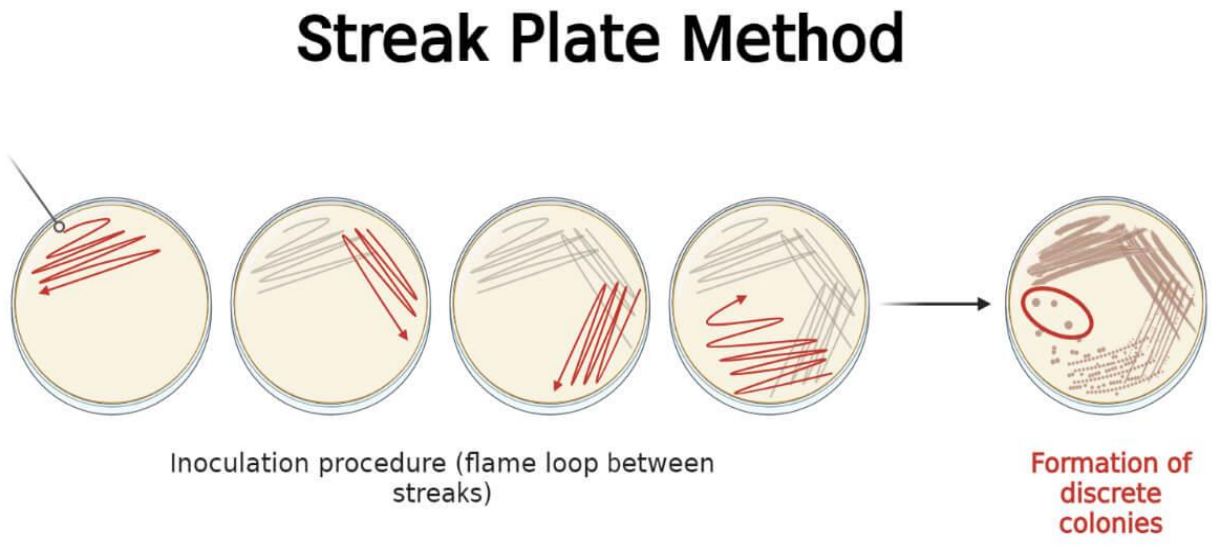
- A sterilized inoculating needle with a loop made up of either platinum or nichrome wire is used for streaking.

- One loopful of specimen is transferred onto the surface of the agar plate in a sterile petri dish and streaked across the surface in the form of a zig-zag line. This process is repeated thrice to streak out the bacteria on the agar plate so

that some individual bacteria are separated from each other.

- The first streak will contain more organisms than the second and the second more than the third and so on. The last streaks should thin so on. The last streaks should thin out the culture sufficiently to give isolate colonies. The successful isolation depends on spatial separation of single cells.

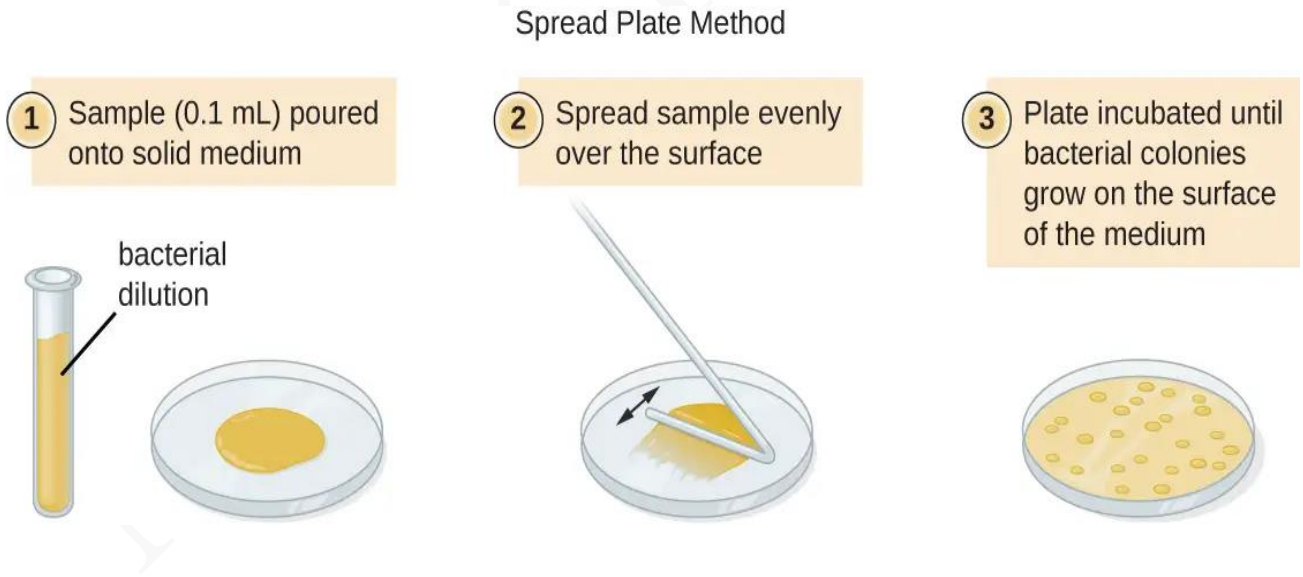
- Each colony usually represents the growth from a single organism when such a plate is incubated colonies will appear on the surface of the medium. Because of the high concentration of water in agar, some water of condensation forms in petri plate during incubation.
- Moisture is likely to drip from the cover to the surface of the agar and spread out, resulting in a confluent mass of growth and running individual colony formation.
- To avoid this, petri plates are routinely incubated bottom side up. Pure colonies can be obtained from well isolated colonies by transferring a small portion of each to separate culture media.



2. Spread Plate Method

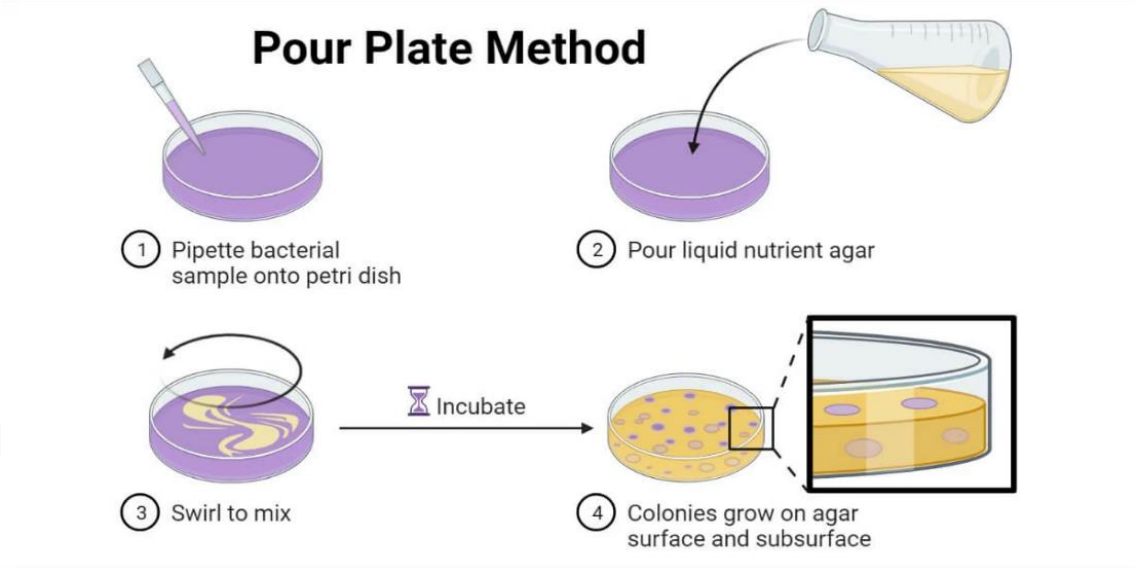
- The spread plate technique is used for the separation of a dilute, mixed population of the microorganisms so that individual colonies can be isolated. means mixed culture of various microorganism.
- In this technique, a small volume (A drop) of dilute microbial mixture is transferred to the center of an agar plate and spread evenly over the surface with a sterile L-shaped bent glass rod, while the petri dish is spun, at some stage, single cells will be deposited with the bent glass rod on the agar surface.
- As a result of separation of individual microorganism by spreading over the drop of dilution liquid on the medium of the plate.
- Incubate the agar plate at 37ºC for 24 hours, in the inverted position. The dispersed cells will develop into isolated colonies. Because the number of colonies will be equal to the number of viable organisms in the sample spread plates can be used to count the microbial population.

- Isolated colonies are picked up and transferred onto fresh medium to ensure purity



3. Pour Plate Method

- The Pour Plate Method is a microbiological technique used to estimate the number of viable microorganisms in a liquid sample and to observe their growth under both aerobic and anaerobic conditions.
- In this method, a measured volume of the liquid sample is mixed with molten agar that has been cooled to approximately 45-50°C.
- The mixture is then poured into a sterile petri dish and allowed to solidify. As the agar solidifies, the microorganisms are embedded within the medium, leading to the formation of colonies both on the surface and throughout the agar.
- This technique allows for the enumeration of microorganisms by counting the colonies that develop, which represent viable cells from the original sample.
- The Pour Plate Method is particularly useful for detecting and counting microorganisms that may not grow well on the surface of an agar plate or that require different oxygen levels for optimal growth.



4. Serial Dilution Method

The **Serial Dilution Method** is a technique used in microbiology and chemistry to reduce the concentration of a substance in a solution step-by-step to obtain a range of concentrations. This method is particularly useful for estimating the concentration of microorganisms in a sample, determining the effective dilution range for assays, or preparing solutions for further experimentation. Here's an overview of the process:

1. **Preparation**:
   - **Stock Solution**: Begin with a concentrated solution or suspension of microorganisms or solute. This is often referred to as the stock solution.
   - **Diluent**: Prepare a diluent (e.g., sterile water or buffer) to use in the dilution process.
2. **Initial Dilution**:
   - Take a measured volume of the stock solution and mix it with a specific volume of diluent in a new container. For instance, if you start with 1 mL of stock solution and add it to 9 mL of diluent, you achieve a 1:10 (or 10^−1) dilution.
3. **Subsequent Dilutions**:
   - From this first diluted solution, take another measured volume and mix it with a fresh volume of diluent to achieve the next dilution level. Repeat this process multiple times to create a series of diluted solutions. Each step typically involves transferring 1 mL of the previous dilution into 9 mL of fresh diluent, resulting in a series of tenfold dilutions (1:10, 1:100, 1:1000, etc.).
4. **Plating and Analysis**:
   - Once the dilutions are prepared, a measured volume of each dilution is plated onto an agar medium using a method such as the pour plate or spread plate technique. This allows the growth of colonies from each dilution to be observed.
   - After incubation, colonies can be counted to estimate the concentration of microorganisms in the original stock solution. The dilution factor and the number of colonies help calculate the concentration of viable organisms per unit volume.
5. **Purpose and Applications**:
   - The Serial Dilution Method is used to accurately quantify microbial populations, assess the effectiveness of disinfectants, prepare standardized solutions, and perform various quantitative assays. It helps in obtaining manageable numbers of colonies that are easy to count and analyze.

Serial Dilution