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Notes

Introduction

A pure culture theoretically contains a single bacterial species. There are a number of procedures available for the isolation of pure cultures from mixed populations. A pure culture may be isolated by the use of special media with specific chemical or physical agents that allow the enrichment or selection of one organism over another. Simpler 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. The purpose of spread plating and streak plating is to isolate individual bacterial cells (colony-forming units) on a nutrient medium. Both procedures (spread plating and streak plating) require understanding of the aseptic technique. Asepsis can be defined as the absence of infectious microorganisms. However, the term is usually applied to any technique designed to keep unwanted microorganisms from contaminating sterile materials.

Isolation of Pure Culture

Microorganisms are generally found in nature (air, soil and water) as mixed populations. Even the diseased parts of plants and animals contain a great number of microorganisms, which differ markedly from the microorganisms of other environments. To study the specific role played by a specific microorganism in its environment, one must isolate the same in pure

culture. Pure culture involves not only isolation of individual microorganisms from a mixed population, but also the maintenance of such individuals and their progenies in artificial media, where no other microorganisms find way to grow. It is necessary to make the colonies well-isolated from each other so that each appears distinct, large and shows characteristic growth forms. Such colonies may be picked up easily and grown separately for detailed study. Several methods for obtaining pure cultures are in use. Some common methods are in everyday-use by a majority of microbiologists, while the others are methods used for special purposes.

1. **Serial Dilution method**

Serial dilution, as the name suggests, is a series of sequential dilutions that are performed to convert a dense solution into a more usable concentration. Serial dilution is the process of stepwise dilution of a solution with an associated dilution factor.The objective of the serial dilution method is to estimate the concentration (number of organisms, bacteria, viruses, or colony forming units) of an unknown sample by enumeration of the number of colonies cultured from serial dilutions of the sample.In serial dilution, the density of cells is reduced in each step so that it is easier to calculate the concentration of the cells in the original solution by calculating the total dilution over the entire series.Serial dilution involves the process of taking a sample and diluting it through a series of standard volumes of sterile diluent, which can either be distilled water or 0.9 % saline.Then, a small measured volume of each dilution is used to make a series of pour or spread plates.

The dilution factor in a serial dilution can be determined either for an individual test tube or can be calculated as a total dilution factor in the entire series.
The dilution factor of each tube in a set:

$$\frac{volume\ of\ sample}{volume\ of\ sample + volume\ of\ diluent}$$

For a ten-fold dilution, 1 ml of sample is added to 9 ml of diluent. In this case, the dilution factor for that test tube will be:

$$\text{Dilution factor} = \frac{1\ ml}{1\ ml + 9\ ml} = \frac{1}{10} = 10^{-1}$$

Example:

For the first tube, dilution factor = 10^{-1} (1 ml added to 9 ml)

For the second tube, dilution factor = 10^{-1} (1ml added to 9 ml)

Total dilution factor = previous dilution × dilution of next tube= total dilution of $10^{-1} \times 10^{-1} = 10^{-2}$

2. Streak plate method

Streak plate technique is used for the isolation into a pure culture of the organisms (mostly bacteria), from a mixed population. The inoculum is streaked over the agar surface in such a way that it "thins out" the bacteria. Some individual bacterial cells are separated and well-spaced from each other.As the original sample is diluted by streaking it over successive quadrants, the number of organisms decreases. Usually, by the third or fourth quadrant, only a few organisms are transferred which will give discrete colony forming units (CFUs).The sample/inoculum is diluted by streaking it across the surface of the agar plate. While streaking in successive areas of the plate, the inoculum is diluted to the point where there is only one bacterial cell deposited every few millimeters on the surface of the agar plate. When these lone bacterial cells divide and give rise to thousands and thousands of new bacterial cells, an isolated colony is formed. Pure cultures can be obtained by picking well-isolated colonies and re-streaking these on fresh agar plates.

3. Pour plate Method:

Pour plate method is usually the method of choice for counting the number of colony-forming bacteria present in a liquid specimen. In this method, fixed amount of inoculum (generally 1 ml) from a broth/sample is placed in the center of sterile Petri dish using a sterile pipette. Molten cooled agar (approx. 15mL) is then poured into the Petri dish containing the inoculum and mixed well. After the solidification of the agar, the plate is inverted and incubated at 37°C

for 24-48 hours.Microorganisms will grow both on the surface and within the medium. Colonies that grow within the medium generally are small in size and may be confluent; the few that grow on the agar surface are of the same size and appearance as those on a streak plate. Each (both large and small) colony is carefully counted (using magnifying colony counter if needed). Each colony represents a "colony forming unit" (CFU).

The number of microorganisms present in the particular test sample is determined using the formula: **CFU/mL= Number of colonies X dilution factor**
Volume of the sample

For accurate counts, the optimum count should be within the range of 30-300 colonies/plate. To insure a countable plate a series of dilutions should be plated.The pour plate method of counting bacteria is more precise than the **streak plate method**, but, on the average, it will give a lower count as heat sensitive microorganisms may die when they come contact with hot, molten agar medium.

4. **Spread Plate Technique**

Spread plate technique is the method of isolation and enumeration of microorganisms in a mixed culture and distributing it evenly. The technique makes it easier to quantify bacteria in a solution.The spread plate technique involves using a sterilized spreader with a smooth surface made of metal or glass to apply a small amount of bacteria suspended in a solution over a plate. The plate needs to be dry and at room temperature so that the agar can absorb the bacteria more readily. A successful spread plate will have a countable number of isolated bacterial colonies evenly distributed on the plate.

5. **Enrichment Culture Method**

Generally, it is used to isolate those microorganisms, which are present in relatively small numbers or that have slow growth rates compared to the other species present in the mixed culture.The enrichment culture strategy provides a specially designed cultural environment by incorporating a specific nutrient in the medium and by modifying the physical conditions of the incubation. The medium of known composition and, specific condition of incubation favors the

growth of desired microorganisms but, is unsuitable for the growth of other types of microorganisms.