Pure Culture Techniques

Introduction

- A population of organisms cultivated in a medium is called as a culture.
- While a culture that only one species of microorganisms is known pure culture or A population of cells arising from a single cell is called as a pure Cuture.
- The natural ecosystems like soil, sewage, milk, urine contain mixed population of several species of microorganisms.
- Pure culture is needed for
  - Identification of species
  - Large scale production of any desired product
  - The Study of molecular Structure and biochemical characters of desired organism.
- Father of microbiology' 'Anton van Leeuwnhook in 1863 First time observed mixed flora in natural samples like faeces, urine, sewage etc.
- In the earliest period microbiologists had faced many problems because of contamination, during their research.
- Later on Joseph Lister a pioneer of aseptic surgery first time developed a method of isolation of single desired bacteria in pure form by Successive dilution of sample using a sterile Fluid.

Methods of Isolation of Pure Culture

I) Streak Plate Method

- This method is used most commonly to isolate pure cultures of bacteria.
- A small amount of mixed culture is placed on the tip of an inoculation loop/needle and is streaked across the surface of the agar medium.
- The successive streaks “thin out” the inoculum sufficiently and the micro-organisms are separated from each other.
- It is usually advisable to streak out a second plate by the same loop/needle without reinoculation.
- These plates are incubated to allow the growth of colonies.
- The key principle of this method is that, by streaking, a dilution gradient is established across the face of the Petri plate as bacterial cells are deposited on the agar surface.
- Because of this dilution gradient, confluent growth does not take place on that part of the medium where few bacterial cells are deposited.
- Presumably, each colony is the progeny of a single microbial cell thus representing a clone of pure culture.
- Such isolated colonies are picked up separately using sterile inoculating loop/needle and re-streaked onto fresh media to ensure purity.

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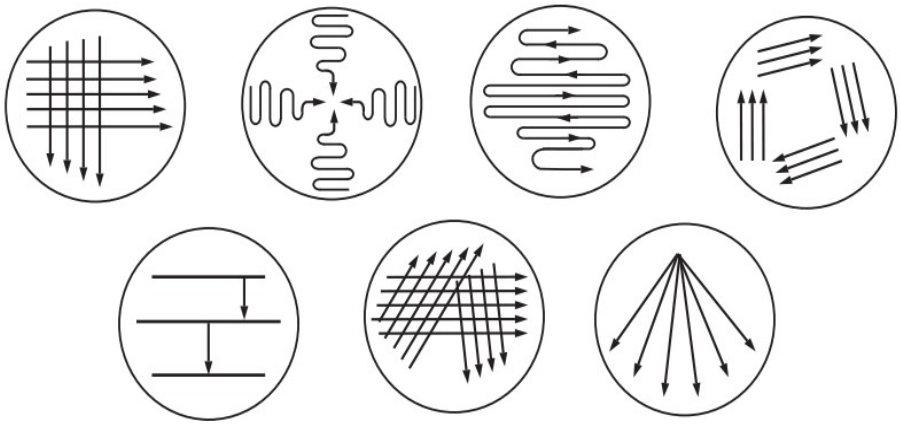
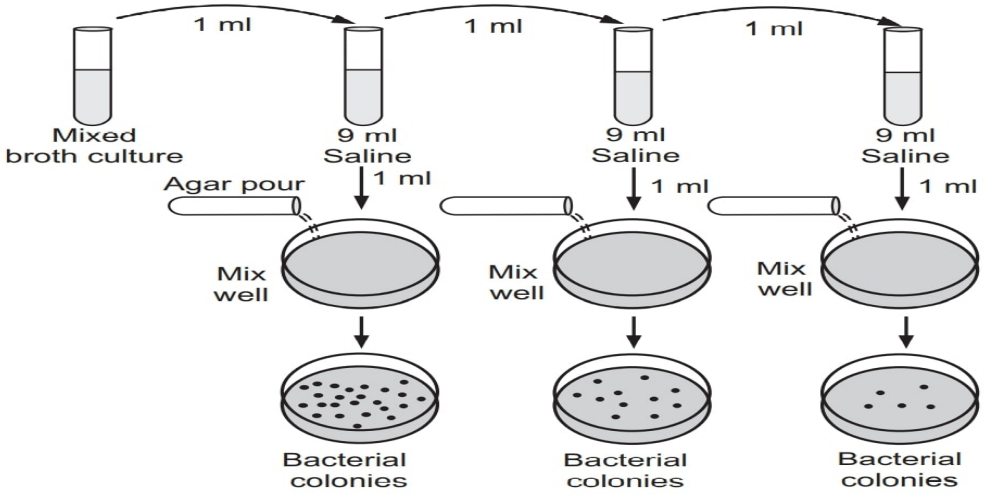


II) Pour Plate Method

- This method involves plating of diluted samples mixed with melted agar medium.
- The main principle is to dilute the inoculum in successive tubes containing liquefied agar medium so as to permit a thorough distribution of bacterial cells within the medium.
- Here, the mixed culture of bacteria is diluted directly in tubes containing melted agar medium maintained in the liquid state at a temperature of 42-45°C (agar solidifies below 42°C).
- The bacteria and the melted medium are mixed well.
- The contents of each tube are poured into separate Petri plates, allowed to solidify, and then incubated.
- When bacterial colonies develop, one finds that isolated colonies develop both within the agar medium (subsurface colonies) and on the medium (surface colonies).
- These isolated colonies are then picked up by inoculation loop and streaked onto another Petri plate to insure purity.



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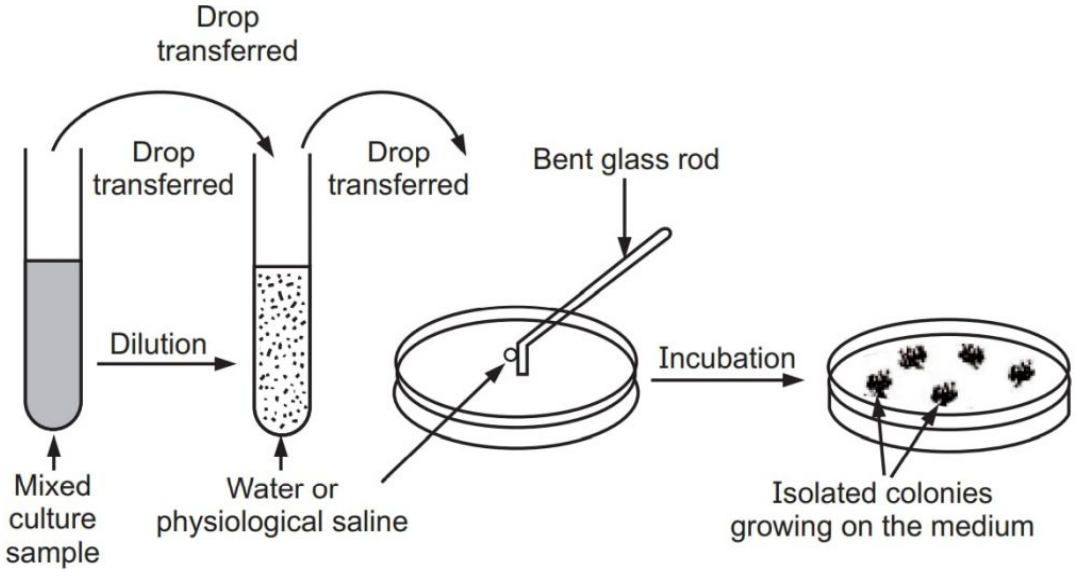
Pour Plate Method has Certain Disadvantages

- The picking up of subsurface colonies needs digging them out of the agar medium thus interfering with other colonies, and
- The microbes being isolated must be able to withstand temporary exposure to the 42-45° temperature of the liquid agar medium; therefore this technique proves unsuitable for the isolation of psychrophilic microorganisms.
- However, the pour plate method, in addition to its use in isolating pure cultures, is also used for determining the number of viable bacterial cells present in a culture.

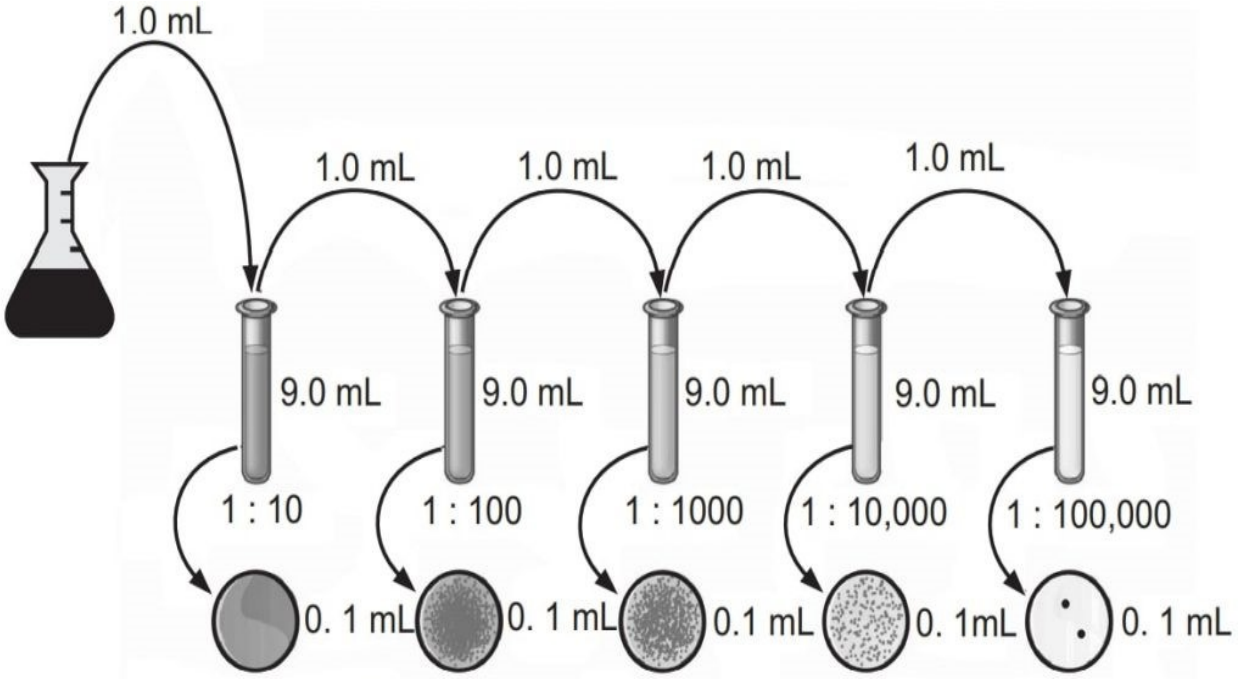
III) Spread Plate Method

- In this method, the mixed culture or microorganisms is not diluted in the melted agar medium (unlike the pour plate method); it is rather diluted in a series of tubes containing sterile liquid, usually, water or physiological saline.
- A drop of so diluted liquid from each tube is placed on the center of an agar plate and spread evenly over the surface by means of a sterilized bent-glass-rod.
- The medium is now incubated.
- When the colonies develop on the agar medium plates, it is found that there are some plates in which well-isolated colonies grow.
- This happens as a result of separation of individual microorganisms by spreading over the drop of diluted liquid on the medium of the plate.
- The isolated colonies are picked up and transferred onto fresh medium to ensure purity.
- In contrast to pour plate method, only surface colonies develop in this method and the microorganisms are not required to withstand the temperature of the melted agar medium.



IV) Serial Dilution Method

- As stated earlier, this method is commonly used to obtain pure cultures of those microorganisms that have not yet been successfully cultivated on solid media and grow only in liquid media.
- A microorganism that predominates in a mixed culture can be isolated in pure form by a series of dilutions.
- The inoculum is subjected to serial dilution in a sterile liquid medium, and a large number of tubes of sterile liquid medium are inoculated with aliquots of each successive dilution.
- The aim of this dilution is to inoculate a series of tubes with a microbial suspension so dilute that there are some tubes showing growth of only one individual microbe.
- For convenience, suppose we have a culture containing 10 ml of liquid medium, containing 1,000 microorganisms i.e., 100 microorganisms/ml of the liquid medium.
- If we take out 1 ml of this medium and mix it with 9 ml of fresh sterile liquid medium, we would then have 100 microorganisms in 10 ml or 10 microorganisms/ml.
- If we add 1 ml of this suspension to another 9 ml. of fresh sterile liquid medium, each ml would now contain a single microorganism.
- If this tube shows any microbial growth, there is a very high probability that this growth has resulted from the introduction of a single microorganism in the medium and represents the pure culture of that microorganism.



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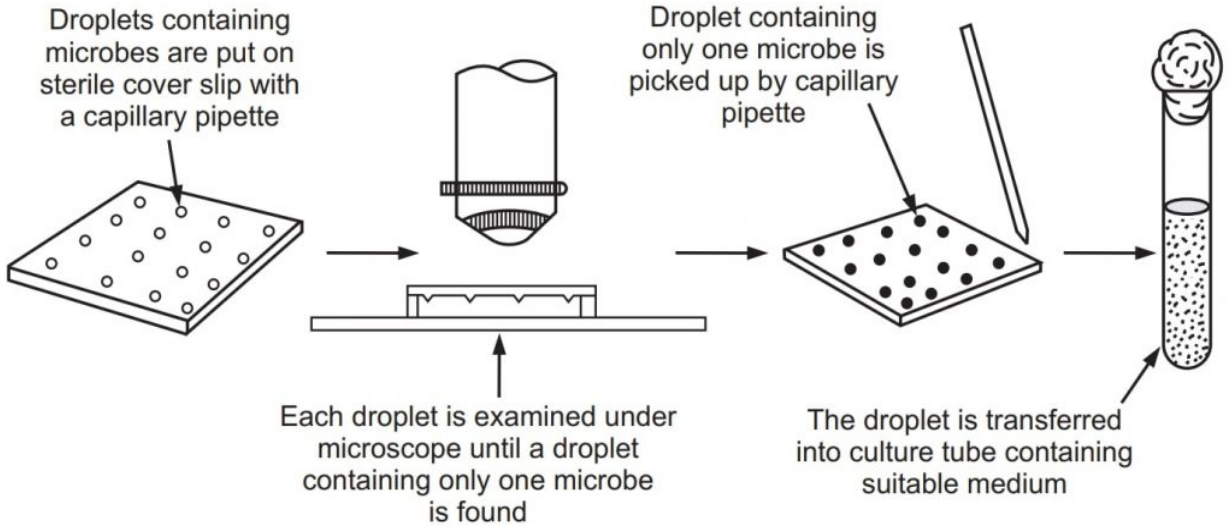
V) Single Cell Isolation Methods

An individual cell of the required kind is picked out by this method from the mixed culture and is permitted to grow. The following two methods are used in single cell isolation methods.

i) Capillary Pipette Method

- Several small drops of a suitably diluted culture medium are put on a sterile glass-coverslip by a sterile pipette drawn to a capillary.
- One then examines each drop under the microscope until one finds such a drop, which contains only one microorganism.
- This drop is removed with a sterile capillary pipette pour to fresh medium.
- The individual microorganism present in the drop starts multiplying to yield a pure culture.



ii) Micromanipulator Method

- Micromanipulators have been built, which permit one to pick out a single cell from a mixed culture.
- This instrument is used in conjunction with a microscope to pick a single cell (particularly bacterial cell) from a hanging drop preparation.
- The micro-manipulator has micrometer adjustments by means of which its micropipette can be moved right and left, forward, and backward, and up and down.
- A series of hanging drops of a diluted culture are placed on a special sterile coverslip by a micropipette.
- Now a hanging drop is searched, which contains only a single microorganism cell.
- This cell is drawn into the micropipette by gentle suction and then transferred to a large drop of sterile medium on another sterile coverslip.

- When the number of cells increases in that drop as a result of multiplication, the drop is transferred to a culture tube having suitable medium.
- This yields a pure culture of the required microorganism.
- The advantages of this method are that one can be reasonably sure that the cultures come from a single cell and one can obtain strains with in the species.
- The disadvantages are that the equipment is expensive, its manipulation is very tedious, and it requires a skilled operator.
- This is the reason why this method is reserved for use in highly specialised studies.

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