Aim 20 To Obtain Pure Culture of Microorganisms

Introduction

The study of microorganism colony characteristics, morphology, staining reactions, biochemical properties, susceptibility to various antimicrobial agents etc. can be done by isolating or separating the specific microorganism from the mixed populations (soil, air, water etc.) and cultivating or growing them in an artificial environment on culture medium.

A mixed culture is a culture that contains more than one kind of microbes.

A culture that has only one type of microbes is called a pure culture.
Pure cultures of microorganisms can be obtained by a number of methods.

The commonly used method is:

1. **Streak plate**
2. **Pour plate**
3. **Spread plate**

Pure culture can be obtained by the appearance of separate, discrete colonies growing on solid media from which transfer can be done.

Pure culture means growth of the colony took place from a single cell or we can say a pure medium consists of a population of only one species of micro organism, all derived from a single parent microorganism. The isolation of one kind of micro organism from a mixture of many different kinds is

called pure culture techniques. To determine the characteristics of a particular species of micro organism, It is essential that the organism should be isolated and grown in lab as pure culture.

(1). Streak plate method

Streak plates are prepared by streaking a little quantity of mixed culture over the surface of the solid medium in petri plates with a nichrome or platinum loop. The sample is streaked in such a way that the successive streaks thin out the culture. Then, plates are incubated at a suitable temperature. A colony is formed by replication of each bacterium that is visible to the naked eye and it may contain up to 10^9 copies of the original bacterium.

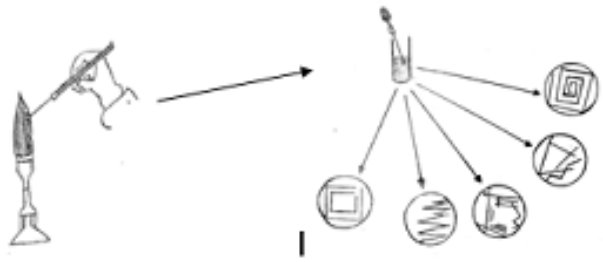
Requirements:

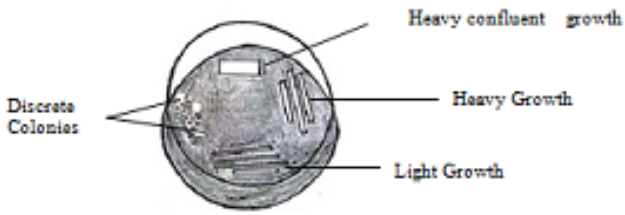
1. Sterile nutrient agar plates
2. Inoculating loop
3. Laminar air flow cabinet
4. Bunsen burner
5. Sample with a mixed population of microorganisms

Procedure

1. With left hand, hold the nutrient broth culture tube.
2. With right hand, hold the loop on the flame to sterilize it, then cool it.
3. With the help of little finger of right hand remove the cotton plug of the tube and flame the rim/neck of the tube.
4. With the help of loop, get out one loop full of culture from the tube.
5. Heat the rim/neck of the tube and replug it.
6. With left hand lift the petri plate cover and hold it an angle of 60°.
7. Put the inoculum on the agar surface and streak the lines across the surface of the agar.

8. Replace the lid of the petri plate.
9. Sterilize the loop by flaming on the burner.
10. At 28°C incubate the plates in an inverted position for 24-72 hours.





Results

At the first streak growth is seen to be dense and less growth has been noticed on the last streak, discrete colonies will be Discrete Colonies d at the end. The contamination is marked on that here the colony is not growing.

The loop softly pressed on agar surface for streaking, if not it will damage the agar surface.

1. Restrict the entery the area where the streaks have been made.
2. Before use inoculating loop should be sterilized and cool.
3. Cover of petri plate should not be lifted completely.

4. Plating of the medium should be done one day in advance of performing this exercise.
5. For preventing the collection of condensation on the agar surface the plates should be inoculated in an inverted position otherwise it becomes difficult to obtain discrete colonies.

(2). Spread plate method

In this, a serial dilution of mixed population inoculum is used that is transferred over the solid medium surface. Then, spread uniformly with a sterile bent glass rod. This technique is also used to separates the aggregates of cells in the sample. The transferred organisms could grow, multiply and produce visible colonies on inoculation. From a single cell, new colony originated, as a result, pure culture is prepared.

Requirements

1. 95% alcohol
2. Laminar air flow
3. Lazy susan turntable.
4. Bunsen burner
5. Sterile microtips
6. Sample of mixed population of microorganism
7. Sterile nutrient agar plates Spreader

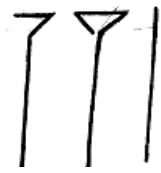
Procedure

1. Dip the spreader into 95% alcohol for sterilizing it. Then, by Bunsen burner flame (after drying, apply flame).
2. Aseptically pour 0.1 ml of diluted inoculums on the surface of the nutrient agar plate.
3. Place the plate on the turntable.
4. With the left hand lift the cover of the petri dish and hold it at an angle of 60°.
5. In the right hand hold the spreader and spin the turntable.

6. With the help of spreader spread the culture over the surface of agar while the turntable is spinning. (rotate the petriplate with left hand, in the absence of turntable)
7. Replace the petri dish cover.
8. Again the spreader is sterilise.
9. For inoculating the other plates, repeat the same process.
10. Keep one plate for control which is uninoculated.
11. All the plates are incubated in an inverted position at 28°C for 24 to 48 hours.
12. Then on agar surface, observe the colonies.

Results

A number of colonies will be discrete, separate or free from each other.



Spreader

Precautions

1. Sterilization of the spreader should be done carefully first apply alcohol than dry it and apply flame.
2. Cover of petriplate should not be lifted completely.
3. Plating of the medium should be done one day in advance of performing this exercise.
4. Dilution must be carried out in a proper way.
5. For each dilution, use separate sterile microtips.
6. The plates should be incubated in an inverted position for preventing the condensation of water vapors on the lid that falls onto agar surface and may cause contamination to culture.

(3). Pour plate method

In this method, serial dilution of the material is added into the sterile petriplates to which is poured melted, sterilized and cooled (~45°C) agar medium and thoroughly mixed by rotating the plates which is then allowed to solidify. After incubation, both surface and subsurface (within the agar) colonies will appear. Some of the colonies will be separate and can be isolated/ transferred for making pure culture.

Requirements

1. A sample of mixed microorganisms population.
2. Sterile test tubes and petri plates.
3. Sterile nutrient agar medium maintained at 45°C.
4. Sterile glass pipettes and micropipette tips.
5. Bunsen burner.
6. Sterile water.
7. Laminar air flow cabinet (LAF).

Procedure

1. Take 1.0 ml/g of material and dissolve it in 9 ml sterile water for preparing the serial dilution of materials. 10^{-1} dilution is labels as a stock solution.
2. Under aseptic condition make various dilutions by transferring 1 ml of stock solution into the second tube containing 9 ml of sterile water and so on.
3. Transfer 0.1 ml of different dilutions into different sterile petriplates.
4. Now in each petri plate add approximately 20 ml of sterile, cool (45°C), molten medium than mix it by gentle rotation of the petri plate.
5. Allow the medium to solidify.
6. Keep one plate as control which is uninoculated.
7. The inoculated plates are inoculated in an inverted position at 28°C for 24-48 hours.
8. Examine the plates for the appearance of individual colonies.

Results

The number of colonies will decrease with increase in dilution of the inoculums and hence colonies can be isolated.

Precautions

1. Dilution must be shaken thoroughly before adding for subsequent dilution.
2. Separate sterile microtips should be used for each dilution.
3. The medium should be maintained at 45°C.
4. The plates should be incubated in an inverted position for preventing the condensation of water vapors on the lid that falls onto agar surface and may cause contamination to culture.