

PREPARATION OF PURE CULTURES OF MICROORGANISMS

Microorganisms isolated from various substrates or sources (soil, air, contaminated water, disease plants, decomposing infected plant material), need to be obtained in pure form as cultures contain millions or billions of individual cells. A culture that contains only one kind of microorganism is called a pure culture. A culture that contains more than one kind of microorganism is called a mixed culture; if it contains only two kinds of microorganisms that are deliberately maintained in association with one another, it is called a two-member culture.

Pure cultures are essential in order to study colony characteristics, biochemical properties, morphology, staining reactions, and immunological reactions or the susceptibility to antimicrobial agents. Two kinds of operations, that is, isolation (the separation of a particular microorganism from the infected host, individually or as the mixed populations that exist in nature) and purification (the growth of a single cell into microbial populations as a culture in artificial environments [culture media] under laboratory conditions), are important irrespective of the kind of microorganism with which a microbiologist deals. Pure cultures of microorganisms that form discrete colonies on solid media may be most simply obtained by one of the modifications of the plating method. This method involves the separation and immobilization of individual organisms on or in a nutrient agar medium. Each viable organism gives rise through growth to a colony from which transfer can be readily made. The three most commonly used methods for obtaining pure cultures of microorganisms are (1) streak plate; (2) pour plate; and (3) spread plate. Other methods for obtaining pure cultures of microorganisms include: (1) use of enrichment media; (2) use of differential and selective media; (3) use of media containing antibiotics; and (4) selected techniques for the cultivation of anaerobes.

BY STREAK PLATE METHOD

The streak plate method offers a most practical method of obtaining discrete colonies and pure cultures. It was originally developed by two bacteriologists, Friedrich Loeffler and George Gaffkey, in the laboratory of Robert Koch. In this method, a sterilized loop or transfer needle is dipped into a suitable diluted suspension of organisms which is then streaked on the surface of an already solidified agar plate to make a series of parallel, non-overlapping streaks. The aim of this exercise is to obtain colonies of microorganisms that are pure, that is, growth derived from a single cell/spore.

Material Required A 24–48-hour nutrient broth of mix or the contaminated colony culture, nutrient agar plates, inoculating loop, Bunsen burner, wax marking pencil

PROCEDURE

With a wax marking pencil, label all the plates on the bottom with the name of the organism(s) to be inoculated. Hold the tube containing the broth of mixed mixture in the left hand. Sterilize the loop holding in the right hand, remove the cotton wool plug using the little finger of the right hand, and immediately flame the mouth of the tube. Introduce the loop into the broth and withdraw one loopful of culture. Flame the mouth of the tube, replace the cotton wool plug, and place the tube in the test tube rack. Lift the Petri plate cover with the left hand and hold it at an angle of 60°. Place the inoculums (the loop containing the droplet of broth) on the agar surface at the edge farthest from you and streak the inoculums from side to side in parallel lines across the surface of area. Reflame and cool the loop and turn the Petri plate to 90°. Touch the loop to a corner of the culture in Area 1 and streak the inoculums across the agar in Area 2 as above (the loop should never enter Area 1 again). The rest of the agar surface is now used to complete the streaking. Replace the lid of the Petri plate after completing the streaking and sterilize the loop by flaming. Incubate all the plates at 28–30°C in an inverted position for 48–72 hours

Observations

After incubation, examine each of the three plates for the growth of the colonies. A confluent growth will be seen where the initial streak was made, the growth is less dense away from the streak, and discrete colonies will be farthest away from the streak (i.e., end of the streak). Any colony not growing on the streak marks is regarded as a contaminant. Select a well-isolated colony from each plate and record their features. If their features agree with the original description, then the two colonies being examined are pure cultures.

Precautions Avoid pressing the loop or needle too firmly against the agar surface as this will damage it. The inoculating loop should be cooled by touching the agar surface before lifting the inoculums for streaking. The Petri plate lid should never be lifted completely. Plating of the medium should be done 24 hours in advance of performing the exercise, so as to detect the contaminated plates to discard them