



Pure Culture Techniques

EXERCISE

9

Learning Outcomes

After completing this exercise, you should be able to

1. Obtain isolated colonies of a mixed culture using the streak-plate method.
2. Obtain isolated colonies from a bacterial culture using the loop dilution and pour-plate method.
3. Evaluate the purity of your isolated colonies by transferring a single colony to an agar slant and obtaining the growth of a single type of organism.

When we try to study the bacterial flora of the body, soil, water, or just about any environment, we realize quickly that bacteria exist in natural environments as mixed populations. It is only in very rare instances that they occur as a single species. Robert Koch, the father of medical microbiology, was one of the first to recognize that if he was going to prove that a particular bacterium causes a specific disease, it would be necessary to isolate the agent from all other bacteria and characterize the pathogen. From his studies on pathogenic bacteria, his laboratory contributed many techniques to the science of microbiology, including the method for obtaining **pure cultures** of bacteria. A pure culture contains only a single kind of an organism, whereas a mixed culture contains more than one kind of organism. A contaminated culture contains a desired organism but also unwanted organisms. With a pure culture, we can study the cultural, morphological, and physiological characteristics of an individual organism.

Several methods for obtaining pure cultures are available to the microbiologist. Two commonly used procedures are the **streak plate** and the **pour plate**. Both procedures involve diluting the bacterial cells in a sample to an end point where a single cell divides, giving rise to a single **pure colony**. The colony is therefore assumed to be the identical progeny of the original cell and can be picked and used for further study of the bacterium.

In this exercise, you will use both the streak-plate and pour-plate methods to separate a mixed culture of bacteria. The bacteria may be differentiated by the characteristics of the colony, such as color, shape, and

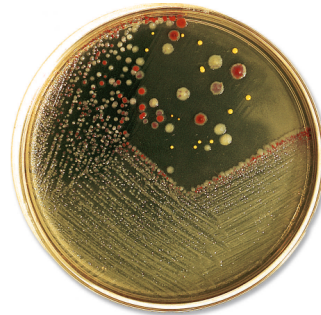


Figure 9.1 A streak plate demonstrating well-isolated colonies of three different bacteria.

other colony characteristics. Isolated colonies can then be subcultured and stains prepared to check for purity.

Streak-Plate Method

The streak-plate method is the procedure most often used by microbiologists to obtain pure cultures. It is simple and allows for economy of materials. However, it requires a certain level of skill which is only obtained through practice. Your instructor may want you to try more than one method or only concentrate on one of the quadrant streak-plate procedures. Figure 9.1 illustrates how colonies of a mixed bacterial culture should be spread out and separated on a properly made quadrant streak plate shown in method B of figure 9.2. Good spacing between colonies on the plate is critical so that a single pure colony can be aseptically isolated from quadrant 4 and used for further testing and study. This will insure that you are not working with a mixed or contaminated culture.

Figure 9.2 shows three procedures for producing a streak plate that will yield isolated colonies. By far the most popular and most utilized procedure is the