Cultivation of Bacteria and Establishing Pure Culture

When microorganisms are cultivated in the laboratory, a growth environment called a medium is used. Microorganisms growing in or on such a medium form a culture. To work with microorganisms in the laboratory, it is desirable to obtain them in pure cultures.

Types of culture According to purity:

1. A mixed culture: is a culture that contains many species.
2. Pure culture (axenic): a culture of a single cell species, without the presence of any contaminants, and it is derived from a mixed culture.
3. Contaminated culture: culture tainted with intruding microorganisms.

Pure cultures of bacteria can be obtained by spreading bacteria out and permitting the individual cells to form masses of growth called colonies. There are three methods commonly used to derive a pure culture:

1. Streak Plate Method

The first step in the identification process is the isolation of individual bacterial species from a sample. A common isolation technique is the streak plate. It is the most important and useful method which depends upon streaking and spreading mixed culture on the surface of media which allows the microorganism to separate from one another.

There are many patterns used in streaking an agar plate, the choice of which depends on the source of inoculum and preference of the microbiologist. These patterns include:

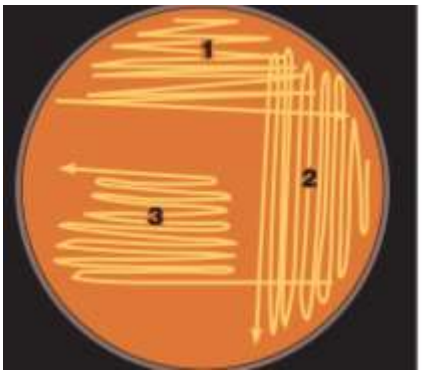
1. **A quadrant streak**: is usually performed for isolation of two or more bacterial species in a mixed culture with suspected high cell density.

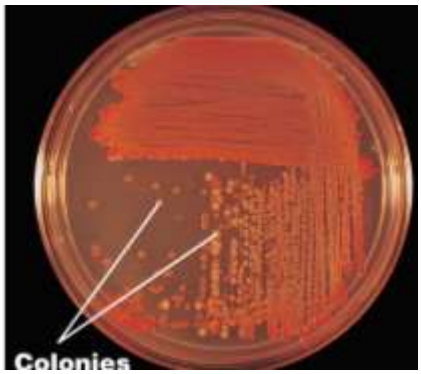
The quadrate streak technique is described below.

a. Mark your agar plate into four quadrants either with your indelible marker or with your imagination.
b. Flame your loop, let it cool, then remove one colony from the mixed culture and streak the first quadrant.
c. Flame your loop and let it cool. Drag your loop from the second quadrant back into the first quadrant three times and streak the second quadrant.
d. Flame your loop and let it cool. Drag your loop from the third quadrant back into the second quadrant three times and streak the third quadrant.
e. Flame your loop and let it cool. Drag your loop from the fourth quadrant into the third quadrant **one time** and then streak the fourth quadrant with one wavy line. Flame your loop, cover your agar plate, flip it, and label.

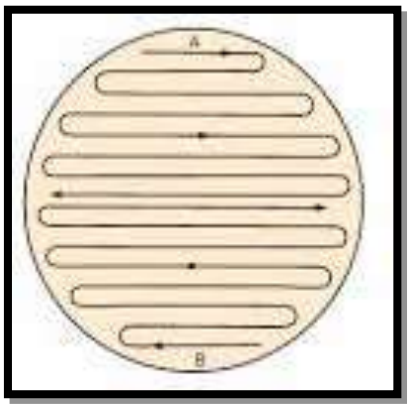
2. **T-Streak Method**
   a. Streak one half of your agar plate with the bacteria, then
   b. Flame your loop. After the loop has cooled, in one of the quadrants left, run your loop into the first region three times, then streak the quadrant.
   c. Flame your loop and let it cool, again. Run your loop through the last quadrant into the last region (quadrant) you just streaked **one time** and streak the last quadrant with your loop.
   d. Flame your loop, cover your agar plate, flip it, and label.

   Note that if you were to have drawn a "T" splitting the plate into two semicircles, then splitting one of the semicircles in half into two quadrants, that this resembles a "T" and is why this is called a "T"-streak.





3. **Continuous Streak Method:** whereas a simple zigzag pattern may be used for samples containing lower cell densities and with pure cultures when isolation is not necessary.
   a. Mark your agar plate into two half-circles. Flame your loop and remove one colony from the mixed culture and streak one of the half-circles in a continuous back-n-forth line.
   b. Rotate the agar plate 180° and do the same thing in the second half-circle **without flaming your loop**.
   c. Flame your loop, cover your agar plate, flip it, and label it.
   d. Place your inoculated agar plates in the incubator for 24-48 hours.



2. Pour Plate Method

3. Spread Plate Method: