Exercise 4: Pure Culture Technique – Preparing a Streak Plate

Objectives

- Employ the streak plate technique to produce individual colonies on an agar plate.
- Practice aseptic technique.

Reading

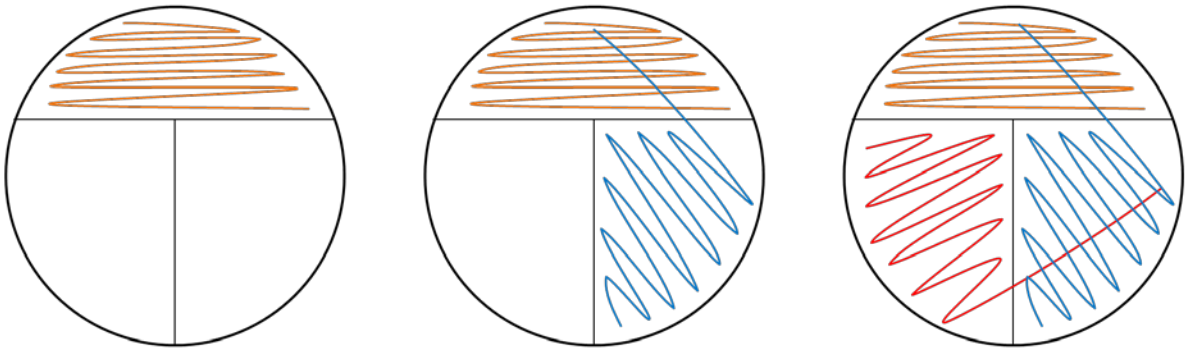
In order to study microorganisms and observe their characteristics it is first necessary to obtain them in **pure culture** (a pure culture is defined as a culture containing only one species of organism). This is very important because it is impossible to study the characteristics of a microorganism when contaminants (unwanted organisms) are present.

One of the most common methods employed in the laboratory to obtain a pure culture is the preparation of a **streak plate**. To properly streak a plate for isolation you must spread out the organism(s) by means of the inoculating loop until single colonies result. Each single colony consists of a cluster of cells that originate by cell division from a single bacterial cell. **Thus each isolated colony represents a pure culture of bacteria.** To do this, you must first learn **aseptic technique**. This is a special set of procedures designed to prevent contamination. This means that you transfer only the microorganism of interest and do not contaminate them with other microorganisms from the surrounding environment.

Rules for Aseptic Technique

- Bacteria are found everywhere, use common sense to avoid contamination.
- You must not allow any part of yourself or any other non-sterile object to touch the growth media.
- Do not remove the lid from your Petri dish completely, instead lift it and hold it above the dish to protect the media from dust.
- Do not put test tube caps or Petri dish lids down on the counter.
- Sterilize your loop or inoculating needle by heating it until it glows. Do this before and after each transfer.

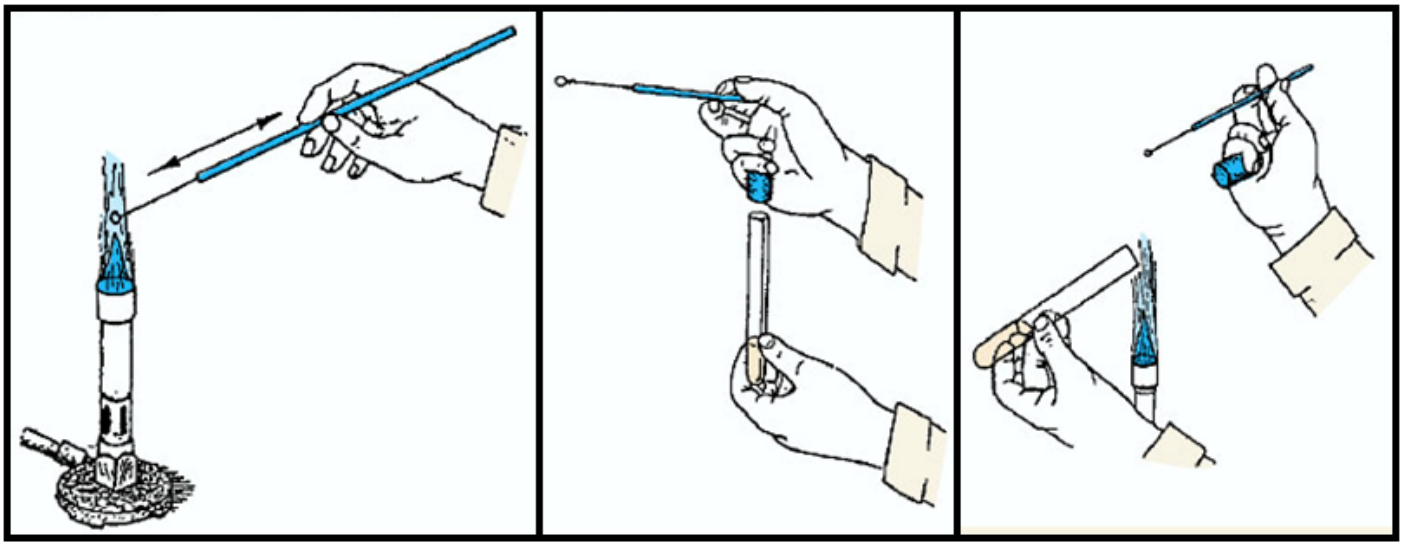
Figure 4.1 Separation of bacteria using the streak plate



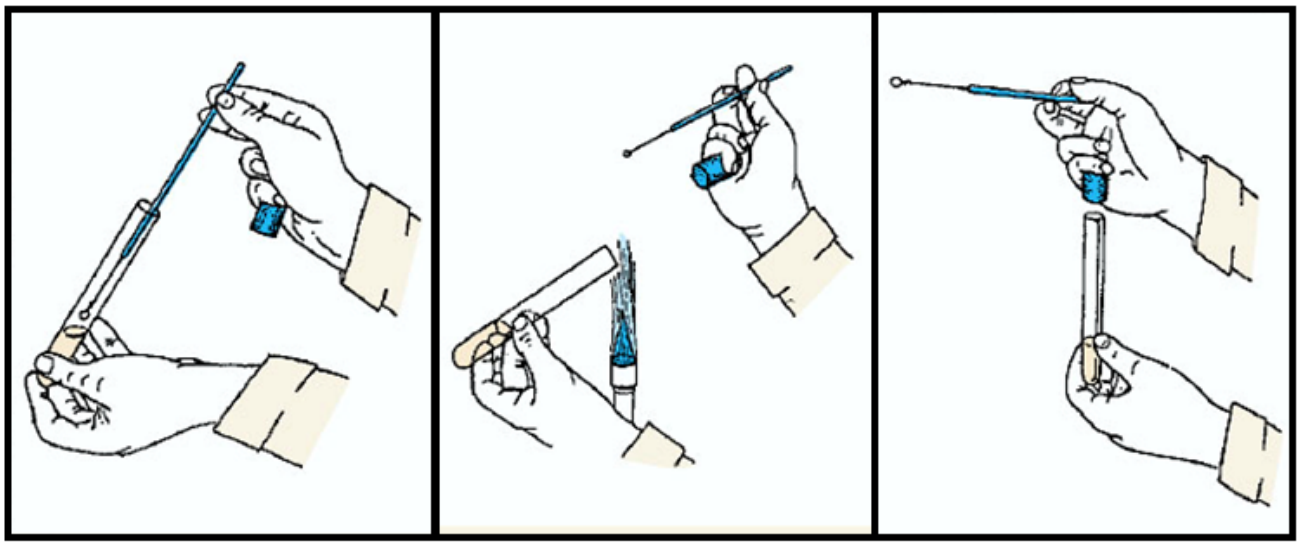
First Streak – Pick up bacteria using a sterilized loop; streak bacteria on the top third of your plate.

Second Streak – Flame the loop. Touch the hot loop to the agar to cool before going across the previous streak once, then streaking the second section.

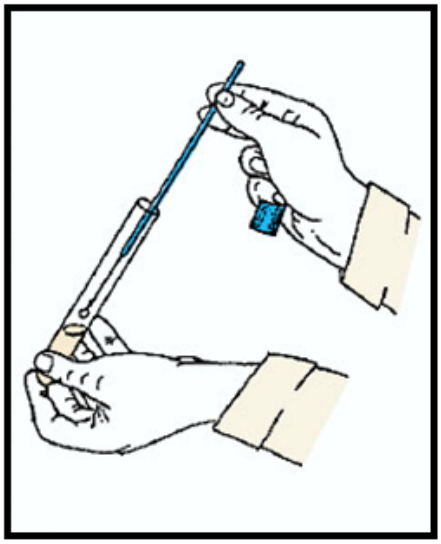
Third Streak – Flame the loop. Cool and streak from section 2 to 3, only going once across your second streak.



Flame Inoculation Loop | Remove Cap | Flame Tube Opening



Get Drop of Culture | Flame Tube Opening | Return Cap



Inoculate new tube or plate using aseptic technique with new tube/plate.

Figure 4.2

EX 4: Streak Plating – Materials

broth culture: mixed culture containing both *Micrococcus luteus* and *Escherichia coli*
inoculating loop
1 nutrient agar (NA) plate (per student)

EX 4: Streak Plating - Methods

1. Follow the procedure in Figure 4.2 to aseptically pick up a loopful of bacterial broth culture. Follow the procedure in Figure 4.1 to spread the loopful of culture onto the agar surface, covering ≈ ⅓ of the surface in a back-and-forth fashion. Keep your loop on the agar surface and don't gouge the agar. In this area you should get confluent growth after incubation.

2. Reflame your loop, killing all of the bacteria on its surface. Touch the hot loop to the agar surface in order to cool it down before going across your first streak once, and then proceeding to streak the second section of the agar. In section two, you should get moderate growth after incubation.

3. Reflame your loop, again killing all of the bacteria on its surface. Touch the hot loop to the agar surface in order to cool it down before going across the area of your second streak once and then proceeding to streak the third section of the agar. In section three, you should get discrete colonies after incubation, because the bacteria will have been diluted out sufficiently from the original culture.

4. Label the agar-side of the plate with your initials/seat # and 'Mix' to indicate the bacterial culture used. Incubate plate agar-side up in the appropriate incubation box until next lab period.

5. Next lab, observe the plates and answer questions in **RESULTS** section.