Exercise 2.3 – Isolation Streak Plate

LEARNING OUTCOMES

1. State the purpose and principle of an isolation streak plate.
2. Identify factors contributing to a poor isolation streak plate.

In microbiology, isolation streaking is a technique used to separate organisms in a mixed sample. The procedure is done by spreading the sample over several sections of an agar surface using an inoculating loop or needle. Following incubation, a colony of interest is picked to create *a subculture* by repeating the process on a sterile agar plate. Colonies on the subculture plate are identical, thus providing a pure culture for further identification and testing.

In this exercise, you will prepare a *quadrant streak plate* to separate two bacteria, *Serratia marcescens* and *Micrococcus luteus,* growing together in broth medium. Both organisms are members of the human microbiome and grow at 37°C. However, while *Micrococcus* colonies appear bright yellow at all temperatures, *Serratia* colonies are red only at lower temperatures. Incubating the streak plate at 25°C rather than at 37°C results in colonies of two different colors, yellow and red (Figure 2.7). This contrast distinguishes or differentiates organisms in a mixed sample and demonstrates if successful isolation of each organism was achieved.

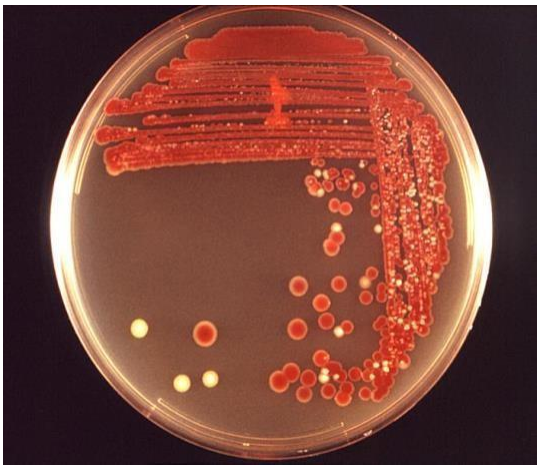

Figure 2.7: Isolation streak plate prepared from a mix of Serratia marcescens (red colonies) and Micrococcus luteus (yellow colonies) following incubation at 25°C.

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OBJECTIVE

Isolate bacteria from a mixed culture using the quadrant streak method.

MATERIALS – STUDENTS WORK INDIVIDUALLY

- EQUIPMENT: Inoculating loop, incinerator, vortex, marking pen, labeling tape
- MEDIA: Trypticase soy agar plate
- CULTURES: Mix of *Escherichia coli* and *Serratia marcescens*

PROCEDURE

1. Using a marker, label the bottom of each plate with your initials, date, and culture mix.
2. Vortex the mix and aseptically obtain a loopful of broth.
3. Heat and close the culture tube before opening the plate.
4. Inoculate the first quadrant by streaking the agar as shown in Figure 2.8.
5. Close the plate and sterilize the loop, allowing it to cool for at least 5-10 seconds.
6. Rotate the plate 1/4 turn and continue the streak into the next quadrant, going back into the first quadrant with the loop several times and then continuing the streak. This separates cells to form isolated colonies.
7. Close the plate and heat the loop, allowing it to cool for at least 5-10 seconds.
8. Rotate the plate another 1/4 turn and continue the streak into the third quadrant.
9. Do not heat the loop. Rotate the plate a final 1/4 turn and streak the last quadrant, bringing the loop into the center of the plate while avoiding touching the first quadrant.
10. Invert the plate and place it in the common rack for incubation at room temperature.

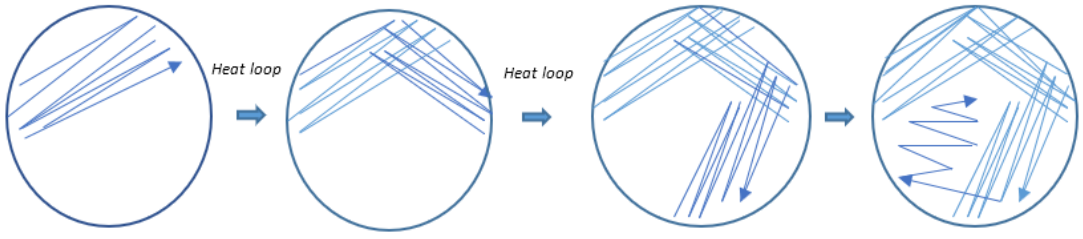

Figure 2.8: Quadrant streak plate technique.