Bacterial dilution series plating practice

Procedure

Each group will receive two bacterial cultures, *Escherichia coli* and *Streptococcus mutans.* You will have 1 ml of each, but the number of bacteria in that culture is unknown. Your mission is to determine the number of colony forming units (c. f. u.) in your 1 ml cultures.

You will perform a serial dilution, or in other words, dilute your bacterial culture more and more until the number of colonies is easy to count.

1. Label all plates and tubes before you start! You will do two dilution series for each culture (these are called replicates, and should be labeled A and B). Tubes should be labeled with the culture type, replicate, and dilution. Plates should be labeled with your group name, dilution, replicate, and culture type.

2. Put 900 µl of LB broth into each tube for each dilution.

3. Take 100 µl of the original inoculum and pipet into the first dilution tube (1:10). Mix the contents of the tube well.

4. Take 100 µl of the previous dilution and pipet into the next dilution and mix well. For example, for the 1:100 dilution, take 100 µl of the 1:10 dilution and mix with 900ul of LB broth in the 1:100 dilution tube.

5. Repeat until all dilutions are mixed.

6. Spread 1 ml of each dilution onto its corresponding plate.

7. Incubate plates overnight at 37°C, and we will count colonies that grow tomorrow!

Materials necessary

Overnight liquid culture of bacteria (*E. coli* and *S. mutans*)
LB both
LB agar plates
200 and 1000 µl pipette tips

Equipment necessary

200 µl micropipette
1000 µl micropipette
Spreader
Bunsen burner