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Chapter 16

Enumeration of soil microbiota: Calculation of colony forming units (CFU) for microbial quantification

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1. Introduction:

Quantifying bacterial cells or determining their density holds paramount importance in various standard procedures and assays within the realm of biology (Tortora, et al.,2021). Understanding cell counts serves as a cornerstone for monitoring cell growth, evaluating resistance during transformation and selection processes, seeding cells for further experimentation, and preparing for cell-centric studies (Alexander, 1977; Tripathi et al., 2018). The accuracy and consistency of bacterial cell counts are pivotal for precise quantitative analysis in subsequent phases (Cappuccino and Sherman, 2014).

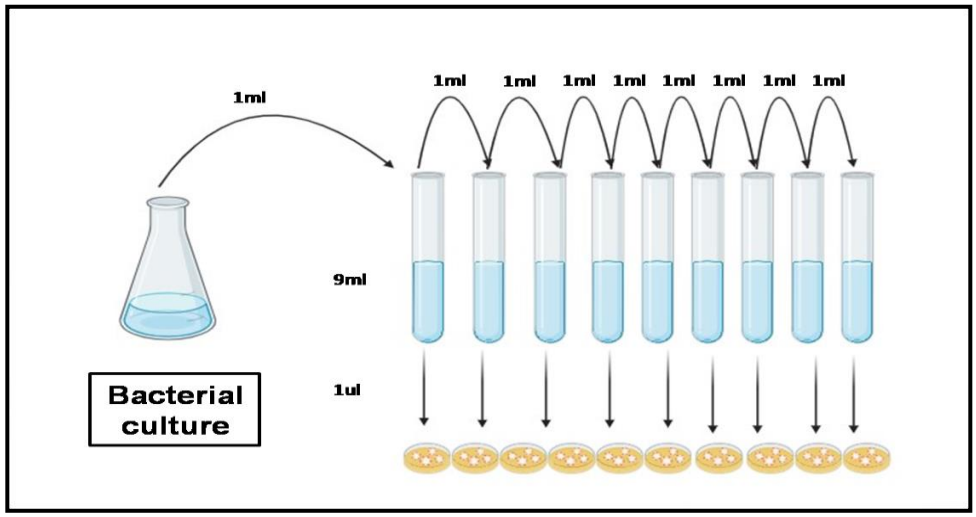

Figure 1: Calculation of CFU using serial dilution method from soil sample

Colony Forming Units (CFU): CFU provides a precise means of assessing living bacterial cells, specifically quantifying the number of distinct colonies formed by any microorganism capable of thriving on a media plate. Biologists commonly utilize CFU (colony-forming unit) as a metric to gauge the quantity of culturable microorganisms

within a specified volume of culture (Reddy et al., 2019; Yadav et al., 2021). This quantification typically involves serial dilution and spread plating techniques, illustrated in the accompanying figure

2. Materials required:

1. Sterile scoop or spatula
2. Sterile containers
3. Sterile saline solution or buffered water
4. Pipettes and pipette tips
5. Sterile spreaders
6. Incubator
7. Nutrient Agar plates
8. Agar powder
9. Antibiotics (if applicable) for selective bacterial growth inhibition
10. Disposable gloves
11. Petri dishes
12. Sterile containers

3. Procedure:

3.1. Sample Collection:

a. Collect soil samples from the desired location using a sterile scoop or spatula.
b. Ensure to gather samples from various points to obtain a representative sample of the area.

3.2. Sample Preparation:

a. The soil sample should be transferred into a sterile container with a defined weight or volume.
b. Add a suitable diluent (e.g., sterile saline solution or buffered water) to create a dilution series.

3.3. Serial Dilution:

a. Mix the soil sample thoroughly with the diluent to create an initial dilution.
b. Transfer a small volume (e.g., 1 mL) of the initial dilution to a new container containing fresh diluent.

3.4 Dilution Series:

Perform sequential dilutions to generate dilutions of varying concentrations (e.g., 10^{-1}, 10^{-2}, 10^{-3}, etc.).

3.5. Plate Spreading:

a. Properly label sterile agar plates with sample identifiers and dilution factors.
b. Pour an appropriate agar medium into the labeled plates and allow it to solidify.
c. Using a pipette, dispense a known volume (e.g., 100 µL) of each dilution onto

separate plates.

d. Spread the liquid evenly over the agar surface using a sterile spreader.

3.6. Incubation:

After inverting the agar plates, incubate them for 24 to 48 hours at a temperature and circumstances that are optimal for bacterial growth.

3.7. Colony Enumeration:

a. Following incubation, carefully inspect the plates for visible bacterial colonies.

b. Count the colonies on plates containing 30-300 well-separated colonies to ensure accuracy.

c. Record the dilution factor and compute the Colony Forming Units (CFU) per gram or per milliliter of the original sample

3.8. CFU Calculation:

Utilize the formula for each plate to determine CFU/ml:

CFU/ml = (Number of colonies x Dilution factor) / Volume of culture plate.

Precautions:

1. Dilute soil samples appropriately to achieve countable CFU numbers.
2. Use a suitable culture medium and incubation conditions specific to the microbes of interest.
3. Spread or pour plate method should be performed carefully to ensure even distribution of microbes.
4. Incubate plates at the correct temperature and time suitable for the microbial growth.
5. Count colonies carefully, ensuring each colony is counted only once and colonies are well-separated.
6. Consider colony morphology and size to distinguish different types of microbes.

4. References:

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