Standard Laboratory Operating Procedure #500

Dilution and Plating Method for Soil Bacteria Enumeration

Laboratory: Biotechnology
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General: Surface soils are a heterogeneous mixture of inorganic and organic particles that combine together to form secondary aggregates. Within and between the aggregates are voids or pores that visually contain both air and water. These conditions create an ideal ecosystem for bacteria, so all soils contain vast populations of bacteria, usually over 1 million per gram of soil. This experiment demonstrates the dilution and spread plating methodology used to enumerate the number of bacteria within a soil sample.

Aseptic technique is used to transfer bacteria from one place to another in a way that avoids contamination from the air, surfaces or other sources of bacteria. Aseptic technique begins with washing hands, then cleaning and sterilizing surfaces and equipment either with 10% bleach solution or 70% ethanol before beginning any transfer. When pipetting for the serial dilution described below, a new sterile pipette tip must be used to transfer the bacterial solution from each tube.

Before plating the bacteria, sterilize metal inoculating loops that transfer bacteria from a dilution tube to an agar plate by flaming over a Bunsen burner until the metal turns red, starting at the end closest to the handle then moving to the loop. Sterile plastic loops may be used, but after each use the loop must be thrown away and a new sterile loop must be used.

Safety: PPE—eye protection, gloves, aprons

Materials

nutrient agar plates (as prepared by SOP #99 or other prepared plates)
distilled water
microtubes
H1000 micropipetter
micropipette tips
metal inoculating loops or sterile plastic loops
soil samples
electronic balance
vortexer
incubator

Procedure

Prepare soil sample

1. Label a microtube with initials and soil sample type.
2. Using 100-1000µL micropipette, add 1000µL of distilled water into labeled microtube.
3. Add 0.1g soil sample to microtube with distilled water and mix for 15 seconds on vortexer. (If you do not have a vortexer, shake the tube.)
4. Repeat steps 1-3 for each soil sample.

Soil Health and Microbes

Set up serial dilutions

1. Label micro tubes 10^1 through 10^7.
2. Use a 100-1000µL micropipette to add 900µL of distilled water into each microtube.
3. Add 100µL from the starting soil sample microtube to tube labeled 10^1 and mix on vortexer.
4. Using aseptic technique, transfer 100µL of sample in 10^1 to microtube labeled 10^2 and mix on vortexer.
5. Using aseptic technique, transfer 100µL of sample in 10^2 to microtube labeled 10^3 and mix on vortexer.
6. Using aseptic technique, transfer 100µL of sample in 10^3 to microtube labeled 10^4 and mix on vortexer.
7. Using aseptic technique, transfer 100µL of sample in 10^4 to microtube labeled 10^5 and mix on vortexer.
8. Using aseptic technique, transfer 100µL of sample in 10^5 to microtube labeled 10^6 and mix on vortexer.
9. Using aseptic technique, transfer 100µL of sample in 10^6 to microtube labeled 10^7 and mix on vortexer.
10. Label corresponding nutrient agar plates for dilution factors 10^1 through 10^7.

Plate sample dilutions

11. Using aseptic technique, pipette 100µL of each sample onto its corresponding plate and use a sterile loop to move sample over entire plate. Make sure to sterilize the loop or get a new sterile loop in between each sample.
12. Seal the top of each plate using parafilm or lab tape. Place in incubator overnight.