LABORATORY 8

SOIL BIOLOGY

I Objectives

Learn procedure for enumerating soil microorganisms.

II Introduction

The soil is home to a wide range of plant and animal life. Roots are the largest form of plant life and certain rodents, insects and earthworms, the largest animals. Though higher plants are the primary producers of chemical energy that sustains the terrestrial ecosystem, plants are dependent on the invisible (other than certain fungal structures) community of soil microorganisms for continued supply of many essential nutrients. Nutrient elements bound in organic combinations would be inaccessible to plants without microbial decomposition of organic matter and mineralization of these elements. Not only would nutrient cycling stop but also biological N-fixation. Soil microorganisms are indispensable to life on earth.

Soil microorganisms live in thin films of water that surround soil particles. These tiny organisms include ***microflora*** (***bacteria***, ***fungi*** and ***actinomycetes***) and ***microfauna*** (***protozoa*** and ***nematodes***). In terms of numbers and biological activity the microflora are dominant. Bacteria are small (about 1 - 10 μm) and occur in three general shapes –rod (bacillus), spherical (coccus) and spiral (spirilla). Bacilli and cocci are more common in soil. Fungi are filamentous and much larger. The branched ***hyphae*** exhibit cell divisions and fungal ***mycelia*** (hyphal mass) are often macroscopic. Actinomycetes are also filamentous and branched but smaller.

Agar Plate Method for Microbial Count

In this method, soil is dispersed in an agar medium so that individual microbial cells, spores or mycelial fragments develop into macroscopic colonies. The procedure involves successive dilutions of soil. Depending upon extent of dilution, plates may be filled with a huge number of colonies or very few. Enumeration of colony-forming units initially present in the soil is from plates in between these extremes. This method requires sterile technique to avoid introduction of extraneous microbes.

Any one of several different growth media can be used but no single growth medium is optimal for all microorganisms that inhabit soil. Thus, growth of certain organisms is favored in the chosen medium and growth of others is stymied. Antagonistic or antibiotic effects may also affect colony development in the agar plate method.

III Procedure

You are provided with a homogenized, field-moist sample of topsoil. You are also provided with bottles containing 90 mL of sterilized water.

1. Add a 10 g sub-sample of topsoil to the bottle of sterilized water. Tightly cap and shake vigorously for 10 minutes to disperse the soil. This is the 10^{-1} dilution.

2. Transfer 10 mL of the 10^{-1} dilution to another bottle of sterilized water. Use a sterile pipette. Take sample from the middle. Tightly cap and shake to uniformly mix. This is the 10^{-2} dilution.

3. Repeat step 2 using the 10^{-2} dilution to make a 10^{-3} dilution and proceed similarly, making 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} dilutions.

4. From the 10^{-7} dilution, transfer 1 mL to each of 2 sterile petri dishes using a sterile 1 mL pipette. Make similar transfers from the 10^{-6}, 10^{-5} and 10^{-4} dilutions.

5. Into each seeded petri dish, pour enough sterile, melted agar to fill dish about ½ full. Immediately swirl it around to ensure good mixing of soil inoculant and agar.

6. After the agar has solidified, invert plates and incubate at 35 ^{o}C for 1 week.

7. Next week, count the number of colonies on plates from the dilution that contains from 30 to 300 colonies. Do not count from those plates that contain colonies larger than 2 cm diameter. Record in Table 1. Multiply by dilution, take the average and correct to oven-dry moisture content of the soil. This gives the number of colony-forming units (CFUs) per gram of soil.

IV Worksheet

Plate No.	Dilution	No. of Colonies	No. CFU / g soil
		Average CFU / g	