

Enumeration of Soil Microorganisms

Actinomycete bacteria (also known as actinobacteria, including actinoplanetes, nocardioform and streptomycetes), other bacteria and filamentous fungi (including *Rhizopus*, *Mucor*, *Penicillium* and *Aspergillus*) are all important members of the soil microbial community. Protozoa, algae, cyanobacteria, nematodes, insects and other invertebrates, and viruses are also important members but will not be studied in this exercise. Each gram of rich garden soil may contain millions of these micro- and macroorganisms.

Since soils vary greatly with respect to their physical features (e.g. pH, general type, temperature and other related factors), the microorganisms present will also vary. For example acid soils will have a higher number of fungi compared to alkaline soils, and rich garden soil will contain more actinomycete bacteria than either the other bacteria or fungi. Not surprisingly, no single techniques is available to count the microbial diversity found in average garden soil. Thus in this exercise, each group of students will try to determine only the relative number of fungi, actinomycetes, and other bacteria in a sample of garden soil using the serial dilution agar plating procedure shown in the accompanying figure.

To support the three different groups of microorganism, you will use three types of media: (1) Sabouraud dextrose agar for the isolation of fungi, (2) glycerol yeast agar for the isolation of actinomycete bacteria (actinobacteria) and (3) tryptic soy agar for the isolation of other bacteria. For the second and third media, cyclohexamide will also be added to the media to reduce the growth of fungi. Cyclohexamide is an antibiotic that inhibits protein synthesis in Eukaryotic cells (e.g. fungi) but not in prokaryotic cells.

Procedure:

1. Place 1 g of garden soil into a 99 ml sterile water blank. Mix the soil and water thoroughly by shaking water-soil mixture vigorously for 3 minutes, keeping your elbow on the lab table. Transfer 1 ml of this mixture to a second water blank and mix as above. Transfer 1 ml of the second mixture to the third water blank and mix as above.
2. Label the plates, indicating the type of plate, the dilution, and the volume plated.
3. To the plates that will have molten Glycerol Yeast Agar or Tryptic Soy Agar added, add the amount of cyclohexamide solution indicated by your instructor. No cyclohexamide should be added to plates that will contain Sabouraud Dextrose Agar.
4. Place the correct volume of diluted soil into the bottom of each plate, according the accompanying figure. Place the inoculum in a separate location from the cyclohexamide solution (if any).
5. Pour the appropriate molten agar deeps into the corresponding plates. Mix the plates gently on the bench top in a figure-8 pattern.
6. Allow the agar to solidify for all plates.

7. Tape the lids of the Sabouraud Dextrose Agar plates to the bottom so that they cannot be accidentally opened.
8. Incubate the plates inverted, at room temperature, for 3 to 7 days. Observe daily for the appearance of colonies. Count the plates with fewer than 250 colonies but more than 25. Designate plates with over 250 colonies as too numerous to count (TNTC) and those with less than 25 colonies as too few to count (TFTC).
9. Use the plate count data, the dilution and volume plated to calculate the number of each type of organism (fungi, actinomycete bacteria, other bacteria) per gram of soil.
10. Observe the various colony morphologies present on each of the plates. Note the filamentous nature of prokaryotic actinobacteria and eukaryotic fungi. Also note the presence of colored colonies and observe carefully for signs of antibiotic formation (zones of growth inhibition around antibiotic-producing colonies).
11. Do not open the Sabouraud Dextrose Agar plates, to prevent mold contamination in the lab. Some people can be quite allergic to mold spores.

