Exp.3: Bacteria and Actinomycetes

Soil bacteria are the most abundant organisms found in surface soils. These organisms are very diverse; all are prokaryotic, but the bacteria can be aerobic, anaerobic of facultative anaerobic. In addition, there are autotrophic and heterotrophic bacteria; Within this prokaryotic group are the filamentous microbes known as actinomycetes.

Bacteria and actinomycetes are important in nutrient cycling and degradation of organic contaminants. In addition, they interact with plants as "rhizosphere" populations in close proximity to plant roots. Finally, soil bacteria can be pathogenic to plants (*Agrobacterium tumefaciens*) and humans (*Clostridium perfringens* and *Bacillus anthracis*).

Both bacteria and actinomycetes are prokaryotic organisms; actinomycetes are considered to be true bacteria; When grown on agar, bacteria produce slimy colonies ranging from colorless to brightly colored orange, yellow, or pink colonies. In contrast, actinomycetes have a filamentous growth habit which makes it possible to visually distinguish them from the bacteria. Actinomycete colonies are chalky, firm and leathery, and will break under pressure. In contrast, bacterial colonies will smear under pressure.

PROCEDURE

- **First Period**

Materials

25 g soil, one plastic cup for each soil type, benchtop balance (±0.01 g), weighing dishes, deionized water, plastic wrap, rubber bands, marking pens and dissecting probe.

Method

1. Weigh out one 25 g sample of each soil into a labeled plastic cup. Amend the soil with deionized water to the moisture content specified by your instructor. Cover the samples with plastic wrap to reduce moisture loss, and secure with a rubber band. Puncturing the wrap several times with a probe allows aeration without substantial moisture loss.

2. Weigh the samples with the plastic wrap and rubber band and record the weights. You will need these values to determine the final soil moisture content. Incubate the samples at room temperature for one week.

- **Second Period**

Materials

incubated soils from Period 1, benchtop balance (±0.01 g), 9 peptone-yeast agar plates per soil type, 9 glycerol yeast extract agar per soil type, 1 sterile, 95 ml water blank for each soil type, 4 sterile, 9 ml water blanks for each soil type, 10 sterile, 1 ml pipettes for each soil type, pipette bulb, 1 test tube rack, glass hockey stick spreader, ethyl alcohol for flame sterilization, vortex, gas burner, pre-prepared R2A agar plates, pre-prepared glycerol-casein agar plates.

Method

Preparation of the Plates

Agar plates already prepared will be provided to you. The medium in the plates consists of R2A.

Preparation of Soil Dilutions for Plating

1. Re-weigh each of the soil samples including the plastic wrap covering, to allow for soil moisture calculation at the time of plating.

2. Prepare a dilution series of each of the soils

3. For each soil, suspend 10 g to a 95 ml water blank. Shake the suspension well.

4. Before the soil settles in the bottle, remove 1ml of the suspension with a sterile pipette and add it to a 9 ml water blank. Vortex well.

5. Repeat the previous step three times, each time with a fresh 9 ml water blank and sterile pipette. Vortex well. This will result in dilutions of ca. 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} g soil ml^{-1} (tubes A thru E).

QUANTIFYING HETEROTROPHIC BACTERIA USING THE SPREAD-PLATE TECHNIQUE

1. Prepare two or three spread plates for each dilution 10^{-3}, 10^{-4}, 10^{-5}, as follows. After vortexing, place a 0.1 ml drop of each dilution (this will increase your effective dilution by a factor of ten) to three separate, labeled peptone-yeast agar plates. Inoculate no more than three plates before spreading, as standing will allow too much liquid to be absorbed into the agar in one spot.

2. Take the glass hockey stick spreader, dip it in ethanol, and flame the spreader in a Bunsen burner just long enough to ignite the ethanol.

3. Moving the spreader out of the flame and holding it just above the first of the inoculated plates allows all of the ethanol to burn off. Then quickly open

the plate, holding the lid nearby in one hand. Touch the spreader to the agar away from the inoculum to cool it, and spread the drop of inoculum around on the surface of the agar until all traces of free liquid disappear (the surface will become somewhat tacky).

4. Replace the lid, re-flame the spreader, and repeat with the next plate. Work quickly so as not to contaminate the agar with air-borne organisms.

5. Incubate the bacteria plates (inverted) at room temperature for one week.

QUANTIFYING ACTINOMYCETES USING THE SPREAD-PLATE TECHNIQUE

1. Use the dilutions 10^{-2}, 10^{-3}, and 10^{-4} from above. Spread plate 0.1 ml of vortexed suspension on glycerol-casein plates as you did above, make three replicates for each dilution.

2. Incubate the actinomycete plates (inverted) at room temperature for two weeks then Counting Actinomycetes (after 2-week incubation).