Finding Antibiotics In Soil

INTRODUCTION
Soil is the major source of microorganisms that produce antibiotics. Considering that soil is densely packed with microorganisms, it is not surprising that many bacterial species have developed ways of inhibiting their neighbors for the benefit of their own growth. An antibiotic made by a microbe can inhibit many other soil microbes. The bacterial genera *Bacillus* and *Streptomyces* are commonly found in soil. The genus *Streptomyces* are the most abundant antibiotic producers and form a unique subgroup of bacteria called the Actinomycetes.

In this experiment you will try to isolate an antibiotic producing bacterium from soil. If you succeed, you will then test that bacterium to determine what organisms might be inhibited by the antibiotic that it makes.

OBJECTIVES:
First Session: To determine see if a variety of organisms live in soil.
Second Session: To identify bacterial antibiotic producer in the soil sample.
Third Session: To determine if an antibiotic is made by the bacteria.

MATERIALS NEEDED:
First Session: Isolate Bacteria
- 1 gram Soil sample (S17715)
- A bottle of 0.9% NaCl (S25542, S99407)
- Graduated cylinder (S01513)
- Balance (S94793A)
- 1 ml pipet (S01760)
- Vortex mixer (S96517)
- MYM agar plate (B21567X)
- Spreader for making spread plates (S67526)
- Sharpie (S02727)
- Incubator (S68781A)

Second Session: Test Antibiotic
- Culture tube of *E. coli* (S20918)
- TSA agar plate (S716981A)
- 1 ml pipets (S01760)
- Sterile inoculating needle (S17356A)
PROCEDURE:
First Session
1. Weigh out 1 gram of soil.
2. Obtain a glycerol yeast agar plate and label it with your period and lab group number.
3. Fill a test tube with 9 ml of 0.9% NaCl solution. Place the gram of soil into the test tube and use a vortex mixer to mix well.
4. Let the soil particles settle after shaking. Place 0.1 ml of the soil solution onto the top of the agar.
5. Place the spreader into the alcohol solution and then stick it into the flame to catch it on fire. DO NOT HOLD THE SPREADER IN THE FLAME. When the fire is out, you have a sterile spreader.
6. Place the spreader on the agar with the 0.1 ml sample and spread the sample all over the agar medium.
7. Incubate the plates at 30C until the next lab session.

Second Session
8. Calculate the CFUs/cell counts for the 1 gram of soil.
9. Inoculate the TSA agar plate with 1 ml of *E.coli* broth culture.
10. From the plate incubated in session 1, choose a colony that looks like it might be *Bacillus or Streptomyces*. Your instructor will show you what these organisms look like on an agar plate to help you with identification.
11. Use the sterile inoculating needle to pick up a single colony and inoculate the *E.Coli* plate using the sectional streak plate technique.
12. Incubate the plates at 30C until the next lab session.

Third Session
13. Examine the 2 plates for evidence of inhibition of *Staph epidermidis and E. coli* growth. If you see inhibition (areas of non-growth), you have probably isolated an antibiotic producer

Sourced from: Fall 2011 - Jackie Reynolds, Richland College, BIOL2421