Microbial Diversity in your Backyard
[a lesson plan for a classroom activity]

Author:
Dwight Baker, Ph. D., Senior Director, Natural Product Discovery, Cubist Pharmaceuticals, Inc., Lexington, Massachusetts 02421 [dwight.baker@cubist.com]

Activity Appropriate for a classroom demonstration or hands-on activity

Abstract

Soil is a biologically diverse, dynamic ecosystem right under our feet. Soil contains inorganic parent materials, such as sand, silt, clay, and rock; but primarily is composed of plant litter, microorganisms and microscopic arthropods (invertebrate animals). Soil is in a constant state of flux as the microorganisms and invertebrate animals digest the organic compounds in the plant litter, and new litter is added seasonally. However, for microorganisms, soil is a nutrient rich environment in which to live, compete with each other, and multiply. The diversity of microorganisms in the soil is estimated to be in the thousands or millions of different species per gram of soil, and this activity will permit the observation of some of the diversity in soils in your local area.

National Science Education Standards Addressed: B & D

Keywords
Biological diversity, microorganisms, fungi, bacteria, soil

Intended Audience: grades 5-8 or 9-12

ACTIVITY

Learning Objectives
This activity is designed to demonstrate the large amount of microbiological diversity present in soil. It will allow students to view fungi, bacteria and actinomycetes on Petri plates. It will also explain how soil is not just "dirt", but a living microcosm of invisible organisms.

Preparation Time
Preparation time will vary depending on whether you choose to prepare and sterilize materials yourself or to purchase pre-sterilized materials. Preparation of the nutrient media in Petri plates will require several hours and the use of an autoclave or pressure canner. If pre-sterilized media and supplies are purchased, preparation time should be less than one hour.

Learning Time
This activity will typically occupy two 1–1_ hour sessions on two days one week apart.

Background
Soil has one of the largest and most diverse populations of microorganisms on the planet earth. It is estimated that populations of microorganisms in a rich, fertile soil sample will be in the range of 10^8 or 10^9 cells per gram. The microorganisms found in soil include the majority of taxonomic groups recognized by microbiologists. In particular, one can readily observe single-celled bacteria,

filamentous bacteria called actinomycetes, and filamentous fungi by placing a very small amount of soil on a nutrient growth medium in the classroom.

Materials

1. 1 liter Erlenmeyer flasks or 1 liter Wheaton bottles for preparing agar-based media – numbers required will depend on amount of media required
2. Triple beam balance or electronic balance for weighing media components and soils – 1 or 2 per classroom should be sufficient
3. 6 X 50 mm glass test tubes with caps – 8 per student or team
4. Test tube rack to hold test tubes – 1 per student or team
5. Sterile Petri dishes – 1 or 2 per student or team to observe soil
6. Petri dishes containing nutrient agar – 5 per student or team
7. Petri dishes containing malt extract agar – 5 per student or team
8. 1 mL disposable sterile pipettes, individually wrapped – 2 or 3 per student or team
9. Pipet filling bulb or pipet-aid – 1 per student or team
10. Sterile plastic plate spreaders (glass rods can also be bent and used for the spreading of suspensions on agar plates, but must be sterilized by flaming between samples and this process must be carefully monitored for students) – 2 or 3 per student or team
11. distilled or deionized water – approximately 10 mL per student or team
12. Resealable (zipper-lock) quart size plastic bags – 2 per student or team
13. Dissecting microscope (if available) – this can be shared by the classroom

Procedure – Instructor Version

1. Prepare the dilution tubes by pipetting into 5 tubes per student (or team) 0.9 mL of distilled water. Fit each tube with a loose-fitting cap.
2. Prepare one additional tube containing 9 mL of distilled water. Fit each tube with a loose-fitting cap.
3. Prepare 150 mL of nutrient agar and 150 mL of malt extract agar for each student (e.g., for 10 students or teams you will need 1.5 L of each of the media). To autoclave the agar-based media you generally must not fill the containers (flasks or bottles) more than half to two-thirds full. Cover the flasks or bottles with loose-fitting caps or a square of aluminum foil wrapped over the top.
4. Sterilize the dilution tubes and agar-based media by autoclaving for 15 minutes at 121 °C [a home pressure canner may be used for this step provided that you use the 15 p.s.i. setting on the pressure regulator]. Allow the pressure in the autoclave or pressure canner to decrease slowly to prevent the contents from the flasks and tubes from boiling over.
5. After autoclaving, allow the agar-based media to cool slightly. Aseptically, dispense 25-30 mL (or until each plate is about half full) of this warm media into each sterile Petri dish, taking care not to create bubbles as you pour. Allow to cool and solidify for 3 to 4 hours or overnight. If plates are not to be used within a day or so, place in plastic bags and store in a refrigerator. Plates may be stored without problems for several weeks to a month if poured aseptically.
6. Collect soil samples from a variety of environments and place in plastic zipper-top bags so that each student or team will have one sample. Alternatively, you might provide each student with a collecting bag in advance of the day of the activity and instruct them to collect a soil sample from their home or in the vicinity of their home. Students should NOT be instructed to collect soils on publicly owned lands (it is not legal to collect biological

materials on public lands) or where they cannot obtain landowner permission. Therefore the instructor should always be prepared to provide some soil samples for those students who cannot or have not provided their own samples.

7. At the beginning of the class, be prepared to demonstrate the proper use of the pipette filling bulb or pipet-aid. If time permits, it may be helpful to have each student practice the proper use of the device with a pipette and water, before getting started on soil suspensions. A variety of types of pipette filling devices are available and each has its own technique for proper use. The most common problem that might be anticipated is for students to overfill the pipette and get water or soil suspension into the pipette filling device. Using cotton plugged pipettes can minimize this, but not eliminate the problem, since fluid can pass through the cotton plug. Any fluid must be removed at once to ensure that the pipette filling device is not damaged. Pipet filling using the mouth (a common practice in the United States until the 1980's) should not be used under any circumstances in accordance with current lab safety practices.
8. Also at the beginning of the class, be prepared to demonstrate aspects of sterile technique, which must be followed to prevent common microbial contamination from hands or non-sterile equipment. The first rule of sterile technique is not to touch with the hands any item that is intended to remain sterile, or allow the sterile item to touch any other object which is not sterile. This can sometimes be a difficult concept to teach students, who are frequently encouraged to touch and feel objects in science classes. Secondly, for laboratory equipment which must be held, there will be some part of it which cannot be touched to maintain sterile technique. For example, a sterile pipette may be held by the suction end (where it is attached to the pipette aid or bulb) but not be touched or be allowed to touch anything at the tip. Because this is initially a foreign concept to grasp, you might have students practice prior to beginning the activity and correct any problems that are observed.
9. Provide plastic safety glasses and gloves if appropriate.

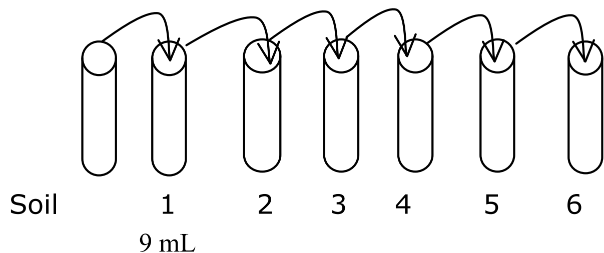
Procedure – Student Version [the text below can be copied and made into a handout, if desired]

In this laboratory activity, you will be investigating the microbial diversity within a soil sample, conducting a serial dilution of the soil sample to determine the number of microorganisms per gram of soil, and isolating some of the microorganisms to observe.

You will have available to you some samples of soil. Take one of the soil samples and observe it. You might place a small amount of the soil in an empty Petri dish so that the particles of the soil can be spread out and observed more clearly. What is the general color of the sample? What can you see within the sample? Are there particles of sand or small stones in your sample? Is there clay or silt? Are there particles of plant tissue? Are there any small animals (insects, arthropods, worms) visible? Make a few notes about your soil sample, so that you might compare it with samples observed by your classmates.

Using a balance, weigh out 1 gram of the soil sample. Try to avoid large stones or debris that might be present. Place the one gram sample into a test tube containing 9 milliliters (mL) of sterile water. You have now made a 1 to 10 dilution of the soil based on a weight to volume calculation (1 milliliter of water weighs 1 gram). Cap your tube and shake the sample well to make a uniform suspension of the soil.

You will have available to you 5 test tubes containing 0.9 mL of sterile water. Label the test tubes 2, 3, 4, 5 and 6. Using a pipetting aid and a sterile 1 mL pipette, withdraw 0.1 mL of the soil suspension and transfer to the test tube labeled 2. Using the pipette, mix this new suspension by pipetting the suspension up and down in the pipette. When the suspension is well mixed, withdraw 0.1 mL of the suspension and transfer to the test tube labeled 3. Again using the pipette, mix this new suspension as you did the first time. Repeat the mixing and transferring until you have transferred some fluid into each of the labeled tubes. You have now completed a ten-fold dilution series of the original soil sample. Any microorganisms in the original sample will be diluted in numbers by inverse of the tube number, for example the number of microorganisms in the tube labeled 4 is 10^{-4} times the original concentration.



You will have available to you 10 Petri dishes containing a nutrient medium which supports the growth of different kinds of soil microorganisms. Five of these plates will contain a selective medium for one type of microorganism, the other five will contain a selective medium for other types of microorganisms. Using a permanent marker, label the bottom of the 5 plates of each type of media with the numbers from 3 to 7, your name and the date.

Using a new pipette and the pipette aid, transfer 0.1 mL of the suspension in the highest numbered test tube (6) unto the surface of the medium in the first Petri dish labeled 7 (the transfer of this sample onto the plate is an additional dilution step, hence the higher number). Repeat this same procedure for the second Petri dish labeled 7. Using the same pipette, transfer 0.1 mL of the suspension in the next highest numbered test tube (5) onto the surface of the medium in each of the plates labeled 6. Repeat this transfer procedure until you've transferred an aliquot of suspension onto each of the labeled plates. Spread the liquid suspension as evenly on the surface of the medium in the Petri dish as possible using a sterile spreader or glass rod. Invert the inoculated Petri dishes and place in a large zipper-lock plastic bag. Place the bag of Petri dishes in a warm dark place (22 – 30 °C) for 7 to 10 days.

After the inoculated Petri dishes have been incubated for about a week, withdraw each Petri dish from the plastic bag and observe by eye or with the assistance of a simple microscope. Are there a variety of microbial colonies visible on your plates? Draw a picture of one or more of your plates illustrating the variety of colonies present.

Lay out all of your plates in two rows, one row for each type of medium, in numerical order. Can you observe a consistent increase in the number of colonies visible on each plate with a lower number? Choose the one plate in each row which appears to have between 30 and 300 colonies on it. Carefully count the number of individual colonies and record along with the number marked on

the plate. The concentration of microorganisms in the original soil sample can be calculated by the formula:

Colonies observed X $10^{(\text{dilution number})}$ = Colony forming units/gram of original soil suspension

For example, if you obtained 44 colonies on the plate labeled 6, then the number of colony forming units (CFUs) per gram of the original soil suspension would be 44 X 10^6, or more typically expressed as 4.4 X 10^7.

Safety Issues

In most areas of the United States soils do not contain appreciable numbers of pathogenic (disease-producing) microorganisms and therefore you should be able to conduct this activity without major concern for exposure to pathogens. However, in some areas of the United States, for example, the mid-west and the arid southwest, the soils contain some significant fungal or viral pathogens and care should be taken to minimize direct exposure to the soil, soil dilutions or isolated microbial cultures. Your instructor may ask you to wear safety glasses, lab coats and gloves while conducting this activity.

Assessment

Students of this age group should be able to follow directions and complete the activity without significant supervision. If they have accomplished the activities correctly, there should be a noticeable gradient of numbers of microorganisms across their dilution series. They should be able to calculate the number of microorganisms in the original soil sample. One possible complication is that one very aggressive microorganism grows rapidly and covers the Petri dish to the exclusion of other microorganisms. This may occur on one or two of the Petri dishes, but shouldn't typically occur on all. A number of the questions posed in the procedure could become requirements for an activity report to be submitted by the students after the activity is completed.

SUPPLEMENTARY INFORMATION

Possible Modifications

Antibiotics can be added to the nutrient media used for isolating microorganisms, thus selecting for specific classes of microbes. If you wish to eliminate eubacteria from the isolations, the addition of trimethoprim (40 μg/mL) and nalidixic acid (30 μg/mL) is useful. If you wish to eliminate fungi from the isolations, the addition of nystatin (40 μg/mL) or cycloheximide (100 μg/mL) is appropriate [nystatin and cycloheximide are eukaryotic poisons and are toxic to humans at high doses, therefore additional care with these is required]. The addition of all four of these antibiotics to one of the media will select for the filamentous bacteria, actinomycetes. The actinomycetes are the most prolific antibiotic producers among the microorganisms, and selective procedures can be used to isolate these for a related activity on testing for antibiotic production.

References and Resources

I. Textbooks

A. Biology of Microorganisms, T. D. Brock

B.

II. Internet websites

A. Cubist Pharmaceuticals, Inc. (www.cubist.com): Cubist conducts isolation of microorganisms for antibiotic discovery and information on microorganisms and their activities can be found here.
B. American Society for Microbiology (www.asm.org): a general resource for microbiology information
C. Soil Science Society of America (www.soils.org): resources are available from the Smithsonian Soils exhibit
D. Society for Industrial Microbiology (www.simhq.org): check out the kid's zone and the Resources and Links for additional information
E. VWR Corporation (www.vwr.com): a supplier of laboratory supplies and sterile items
F. Fisher Scientific (www.fishersci.com): a supplier of laboratory supplies and sterile items

Appendices

1. Recipe for Nutrient agar

Beef extract	3	g/L
Peptone	5	g/L

2. Recipe for Malt extract agar

Malt extract	20	g/L
Glucose	20	g/L
Peptone	1	g/L
Agar	20	g/L

Both of these microbiological media are typically available from commercial vendors as dry powder stocks. However, each can be prepared from their separate components if desired.