MINNESOTA ACADEMY OF SCIENCE

Lesson Title / Topic

Searching for antibiotic-producing bacteria in soil around town

Content Areas

Biology, Microbiology, Biomedical Sciences

Grade Level(s)

9-12

MN Science Standards

1.1.1 Students will be able to ask questions about aspects of the phenomena they observe, the conclusions they draw from their models or scientific investigations, each other's ideas, and the information they read.

1.2.1 Students will be able to design and conduct investigations in the classroom, laboratory, and/or field to test students' ideas and questions, and will organize and collect data to provide evidence to support claims the students make about phenomena.

3.2.2 Students will be able to use their understanding of scientific principles and the engineering design process to design solutions that meet established criteria and constraints.

Student Objectives

Students will be able to:

- Observe and interpret scientific results.
- Develop lab techniques and experience working in the lab.
- Classify and categorize based on results.

Materials

- 3 Petri Dishes per lab group - a few extra just in case (Petri Dishes should be prepared with LB media prior to the lab)
- 1 50mL tubes per lab group
- 1 trowel per lab group
- 1 ruler per lab group
- 3 Test tubes per lab group
- 1 Beaker per lab group

- Pipettes
- Sterile Toothpicks
- Sterile Hockey-Stick Cell Spreaders
- Samples of *B. Subtilis*
- PBS Buffer (Water can be substituted)
- Bunsen burner/cleaner to sterilize lab space (for instructor use)

Time Required

5 class periods, 45-50 mins each

Notes

- Prior knowledge of bacteria (what bacteria is, where it can be found) is needed.
- Sterilize surfaces between each step of the procedure to avoid contamination.
- Use a bunsen burner to further sterilize the lab workplace.

Procedure

DAY 1

1. Tell the class they will be searching for soil that they think has more bacteria, taking samples, and bringing them back to the classroom to culture their samples. Eventually, we will see if any of these bacteria can produce antibiotics.
2. Have the class decide on a depth at which to collect the soil. Any depth will work, but it should be consistent across the class. A depth of 2-5 inches is recommended.
3. Divide the class into small lab groups. Each lab group should decide on a spot around the school to collect soil samples. They should be able to provide some reasoning as to why they think their spot will yield more antibiotic-producing bacteria. Examples: by a road, by the parking lot, near the front school door.
4. Have students go outside and collect soil samples. To do this, give lab groups a towel, a ruler, and a 50 mL tube. Have students dig a hole with the trowel and take a sample from the correct depth using the ruler. Fill the 50 mL tube with soil as best as they can.
5. Once back in the classroom, store the samples at room temperature until the next day.

DAY 2

1. Watch this Small World Initiative video as a class to go over the process of serial dilution: https://www.youtube.com/watch?v=nlQZ3iz4m8U
2. Have students perform serial dilution 3 times on their soil samples. Ideally, use a PBS buffer for dilution. If unavailable, use water.

   Serial Dilution Instructions:
   a. Measure out 1 g of soil sample, pour into a beaker, and fill with PBS buffer (or water) until the soil looks suspended.
   b. Set up 3 test tubes filled with 9 mL of PBS buffer (or water), labeled 1, 2, and 3,

    c. Into test tube 1, pipette 1 mL of the suspended soil, and mix with the pipette to combine.
    d. Into test tube 2, pipette 1 mL of test tube 1, and mix to combine.
    e. Into test tube 3, pipette 1 mL of test tube 2, and mix to combine.
3. Use hockey-stick cell spreaders to plate the dilutions of each soil sample in their own Petri dish, and label.
4. Incubate the plates overnight. The ideal temperature would be 25 degrees Celsius, but use room temperature if this is not available.
5. As a class, discuss what students know about antibiotics. Then watch this Nathan Winch video on what antibiotics are together: https://www.youtube.com/watch?v=OqQxtFcXNGk
6. Discuss how some bacteria naturally produce antibiotics that can kill other bacteria.
7. Tell students that next they will see if any of the bacteria in the collected soil samples produce antibiotics.

DAY 3

1. Each lab group should look at the incubated plates, which should now contain colonies of bacteria. Make observations and take notes, if desired.
2. Using sterilized toothpicks, create a 5 by 5 “grid” on a new plate.
3. One by one, scoop up colonies from the original plate and place them on a new one. Try to scoop a variety of colonies (different shapes/colors depending on what grew). Use a new toothpick between every colony. This process is called “patching.”
4. Have lab groups label and leave this new plate - the “masterplate” - to incubate. It will serve as a control, showing that all the colonies grew when patched onto a new plate.
5. Each lab group should also use hockey-stick cell spreaders to grow a layer of *B. Subtilis* on a separate plate. Leave these to incubate overnight, as well.
6. As a class, review what was learned about antibiotics yesterday and make predictions for what the plates will look like the next day

DAY 4

1. Use the same “patching” method to transfer colonies from the masterplate onto the *B. Subtilis* plates. Keep the colonies in the same position on the plate that they were in before. Label, and leave them to incubate overnight.
2. Since this activity will not fill a whole class period, possibly take this time to have students start a lab write-up, or delve further into antibiotics.

DAY 5

1. Take a look at the *B. Subtilis* plates. A ring of no growth around a colony indicates antibiotic production against *B. Subtilis*. This ring is called a “zone of inhibition.”
2. Use a permanent marker to mark which colonies were producers. See which lab group - and locations - ended up with the most producing colonies!

Here you can see that colonies marked with a black dot are producers! They created a zone of inhibition by killing the *B. Subtilis* surrounding them.

Additional Suggestions

Perform characterizing tests on any producers found. For example:

- MacConkey Agar Test (Gram-positive/negative and lactose fermentation)
- Starch Hydrolysis Test
- Antibiotic Resistance Test

Credit

Elizabeth Levinshteyn, Spring Lake Park High School (Spring Lake Park, MN), Class of 2025, developed this lesson. Elizabeth's science fair project, "*A Look into the TINY Earth: Finding Antibiotic-Producing Bacteria in Yellowstone Soil*" advanced to the Regeneron International Science & Engineering Fair in 2022. Elizabeth is a 2022-23 Minnesota Junior Academy of Science officer.