Worm Hunt: Isolating Soil Nematodes from Your Backyard

https://www.sciencebuddies.org/science-fair-projects/project-ideas/Zoo_p045/zoology/isolating-soil-nematodes-from-your-backyard (http://www.sciencebuddies.org/science-fair-projects/project-ideas/Zoo_p045/zoology/isolating-soil-nematodes-from-your-backyard)

Procedure PDF date: 2021-06-02

Experimental Procedure

Working with Biological Agents

For health and safety reasons, science fairs regulate what kinds of biological materials can be used in science fair projects. You should check with your science fair's Scientific Review Committee before starting this experiment to make sure your science fair project complies with all local rules. Many science fairs follow Intel® International Science and Engineering Fair (ISEF) regulations. For more information, visit these Science Buddies pages: Project Involving Potentially Hazardous Biological Agents (http://www.sciencebuddies.org/science-fair-projects/competitions/biological-agents-regulations) and Scientific Review Committee (http://www.sciencebuddies.org/science-fair-projects/competitions/scientific-review-committee-src). You can also visit the webpage ISEF Rules & Guidelines (https://www.societyforscience.org/isef/international-rules/) directly.

This science fair project involves the use of the bacteria *E. coli*. While *E. coli* is not considered a biohazardous or dangerous bacteria, it is important to always properly clean and dispose of bacteria and supplies that come in contact with it. See the Bacterial Safety (#bacterial-safety) guidelines below for more details on how to handle bacterial cleanup and waste.

Prepare the Bacterial Plates

1. Take out your *E. coli* culture and follow the instructions in the kit to reconstitute the dried bacteria. Wait 5 minutes after reconstituting, then mix the gently shake to mix the bacterial suspension one more time.
2. Put 2 drops *E. coli* culture on the surface of an agar plate. Try to put the culture in the center of the plate.
3. Use a sterile cotton swab to spread the bacteria around the entire surface of the agar plate as shown in Figure 2.
    a. Streak a vertical line across the surface of the agar plate.
    b. Starting from the top of the plate and moving downward, spread the streak back and forth (to the left and right), from edge to edge. Stop when you reach the bottom of the plate.
    c. Rotate the plate 60 degrees clockwise and repeat step ii.
    d. Rotate the plate another 60 degrees clockwise and repeat step ii.
    e. This approach will make sure the bacteria are spread evenly across the plate.
    f. *Tip:* Try not to pierce, or rip, the surface of the agar.
    g. Put the lid on the plate as soon as you are done.
4. Repeat for the rest of the agar plates.

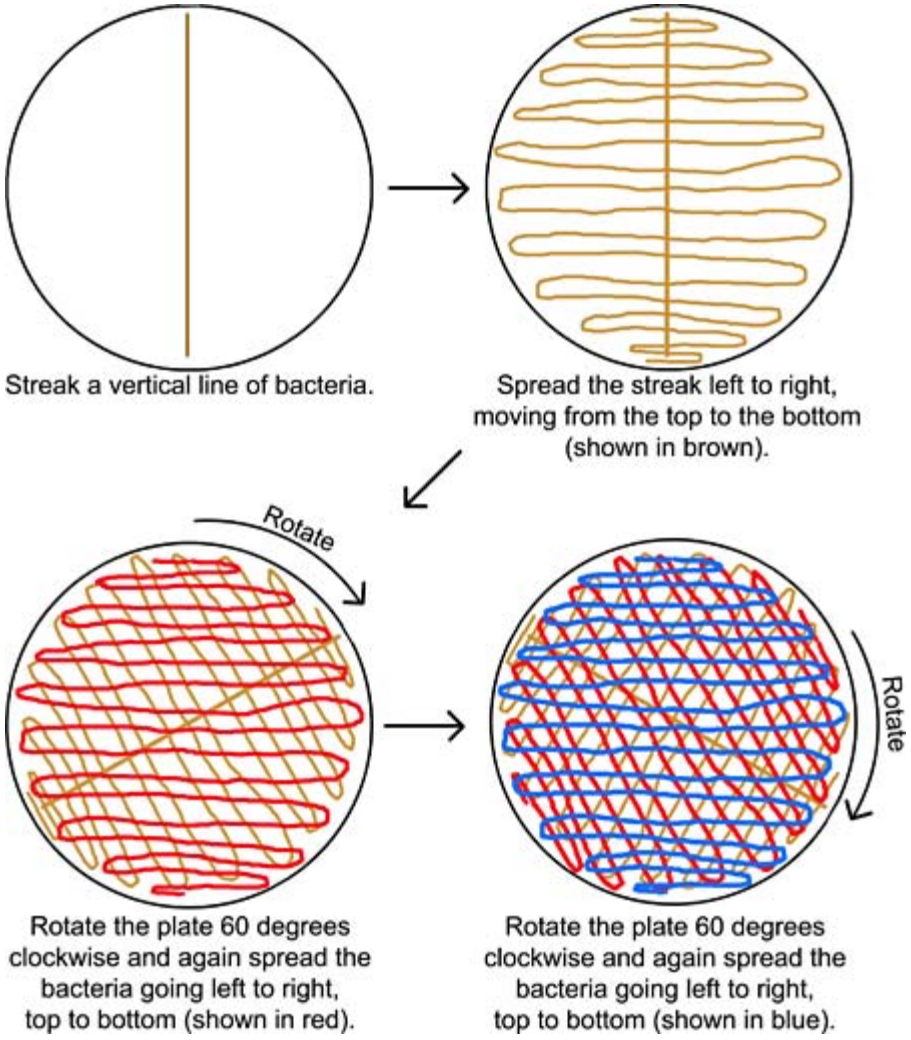

Figure 2. On each agar plate, streak the bacteria by first making a vertical line, then spreading this left to right (and top to bottom), rotating the plate 60 degrees clockwise and again spreading the bacteria left to right (and top to bottom), and then rotating the plate another 60 degrees clockwise and spreading the bacteria again. *Note:* The bacteria should all be the same color on your plates. Color has been added in this diagram to help clarify the procedure.

5. Incubate the plates, with their lids on, for 72 hours at room temperature for the *E. coli* lawn to grow. Avoid keeping the plates in direct sunlight or they will dry out. Alternatively, if you have access to an incubator that will maintain a temperature of 37°C, the *E. coli* lawn will grow within 24 hours if the plates are placed in there.

Isolate Nematodes

1. Using a tablespoon, collect 2 Tablespoons (Tbsp.) each of several soil samples, keeping them all separate in plastic baggies. Using a permanent marker, label each baggie with respect to the type of soil sample and replicate. For example, *Playground A.* Try diverse locations and/or soil types. Make sure to collect the samples in triplicates so that you have replicates for your experiment. For example, if you collect compost soil and playground dirt, you should have compost soil samples A, B, and C, as well as playground dirt samples A, B, and C. At least one of your samples should be a nutrient-rich soil, like compost or fertile garden soil.
2. Once you are back inside, slightly dampen each soil sample with ¼ teaspoon of water, while the soil is still in the baggies.
3. Using a permanent marker, label each agar plate with respect to the type of soil sample and replicate. For example, *Playground B.* (*Note:* Always label the bottom or sides of agar plates, rather than the lids. That way you will not get your samples mixed up if you take off the lids of more than one plate at once.) Distribute the soil samples in a ring around the *E. coli* lawns, as shown in Figure 2 below. Each sample should go on a separate agar plate.

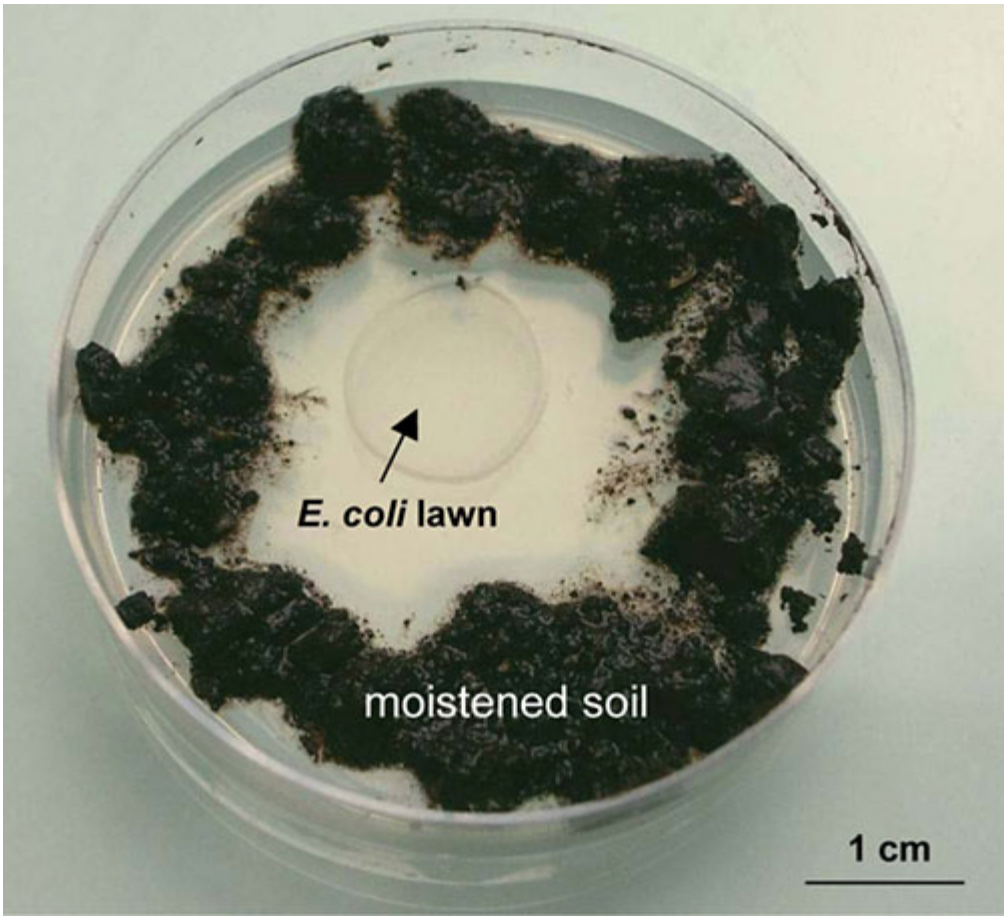

Figure 3. In this nematode isolation setup, the damp soil is placed around the *E. coli* lawn. If there are any nematodes in the soil sample, they will crawl toward the *E. coli.* (Wormbook, 2007.)

4. After 30 minutes, examine the plates using the magnifying glass. Which plates have nematodes on the *E. coli* lawn? How many nematodes do you see per plate? Using your lab notebook, record your findings in a data table like this:

Soil Sample	Replicate	# of Nematodes
Playground	A	
	B	
	C	

5. From your three replicates, calculate the average number of nematodes per soil sample. Advanced students may want to calculate the standard deviation. Which types of soil or soil locations have larger populations of nematodes?

Bacterial Safety

Bacteria are all around us in our daily lives and the vast majority of them are not harmful. However, for maximum safety, all bacterial cultures should always be treated as potential hazards. This means that proper handling, cleanup, and disposal are necessary. Below are a few important safety reminders.

- Keep your nose and mouth away from tubes, pipettes, or other tools that come in contact with bacterial cultures, in order to avoid ingesting or inhaling any bacteria.
- Make sure to wash your hands thoroughly after handling bacteria.
- **Proper Disposal of Bacterial Cultures**
  - Bacterial cultures, plates, and disposables that are used to manipulate the bacteria should be soaked in a 10% bleach solution (1 part bleach to 9 parts water) for 1–2 hours.
  - Use caution when handling the bleach, as it can ruin your clothes if spilled, and any disinfectant can be harmful

if splashed in your eyes.
    - After bleach treatment is completed, these items can be placed in your normal household garbage.
- **Cleaning Your Work Area**
    - At the end of your experiment, use a disinfectant, such as 70% ethanol, a 10% bleach solution, or a commercial antibacterial kitchen/bath cleaning solution, to thoroughly clean any surfaces you have used.
    - Be aware of the possible hazards of disinfectants and use them carefully.