K-12 IYS Activity

Soil Science Society of America

Unlocking the Untapped Antibiotic Potential of Soil Microbes:

Detection of Antibiotic Activities in Soil Microbes

Summary

This activity provides an introduction to the concepts of antibiosis, hormesis, and microbial ecology, the types of microbe-microbe interactions, the history of the discovery of antibiotics, and the need for new antibiotics.

Learning Objectives/ Outcomes

- To define terms *antibiotic*, *antibiosis*, *statis*, *drug resistance*, and *hormesis*
- To identify key concepts in microbial ecology: a range of microbe-microbe interactions, microbe-microbe communications, and the consequences of microbe-microbe interactions at local and global scales.
- To develop protocols for identifying antibiotic interactions between organisms

The experiment content objectives are:

- To learn how to culture microorganisms
- To learn sterile technique
- To compare microbial activity of various types of soils

Materials (per student, group etc.)

- Ziplock bags for soil collection
- Pre-poured laboratory media. LB agar plates are available from commercial suppliers. Note that if dehydrated media (rather than the pre-poured plates) are ordered, autoclaving will be required.
- A culture of avirulent strain of *E. coli* K12 (available from American Type Culture Collection).
- Culture broth to cultivate *E. coli* K12
- Bacti-cinerator loop sterilizer (preferred), or alchohol or a gas burner (if approved by EHS)
- Bacterial loop (metal). Disposable plastic loops are also available from commercial suppliers and do not require a bacti-cinerator.
- EZ-spread glass plating beads
- Pipettors capable of dispensing 100 microliters
- Samples of soil collected at the 2-3-inch depth (approximately 1 tsp. per agar dish needed).

Ages of Audience

1. High School biology, AP biology
2. Adults

Recommended group size?

20-36

Where could you offer this?

1. Your university
2. Local school
3. Summer programs

What type of room do you need?

1. Laboratory with benches, approved for work with biological agents (BSL-1 or BSL-1+). Depending on the regulations of the EHS office, this work may require BSL-2 level approval.
2. An incubator approved for handling biological samples will be required
3. Protocols for safe disposal of biological material may need to be developed

Type of Lesson (may be more than one)

1. Hands-on
2. Indoor
3. Experiment (follow procedure, get results, interpret results)
4. Small group exercise/discussion critical thinking
5. Video

Time Needed

1. Scientist prep time + clean up time: 1 hour
2. Participant/class time: 2-3 hours
3. Observations will require an incubation period of up to 4 days.

If the activity costs money, how have you funded this in the past/suggestions for others?

The cost of supplies (pre-poured agar plates) is approximately $5-$10 per student. Permanent equipment for culturing microbes (incubator, shaker) and protocols for disposal of biological wastes are required.

Methods/Procedures

1. Have students collect soils from yards in their neighborhood, gardens, area parks, the school field, etc., in Ziplock bags, labeling the source of each soil sample. They will need about 1 tsp. for each dish they will prepare and should collect them from a depth of 2-3 inches, where most microbial activity takes place.
2. Grow an overnight culture of *E. coli* K12. This strain is not virulent. When inoculating the LB broth culture, maintain sterile technique to avoid contaminating the overnight culture.

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3. Using sterile technique, dilute the overnight *E. coli* culture approximately 1000-fold in sterile LB broth. Spot 100 microliters of the diluted culture onto LB agar plates. Use EZ-spread plating beads to spread the culture over the plate surface (see video on http://www.genlantis.com/ez-spread-beads.html for instructions).
4. Let the liquid absorb into the agar for 15–20 minutes.
5. Place a sample (0.1–1 gram) of soil in the middle of the plate, over the *E. coli*. Let the plate incubate at least overnight. Do this for each soil collected, and label the plate with the location where the soil was collected.
6. Upon completion of the incubation, a dense bacterial growth should be observable on the plate and there should be a zone of clearing surrounding the soil if it contains antibiotic-producing bacteria (for an example of the expected results, see Fig. 4 of Lingakumar et al., 2011).
7. If there is evidence of antibiotic production, an advanced follow-up activity can focus on identifying the bacteria responsible for the antibiotic production (see protocol of Lingakumar et al., 2011).
8. Students should use common lab reporting methods to report their results to the class.

Discussion Questions

- What are the types of interactions in soil bacterial communities?
- Which soils showed the most antibacterial activity? What might some reasons for the differences be?
- Why is there a need for new antibiotics?
- What are multi-drug resistant pathogens?
- What are the most common types of interactions between organisms?
- Compare and contrast antibiosis and hormesis.
- Why is biological diversity in soils important?
- Besides as a source of new antibiotics, how do you think biological diversity in soils might help support plant and human health?

References

https://explorable.com/history-of-antibiotics

http://www.acs.org/content/acs/en/education/whatischemistry/landmarks/flemingpenicillin.html

http://bioresonline.org/article/isolation-and-characterization-of-antibiotics-producing-actinomycetes-from-soil-samples-of-senbagadaruvi-in-western-ghats-2/