MINING FOR NEW MEDICINES

Part II: Lab

Introduction: Discovery of New Cancer Drugs from Natural Products

How are new drugs discovered? Jim Allison wondered about the effectiveness of asparaginase and its variations on childhood leukemia. To find variations of this potential life-saving enzyme he looked to a number of natural sources of this in nature. What other life-saving drugs can be found in natural environments? Natural products have been important in drug discovery for decades. It has been estimated that more than half of all cancer drugs and antibiotics originated from a chemical compound discovered in a natural product. For example, Paclitaxel (Taxol) (*cancer.gov/about-cancer/treatment/drugs/paclitaxel*), one of the most commonly used chemotherapy drugs, was derived from the bark of the Pacific yew tree; penicillin (*cancer.gov/publications/dictionaries/cancer-terms/def/penicillin*), one of the first antibiotics, from a mold; and drugs to lower cholesterol from compounds found in fungi. Let's become Medicinal Miners. Let's look for a "new" natural antibiotic. This activity will act as an introduction to antibiosis, microbial ecology, and various types of microbe–microbe interactions, as well as the discovery of antibiotics, and the need for new antibiotics.

Soil microbes produce many types of chemicals that could potentially be turned into antibiotics, drugs or industrial chemicals

Learning Objectives/ Outcomes

- To define terms *antibiotic*, *drug resistance*,
- To identify key concepts in microbial ecology: a range of microbe–microbe interactions, microbe–microbe communications, and the consequences of microbe– microbe.
- To develop protocols for identifying antibiotic interactions between organisms
- *The experiment content objectives are:*
  - To learn how to culture micro- organisms
  - To learn sterile technique
  - To compare microbial activity of various types of soils

Please refer to the Tips for the Safer Handling of Microorganisms in the Science Laboratory on the NSTA website (static.nsta.org/pdfs/TipsForSafeHandlingOfMicroorganisms20160412.pdf)

Materials (per research group etc.)

- Plastic zip close bags for soil collection (1 quart)
- Sterile LB agar plates poured prior to lab or pre-poured plates that are purchased from science suppliers.
- A culture of non-pathogenic strain of *E. coli* K12 (available from American Type Culture Collection or commercial science supplier.)
- Culture broth (LB) to cultivate *E. coli* K12

- Bacterial loop (metal). Disposable plastic loops are also available from commercial suppliers and do not require flaming.
- A gas burner to flame a metal inoculating loop.
- EZ-spread glass plating beads or a glass bacteria spreader or a sterile loop to spread *E.coli* on plates
- Pipettors capable of dispensing 100 microliters or automatic pipettors set at 100 microliters
- Samples of soil collected at the 2–3-inch depth (approximately 1 tsp. per agar dish needed)
- An incubator approved for handling biological samples
- Protocol for safe disposal of biological matter (autoclave, Clorox bleach, etc.)

Time Needed

1. Teacher 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.

Methods/Procedures

1. Have students collect soils from yards in their neighborhood, gardens, area parks, the school field, etc., in plastic zip 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. (Or other non-pathogenic E. coli strain.) When inoculating the LB broth culture, maintain sterile technique to avoid contaminating the overnight culture.
3. Using sterile technique, dilute the overnight E. coli culture approximately 1000-fold in sterile LB broth.
4. Pipette100 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), or spread the pool of E. coli solution with a bacteria spreader or a sterile loop, cover the entire surface of the plate.
5. Let the liquid absorb into the agar for 15–20 minutes.
6. 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.
7. Upon completion of the incubation, a dense bacterial growth should be observable on the plate and there
8. should be a zone of clearing surrounding the soil if it contains anti-biotic-producing bacteria (for an example of the expected results, see Fig.1.
9. 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 – https://www.soils.org/files/sssa/iys/antibiotics.pdf).

Figure 1

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 did you observe?
- Which soils showed the most antibacterial activity? What might some reasons for the differences be?
- What in the soil might be keeping the *E. coli* in check?
- Why is there a need for new antibiotics?
- What are multi-drug resistant pathogens?
- What are the most common types of interactions between organisms?
- 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?
- How does this exploration of antibacterial activity in soils relate to Jim Allison's search for a cancer drug research?
- What did Dr. Allison search for various forms of asparaginase?

Extension Activities

- Explore other types of natural materials for antibiotic activity—mushrooms and other fungi types, plant leaves, roots, etc.
- Explore various environments—forest, seashore, fields, streams, etc. ,

References

- **Discovering New Cancer Drugs from Nature**, *National Cancer Institute,* www.cancer.gov/news-events/cancer-currents-blog/2019/cancer-drugs-natural-products-nci-program
- **Drugs from dirt**, *Searching in soil, scientists find a new way to combat tuberculosis – ScienceDaily,* www.sciencedaily.com/releases/2018/11/181101133828.htm
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- **Seeking New Antibiotics in Nature's Backyard**, *Cell,* www.cell.com/fulltext/S0092-8674(06)01477-2
- **Students search the soil for new antibiotics**, https://phys.org/news/2015-11-students-soil-antibiotics.html
- **Tiny Earth – studentsourcing antibiotic discovery**, https://tinyearth.wisc.edu/
- **https://explorable.com/history-of-antibiotics**
- **http://www.acs.org/content/acs/en/education/whatischemistry/landmarks/flemingpenicil- lin.html**
- **http://bioresonline.org/article/isolation-and-characterization-of-antibiotics-producing-acti-nomycetes-from-soil-samples-of-senbagadaruvi-in-western-ghats-2/**

This activity is adapted from Soil Science Society of America lab—"Unlocking the Untapped Antibiotic Potential of Soil Microbes."