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Symbiosis in the Soil: Citizen Microbiology in Middle and High School Classrooms †

Erin McKenney[1], Taylar Flythe[2], Courtney Millis[3], Jennifer Stalls[4], Julie M. Urban[5], Robert R. Dunn[6], and Julia L. Stevens[5,6]*

[1]*Department of Biology, Duke University, Durham, NC 27708,* [2]*Ligon Middle School, Raleigh, NC 27601,* [3]*Lakewood Montessori Middle School, Durham, NC 27707,* [4]*C. M. Eppes Middle School, Greenville, NC 27858,* [5]*Genomics and Microbiology, North Carolina Museum of Natural Sciences, Raleigh, NC 27601,* [6]*Department of Applied Ecology, North Carolina State University, Raleigh, NC 27695*

Microorganisms are vital to environmental health, yet their association with disease often overshadows these benefits. Building citizen-science activities around the positive role of microorganisms and an understanding of their ubiquity can begin to dispel misconceptions while simultaneously engaging the public in research. Here, we describe a citizen-science microbiology project geared toward implementation in middle and high school classrooms. Students culture environmental microorganisms and document microbial diversity of plant root systems compared with adjacent bulk soil. Results contribute data toward research on microbiome recruitment of weeds and other successful plants while addressing core topics in science education.

INTRODUCTION

Citizen science puts research into the hands of nontraditional scientists and introduces the process of discovery to a wider audience (6). Because primary and secondary level science classes typically include hands-on laboratory lessons, this presents an opportunity to engage middle and high school students in citizen science. Current hands-on laboratory activities for middle and high school students are largely limited to "tried and true" modules repeated each year with results staying in lab notebooks. These modules are often described as experiments, but in truth, they contain few true elements of experiments. Specifically, they are typically unreplicated and tend to encourage students to search for an answer that is already known. By implementing lessons engaging in citizen science, students can participate in data collection and contribute to real, replicated research in which the result of the project is not yet known. This lesson provides a framework for generating reliable, usable data on soil microorganisms (2).

The dependence of plants on the organisms that live around and on their roots (1) and limited knowledge of how and whether plants recruit the species that inhabit these zones led to the development of this citizen-science microbiology project. Specifically, this project is interested in the recruitment of microorganisms by weedy plants, which maintain consistently abundant populations of beneficial microbes, ultimately improving soil conditions and leading to increased crop production (5, 7). That weeds persist in often nutritionally poor, degraded soils requires exploitation of microbial nitrogen fixation (3), necessitating efficient recruitment of beneficial bacteria. Therefore, understanding the rhizosphere microbial dynamics and function of weeds could have important implications for agriculture and future soil health (3, 5, 7). Here, students compare the diversity of culturable microorganisms in plants and bulk soil. Results of this citizen science are then reported through a user-friendly online form (http://tinyurl.com/SoilMicrobes) to researchers studying rhizosphere recruitment dynamics of weeds and other successful plants. Students will learn about soil microorganisms and the effects plant roots have on their diversity (i.e., the different types of microorganisms inhabiting the zone). In doing so they will address key curricular concepts including symbiosis, soil stewardship, plant health, and diversity, while contributing to authentic research.

PROCEDURE

The goal of this project is to describe the diversity of bacterial and fungal cultures that grow from soil not associated with a plant (bulk soil), from soil associated with plant roots (rhizosphere), and within the roots (as endophytes)

*Corresponding author. Mailing address: 11 West Jones St., Genomics and Microbiology, North Carolina Museum of Natural Sciences, Raleigh, NC 27601. Phone: 919-707-8096. E-mail: julia.stevens@naturalsciences.org.

†Supplemental materials available at http://jmbe.asm.org

of weeds and other successful plants. This procedure was designed in three sections: making culture media, sample preparation and plating, and data collection. Sample preparation and plating procedures are supplemented by video protocols and flowcharts as additional professional development to improve teacher confidence (4).

Culture media preparation

In order to grow bacteria and fungi in culture, agar plates were made as a nutritional source and growth surface. These agar plates were made up to three weeks in advance and stored at 4°C. In repeating this project, leaders have two options for growth media. The first, as described herein, uses laboratory-grade agar supplies. The second uses materials available in typical consumer markets and is described in detail in Appendix 1. Rose Bengal agar infused with 100 mg/L of chloramphenicol was made to suppress bacterial growth and enhance growth of filamentous fungi and yeast. Nutrient agar was made with nutrient broth and 1.5% agar infused with 100 mg/L of cycloheximide to suppress fungal growth and enhance growth of bacteria.

Sample preparation and plating

Students selected plants that were growing dominantly as weeds (i.e., not ornamentals or grasses). After describing the collection area, students identified and collected the plant and collected unplanted bulk soil at least 15 cm from the plant.

In the laboratory, students mixed 0.1 g of bulk soil in 1 mL of sterile water (Tube 1) and, in a separate tube, 0.1 g of root material in 1 mL sterile water (Tube 2). Both tubes were shaken to fully mix samples in water and allowed to settle for two minutes. Tube 1 had the solution of microbes from bulk soil and tube 2 contained the solution of microbes from the rhizosphere. To make the endophyte solution, the roots were removed from tube 2 and soaked in 10% bleach solution for three minutes to sterilize the root surface. After sterilization, the roots were rinsed, finely chopped, and added to a third tube with 1 mL sterile water. This third tube was also shaken to fully mix and allowed to settle for two minutes and now contained the solution of endophytes. Each solution (Tube 1 – bulk soil, Tube 2 – rhizosphere, and Tube 3 – endophytes) was plated on both fungal and bacterial plates, after dilution to 10% and 1%, respectively, to determine the diversity of culturable microbes of each. A step-by-step procedure with flowcharts and links to video protocols is included in Appendix 1.

Data collection

After 72 hours of growth, students described and counted distinct cultures of bacteria and fungi to determine microbial diversity (i.e., number of different colony morphologies) and evenness (i.e., how many of each morphology were present). Using the student worksheet in Appendix 2, plates were scored individually, with notes made of whether bacteria or fungi were being measured and whether the sample was bulk, rhizosphere, or endophyte. Each distinct colony type was described according to the key on the worksheet and total percent coverage of the plate was measured. Where possible, the number of each colony type was directly counted rather than reporting percent coverage.

Using the class record sheet in Appendix 2, data on bacteria and fungi growing in the bulk soil, in the rhizosphere, and as endophytes were combined from student worksheets. Combining these worksheets in this way facilitated filling out the online form used to report the data to researchers. A possible extension activity is included in Appendix 3, where students also report the number of fluorescent, glowing *Pseudomonas* sp. colonies found in the bulk soil, rhizosphere, and as endophytes.

Safety issues

Environmental microorganisms in this activity are grown in culture. Therefore basic laboratory precautions for BSL1 organisms as outlined in ASM's Biosafety Guidelines (available at www.asm.org) should be followed. Plates should remain sealed while students are collecting data. To dispose of environmental cultures without an autoclave, cover the agar surface with a 10% bleach solution and incubate for at least an hour at room temperature. After this incubation, plates can be disposed of in the regular waste.

CONCLUSION

In general, this lab was received well: students appeared to enjoy studying the world around them and expressed excitement about contributing to ongoing research. Several individuals expressed gratitude for hands-on experience, which they "would never have gotten at school back home." From the teacher's perspective, this protocol is relatively straightforward to explain to students and fosters a dynamic learning environment. The protocol has been adapted to both the traditional, more verbose format, as well as graphic representation in the form of a flowchart.

Students can help to prepare their materials as they read over the protocols. This group preparation helps the instructor to identify and correct misunderstandings before the lab takes place. Experience handling the materials engages kinesthetic learners and fosters confidence as students interact with otherwise foreign research equipment, enriching the overall laboratory activity.

Results from this citizen-science project contribute to ongoing research at the North Carolina Museum of Natural Sciences exploring the recruitment of microbial diversity from bulk soil to rhizosphere and, ultimately, microorganisms living as endophytes. With citizen scientists tracking these shifts in diversity, results will not only engage students in the process of authentic research, but will also provide valuable data toward understanding the recruitment of symbiotic microorganisms by a wide diversity of plants.

SUPPLEMENTAL MATERIALS

Appendix 1: Detailed protocols
Appendix 2: Classroom worksheets
Appendix 3: Extension protocol

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