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Scientific Citizenship

The Search for Violacein-Producing Microbes to Combat *Batrachochytrium dendrobatidis*: A Collaborative Research Project between Secondary School and College Research Students †

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In this citizen science–aided, college laboratory–based microbiology research project, secondary school students collaborate with college research students on an investigation centered around bacterial species in the local watershed. This study specifically investigated the prevalence of violacein-producing bacterial isolates, as violacein has been demonstrated as a potential bioremediation treatment for outbreaks of the worldwide invasive chytrid, *Batrachochytrium dendrobatidis* (*Bd*). The impact of this invasion has been linked to widespread amphibian decline, and tracking of the spread of *Bd* is currently ongoing. Secondary school students participated in this research project by sterilely collecting water samples from a local watershed, documenting the samples, and completing the initial sample plating in a BSL1 environment. In the second phase of this project, trained college students working in courses and as research assistants in the academic year and summer term in a BSL2 laboratory facility were able to use physiological, biochemical, and molecular techniques to further identify individual isolates as well as characterize their properties. Collaboration between these learning spaces provides an increased interest in the community for environmentally relevant research projects and allows for an expansion of the research team to increase study robustness.

INTRODUCTION

Aquatic ecosystems serve as reservoirs for plant, animal, and microbial species. The composition of these communities can change over the course of seasons and years, and the census of many of the organisms can be conducted and recorded by trained observers. Microbial communities are as critical to the wellbeing of an environment as its macroscopic organisms. To begin to identify the composition of the strains within a microbial community in an aquatic environment such as a river watershed, samples can be easily collected and cultured year round, when following appropriate BSL1 and BSL2 safety precautions for working with environmental isolates. The ease of access to water sources and technique simplicity provide a potentially rich learning opportunity for students of all ages and backgrounds. Moreover, by expanding the number of participants in a research project through citizen-science outreach in local classrooms, the numbers of samples and study sites can be increased, improving data robustness. The mechanism of sampling via crowdsourcing techniques has been effective in other microbial systems, including the American Gut Project (6), uBIOME (http://ubiome.com), and the Home Microbiome Project (http://microbe.net/citizen-science-2/citizen-science-sampling-protocols/).

In the microbial system described in this tool, water samples are collected from freshwater streams, and plated samples are examined for the presence of purple/violet microbial colonies. This purple pigmentation is typically indicative of bacterial strains that produce the indole-based pigment violacein. Violacein is produced biologically via the five-gene *vio* operon in organisms including *Chromobacterium violaceum* and *Janthinobacterium lividum* (1, 10). Violacein has a number of documented biological properties, including antibacterial effects (8, 9), chemotactic effects on nematodes (2), changes to human cell lines (7, 11, 4), and parasite killing (12). Critical to our purpose, it also affects chytrid species, including *Batrachochytrium dendrobatidis* (*Bd*), the causative agent of widespread amphibian decline worldwide (3, 5). The initial goal of this study is to examine whether a pattern of violacein-producing strains could be detected in a region of

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comparatively low *Bd* infection rates (http://www.bd-maps.net/maps/) such as the Hudson River Valley Watershed area.

METHODS

This pilot project began with outreach to three local schools in two local school districts. The students participated in the collection of water samples and primary analysis of the microbes native to the local watershed. At the beginning of this project, a research faculty member associated with a local college visited local school classrooms, at the invitation of the classroom teacher, to introduce the research question being investigated. The students participating ranged in age from 6th to 12th grade and included all ability levels. Procedural and safety training workshops were completed during the 45-minute class periods. In these sessions, the visiting researcher gave a short presentation of the background and importance of the question being asked in the study, specifically, whether and to what extent violacein-producing microbial strains are found in the local watershed (Appendix 1). The students were subsequently instructed on how to perform water collection and simple plating techniques. All procedures were conducted according to ASM Biosafety Guidelines and the ASM Appendix to the Guidelines for Biosafety in Teaching Laboratories (available at www.asm.org). The plating of environmental water samples could result in cultivation of potential BSL2 microorganisms, and as such, microbes were not subcultured in any laboratory that did not meet BSL2 standards. The instructions to the students included how to collect an environmental water sample using a sterile 15-mL conical tube (provided to the students) and how to collect the metadata on the tube, as well as photographing the site (noting date, time, location, and initials on the collection tube). Students were also taught how to directly pipette the water sample on R2A 1.5% agar plates (using sterile single-use plastic pipettes and prepared R2A agar plates provided to the students) and how to distribute the water sample evenly over the surface of the plate using pre-sterilized glass beads (subsequently disinfected in Wescodyne solution, provided). Plates were immediately sealed with Parafilm, inverted, and incubated at room temperature (22 to 25°C), prior to visual examination. All waste generated was collected in biohazard waste bags and autoclaved using appropriate heat and pressure waste setting prior to disposal (Appendix 2).

Middle and high school students in three schools and ten classrooms were able to collect and culture over 75 water samples throughout the watershed area. Following room temperature incubation of the water samples on these plates within the classroom setting, students examined the sealed plates and made visual observations of presence, absence, and abundance of purple microbial colonies. All agar cultures and water samples were transported in biosafety containers to the BSL2 laboratory at the local college, where the second phase of microbial characterization was carried out.

Utilizing the more than 75 samples and data collected during the outreach phase of this research project, college research students were able to continue the analysis of the violacein-producing microbial isolates. All participating college students completed at least one laboratory course in microbiology techniques or were individually trained by the faculty member as part of the orientation of the research program, with all students completing BSL1 and BSL2 safety training. Students analyzed the violacein-expressing isolates and verified the phenotype of the initial samples (Fig. 1). Strains of interest were profiled for their growth on various types of media (R2A, luria broth [LB], 1% tryptone), tested for temperature growth range, examined for violacein pigment production, observed for cell motility, and analyzed for 16S rRNA sequences using BLAST (Appendix 3, Fig. 1). Thus far, strains of *Janthinobacterium, Duganella, Iodobacter,* and *Massilia* species have been isolated and characterized from the waterways of interest.

Work in the college research lab is still ongoing and involves sequencing, de novo assembly, and annotation of whole genomes isolated from the environment. Additionally, studies are underway to use random genomic mutagenesis to identify physiological pathways essential to fungal and bacterial killing and other phenotypes of interest. Currently, in the second year of this study, presentations to secondary students beginning the project now incorporate data collected from the previous investigations. Students are learning about the impact that their contribution can make to a larger environmental study and can learn about the overall arc of a long-term research project.

SAFETY ISSUES

All procedures followed ASM Biosafety Guidelines (available at www.asm.org), and all microorganism subculturing took place in BSL2 facilities, with BSL2-trained students. All water samples, plates, pipettes, and waste used or produced in this study were autoclaved using appropriate settings for pressure and temperature. Culture plating procedures were adapted to use pre-sterilized materials to eliminate the need for flame and alcohol sterilization techniques.

POTENTIAL ADAPTATIONS AND EXTENSIONS

As this project continues, additional participation of local schools can be added to the study cohort, as desired, increasing both student numbers and extent of participation. Middle and high school students interested in continuing work on the project were invited to participate in summer and after school research in the BSL2 college laboratory (following appropriate BSL1 and 2 biological safety training) and worked under the supervision of the faculty member and in conjunction with the college research students. During this experience, both secondary and college students were able to learn and practice microbiological methods.

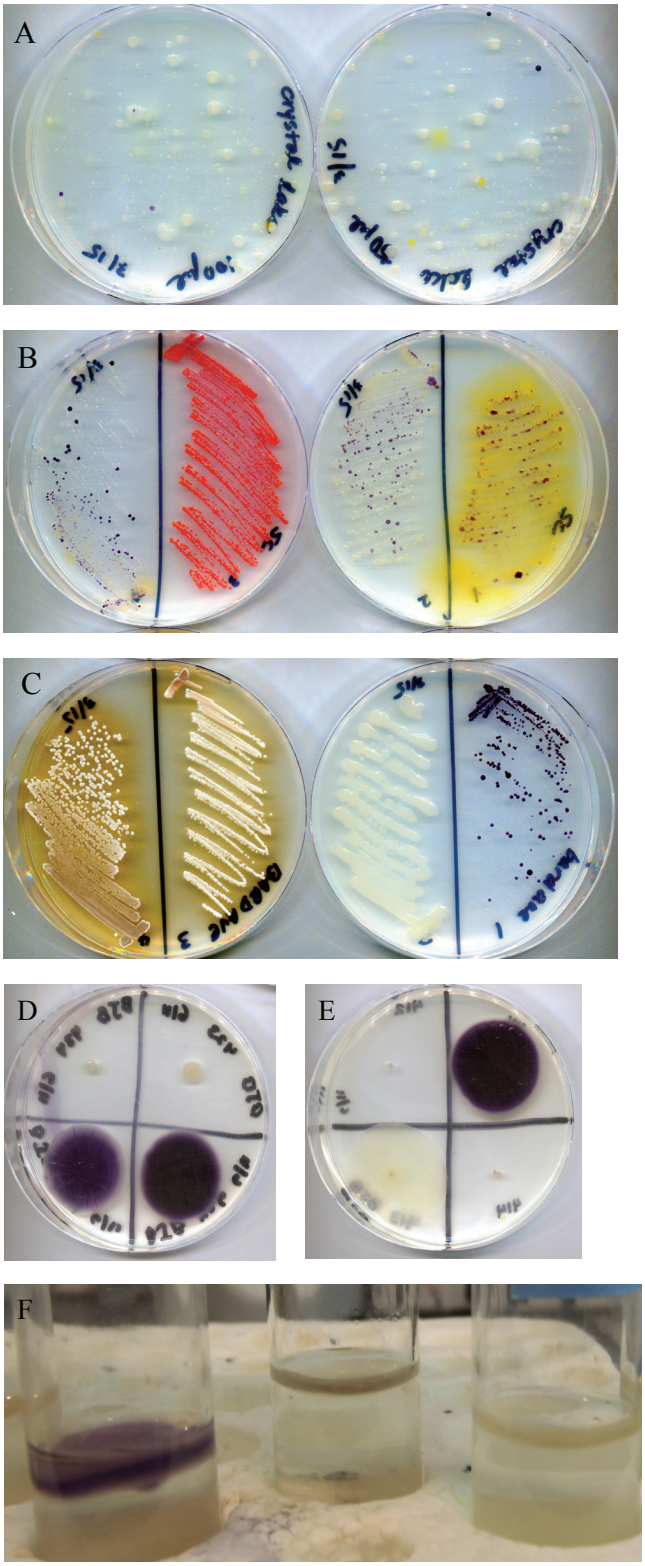

FIGURE 1. Results from water sampling assays. (A) Colonies resulting from pipetting 200 µL of water onto R2A agar, incubated at 22°C for 24 to 28 hours. (B–C) Purification of single colonies onto R2A agar, incubated at 22°C for 24 to 28 hours. (D–E) Isolate growth on 0.3% R2A swimming motility agar incubated at 22°C for 24 to 28 hours. (F) Biofilm production of isolates in liquid R2A broth incubated at 22°C for 24 to 28 hours.

CONCLUSION

Preliminary data from our current study region of the Hudson River Valley watershed indicate a large number and variety of violacein producing bacterial cells in the water samples (data not shown). It is possible that the prevalence of this compound could alter the level of regional chytrid outbreaks, and it possibly protects the native amphibian populations. Although a large-scale study of this question has not yet been conducted, due to the ease of sample collection, microbial cultivation, and strain characterization, this pilot project provides a wealth of investigative opportunities for learners throughout the community. By including learners of a range of ages in an inquiry-based research project and keeping tasks clear, manageable, and goal oriented, gains can be made in the research project, and interest and activism of the surrounding community for this and other citizen science–based projects may be increased.

SUPPLEMENTAL MATERIALS

Appendix 1: PowerPoint presentation introducing project
Appendix 2: Site sampling instructions
Appendix 3: Recipes for media and PCR details and methods

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