Seeking to Understand the Microbiome of Caves Using New Tools and Techniques

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Introduction

The Texas A&M Science Influencers Program enabled me to pursue my passion researching cave microbiology. The highly unexplored potential of these mysterious ecosystems has always intrigued me. While studying biological and agricultural engineering, I discovered a way to combine my interest in microbiology and my hobby of cave exploration. With support from the Science Influencers Program and guidance from Drs. Holli Leggette, Gary Wingenbach, and Barbara Gastel, I pursued a summer research internship with Dr. Hazel Barton at the University of Alabama. Throughout the internship, I learned all I could about cave microbiology and the tools and techniques essential for research.

Background

I assisted each graduate student in the lab, learning all I could about their projects. My primary focus was helping Max Koether research the formation of caves within Gunung Mulu National Park on the island of Borneo. I began my research by studying the basic formation of limestone caves through groundwater (Palmer 1991).

The caves in Mulu are unique. They are enormous compared to caves in other parts of the world (Waltham 1980). The caves may be formed in part by microbial processes being driven by bird and bat droppings in the caves (Barton 2024). The high nitrogen content of these droppings contributes to a unique nitrogen cycle in the caves potentially contributing to their large size (Barton 2024).

By analyzing isotopes, we aimed to understand how nitrogen is cycling in these caves, similar to studies done on marine environments (Dähnke 2013). Microbial interactions could have an important role in the nitrogen cycle in caves (Li 2024). My primary goal for the internship was to assist in demonstrating that microbial processes significantly contributed to the formation of Mulu's extensive cave system.

Objectives

The objectives of the internship began with mastering basic lab procedures used in microbiology. Once comfortable in the lab, I helped Max Koether prepare samples for isotope ratio mass spectrometry. We then worked to find a nuanced method of genomic extraction of bird and bat guano using various genomic extraction kits.

Additionally, I assisted Isuru Silva with his cyanobacteria research and worked on my own to develop a procedure to treat microscopic fibers used in caving rope. Near the end of the internship, I gained networking and communication skills by attending the National Speleological Society National Convention with the lab cohort.

Methods

Early in the internship we used isotope ratio mass spectrometry to analyze samples collected from caves in Borneo (Muccio 2009). We then tested various DNA extraction kits on bird and bat guano taken from the caves in Borneo. Many kits failed to work, but one kit showed positive DNA counts and a good gel analysis. While using the DNA kits I was trained to use a centrifuge, bead beater, micropipette, Bunsen burner, water bath, autoclave, fume hood, gel electrophoreses, a Qubit fluorometer, and other standard lab equipment, while adhering to sterile techniques (Balakrishnan 2013).

I assisted Isuru Silva with microbial collection of cyanobacteria, creation of growth media, and sonication of cells for DNA extraction. While I developed my experimental procedure to test rope fibers, I learned how to safely mix solutions of various acids and use a variety of lab equipment, including a fiber tensile testing machine.

Results

While gaining a variety of lab experience, we accomplished many of our goals. The Borneo cave samples that were prepared for isotope ratio mass spectrometry were analyzed and showed good results. We showed that a DNA extraction kit could produce viable DNA for analysis. Gel electrophoresis and a Qubit fluorometer showed that the DNA extracted was viable for analysis. The growth media I created produced microbial growth. The microbial samples I helped collect were cultured successfully. I produced a nuanced method for treating rope fibers that will greatly advance the research project. I created instructional videos on how to prepare nutrient media and prepare samples for isotope ratio mass spectrometry which were published on the science influencers social media pages. Lastly, I conducted and published a cave survey tutorial and interviews with each student in Dr. Bartons lab, which are posted on YouTube (Crubaugh 2024).

Conclusions

This internship was filled with valuable learning experiences that will shape my future career. The time spent working on cave microbiology with mentors and peers taught me more than any classroom setting.

Our results from the isotope ratio mass spectrometry and genomic extraction kits greatly advanced the Borneo cave research. The results contributed to the hypothesis that the caves are formed through microbial processes. The procedure I developed for treating rope fibers will be used throughout the project, and all the various projects I assisted with have set the perfect foundation for my future research in microbiology and cave science.

I am so grateful to the project managers Dr. Holli Leggette, Dr. Gary Wingenbach, and Dr. Barbara Gastel for making this program such a great experience. Additionally, I am thankful to the students and professors I met over the summer that made this experience so remarkable. Thanks so much to Dr. Hazel Barton, Max Koether, Jaden Waddell, Nathaniel Friedrich, Isuru Silva, Reilly Blackwell, and all the others I met in the Geological Sciences department. This experience has set the perfect foundation for my future research endeavors.

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