Molecular and Metabolic Mechanisms of Carbon Sequestration in Marine Thrombolites
Graduate Student Researcher: Jennifer Mobberley, Ph.D. candidate
NASA - John F. Kennedy Space Center
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Overview of Research Project: The overall goal of my dissertation project has been to examine the molecular processes underlying carbon sequestration in lithifying microbial ecosystems, known as thrombolitic mats, and assess their feasibility for use in bioregenerative life support systems. The results of my research and education efforts funded by the Graduate Student Researchers Program can be summarized in four peer-reviewed research publication, one educational publication, two papers in preparation, and six research presentations at local and national science meetings (see below for specific details).

Peer Reviewed Publications


Educational Learning Module


Publications in Preparation


Presentations of Research Results:

**Project Summary:**

Bioregenerative life support systems (BLSS) that improve the recycling and sequestration of carbon dioxide (CO₂) are needed for long duration space flight. One ecosystem that has the potential to be used for BLSS are thrombolitic microbial mat communities. These microbial communities have been shown to undergo robust recycling of CO₂ and are highly resilient to environmental flux (Nelson, 2008). Thrombolitic mats are self-sustaining microbial communities that undergo extensive biologically induced precipitation of CO₂ as calcium carbonate (CaCO₃). Thrombolitic mats are ideal model systems for examining the mechanisms of carbon sequestration, as they function as semi-closed environments in which carbon is cycled with great efficiency (Canfield and DesMarais, 1993) and they have rates of productivity that are comparable to hardwood forests and grasslands (Canfield et al., 2004). One of the most well-studied thrombolitic mat ecosystems is found on the island of Highborne Cay, Bahamas. Microbial diversity and biogeochemical studies of these Bahamian thrombolitic mats revealed complex microbial consortia contributing to daily metabolic cycling of carbon (Myshrall et al., 2010; Mobberley et al., 2012). The net mat microbial metabolisms create biochemical gradients, such as pH and oxygen, which influence the extent of CaCO₃ precipitation (Visscher et al. 2005; Dupraz et al., 2009). There is a significant gap in knowledge of the functional genetic mechanisms that contribute to CO₂ sequestration within thrombolitic mats.

The goal of my project has been to understand the metabolic processes that contribute to community functioning and carbon sequestration within thrombolitic mat communities. The overall objective of this research plan was to assess and delineate functional gene expression of modern lithifying microbial mat ecosystems. The results of this research project are expected to have a positive impact on understanding the active role microbes play in coordinating metabolisms within thrombolitic mat communities and may also lead to the identification of novel metabolic pathways that could be utilized in future bioregenerative life support systems.

**Undergraduate Training and Educational Outreach:** In addition to my research training I also gained experience in mentoring and training others students in STEM education. Over the past three years I have participating in the following activities:

- Mentored two undergraduate interns working at the Space Life Sciences Lab with Dr. Jamie Foster. These students were Maya Ortega, a NASA MUST intern from University
of Colorado and Steven Ahrendt, a Florida Space Grant intern from University of California Riverside.

- Served as a science fair judge for the 2011 Brevard County Regional Science Fair.
- Assisted the NASA Kennedy Space Center Education Office by providing incoming interns with a tour of the University of Florida research facilities at the Space Life Science Lab.

Major Research Findings and Accomplishments as a result of GSRP funding:

- **Microbial diversity of modern marine thrombolites are distinct from other lithifying microbial mat ecosystems.** The results of my research indicated that the there are four different thrombolitic mat types at Highborne Cay, The Bahamas. Additionally, my work revealed that the microbial diversity contained the unlaminated thrombolitic mats of Highborne Cay is distinct from other lithifying microbial ecosystems. I examined the bacterial, cyanobacterial and archaeal populations within the various mat types. These results suggest that the unlaminated microstructure characteristic thrombolites may be formed by the metabolic activities of a distinct population of microbes dominated by the cyanobacterium *Dichothrix* spp. An example of the bacterial derived from the different thrombolitic mat types (i.e., black, beige, pink, and button mats) is seen in Figure 1.

![Bacterial Phylo/Classes](image)

- **Established the metabolic potential of thrombolitic mat through metagenomic sequencing.** The range of biochemical and molecular pathways that contribute to thrombolitic mat functional gene complexity were delineated through the sequencing of total community DNA extracted from the thrombolitic mats of Highborne Cay, Bahamas (Mobberley et al., 2013). The metagenome was annotated using the SEED genome database and sets of related genes involved in metabolic pathways (i.e., subsystems) were compared using the metagenome statistics program, R. The results indicated that the metagenomes of the thrombolitic mats are distinct from previously sequenced lithifying microbial ecosystems suggesting there are potentially unique processes associated with the carbonate structure formation (Figure 2). The metagenome was dominated by bacterial metabolisms, with overrepresented subsystems associated with Protein, Phosphorus, Nitrogen, Carbohydrate, Mobile Elements, and Amino Acid Metabolisms (Figure 3A). These results are the first metagenomic analysis of the Bahamian thrombolites and suggest that the microbes associated with these communities are capable of a wide range of metabolic activities that may influence carbon sequestration and carbonate mineralization.
**Generated first metatranscriptomic gene expression profile of modern marine thrombolites.**

To complement the metagenomic data, I also examined the metatranscriptome of the thrombolic mats using direct transcriptome sequencing. Metatranscriptomics is the profiling of gene expression via the analysis of message RNA (mRNA) transcripts found in multispecies communities and has advanced our understanding of complex microbial ecosystems at the molecular level. To examine the metatranscriptome and generate a spatial profile of gene expression in the thrombolic mats I horizontally sectioned the mats collected at the peak of solar activity (12PM) into three discrete zones (0 – 3 mm; 3 – 5 mm; and 5 – 9 mm) based on *in situ* biogeochemical measurements of oxygen production in the mats using a Clark microelectrode (Figure 4).

![Figure 2](image-url)  
**Figure 2** Comparison of thrombolite metagenome with metagenomes of previously sequenced lithifying and non-lithifying microbial mat ecosystems. a Principle Component Analysis of SEED subsystems (Level 1) derived from several distinct habitats. These habitats include the hypersaline non-lithifying mats of Guerrero Negro, Mexico; thermophilic microbial mats of Octopus Spring, Yellowstone National Park (YNP); freshwater microbial mats of Los Venados and Pozas Azules found in Cuatros Cienegas Basin, Mexico (CCB); lithifying freshwater oncolites and thrombolites of Rio Mesquites and Pozas Azules in CCB; non-lithifying (Type 1) and lithifying (Type 3) stromatolites of Hightorne Cay, The Bahamas and the un laminated thrombolites of this study. A metagenome from the Sargasso Sea was used as an outgroup. The first two principle components represent 92.85% of the variation between samples. The red biplot lines represent the directionality of the six most important subsystems driving differences between the metagenomes included in the analysis. (taken from Mobberley et al., 2013)

![Figure 3](image-url)  
**Figure 3** Distribution of recovered functional genes of the thrombolitic mats. All samples were collected at 12 PM. (A) Metagenome represents sequencing of community DNA extracted from the three combined layers. Metatranscriptomic libraries of total RNA from (B) Layer 1 (0 – 3 mm); (C) Layer 2 (3 – 5 mm); and (D) Layer 3 (5 – 9 mm).
I generated ~3 Gbp of metatranscriptomic sequencing data for each layer of the thrombolitic mat. For control purposes I also sequenced the total RNA to determine the efficiency of commercial mRNA purification kits. I determined that the mRNA enrichment step, which was intended to remove ribosomal RNA (rRNA), was not effective for microbialitic mat samples and sequencing total RNA not only minimized sequencing bias but was a faster and less expensive approach for future metatranscriptomic analyses. I also developed a metatranscriptome analysis pipeline using pre-existing software and custom Perl and Python scripts to classify potential functional genes from the recovered mRNA transcripts. Additionally, I analyzed the recovered rRNA sequences, which will serve as proxies for those metabolically active species within the thrombolitic mat. The results of the functional gene analyses are listed below.

• Metatranscriptomic sequencing revealed differential gene expression within thrombolitic mats. The annotated metatranscriptomic data from each of the three layers was compared to the metagenome to assess the differential expression of genes associated with key metabolic pathways (Figure 3). Not surprisingly key differences in the relative abundance of gene expression were detected between the metagenome and metatranscriptome in several metabolic pathways. The largest difference occurred in genes associated with photosynthesis. Although photosynthesis comprised only 4% of the metagenome, between 13 and 60% of the recovered transcripts were photosynthetic genes. Taxonomic analysis indicated that most of the primary production was done by cyanobacterial oxygenic photosynthesis with some contribution from eukaryotic algae. These molecular results also correlated to the increase in oxygen production that occurred at those depths (Figure 4). These results provide further evidence that photosynthesis is a major metabolism of the thrombolitic mats and may be driving the biogeochemical cycling within the thrombolitic mat. Photosynthesis is also an important metabolism in carbonate mineralization (Dupraz et al., 2009), and the extensive up-regulation of photosynthesis pathways may be facilitating the precipitation of carbonate in the thrombolitic microbialites.

References:
Mobberley, J.M., Khodadad, C.L.M. Foster, J.S. (2013) Metabolic potential in cyanobacterial-


