

MICROBIAL AND FUNCTIONAL GENE DIVERSITY IN THE THROMBOLITIC MATS OF HIGHBORNE CAY, BAHAMAS. J.S. Foster and J.M. Mobberley, University of Florida, Department of Microbiology and Cell Science, Space Life Science Lab, Kennedy Space Center, FL 32899, jfoster@ufl.edu.

Introduction: Thrombolites are unlaminated organosedimentary structures that result from the interactions between microbial mats and their surrounding environment. Much like their laminated stromatolitic counterparts, thrombolites represent an important milieu for the evolution of life. Thrombolites have a long geological record with definitive fossils dating back to 1.92 Ga and represent one of the earliest organosedimentary depositing ecosystems [3]. Although once prevalent on the ancient Earth, modern accreting thrombolites are rare. One of the few modern sites of active thrombolitic development is the island of Highborne Cay, located along the west margin of Exuma Sound, Bahamas.

In this study we examined the microbial diversity and the functional gene complexity of the microbial mats associated with the Highborne Cay thrombolites. This is the first pyrosequencing diversity analysis of a modern marine thrombolitic mat community. The metagenomic sequencing approach was coupled with both morphological and ARISA profiling. Five spatially and morphologically distinct mat types were identified from the Bahamian thrombolitic mats: black, beige, pink, button and turf. Of the five, only four were dominated by prokaryotes and were the subject of this investigation.

Ecotype differences in the thrombolitic mat types were investigated by pyrosequencing of oligonucleotide barcoded 16S rRNA genes. DNA from each mat type was extracted in triplicate and PCR amplified with three distinct primer sets that targeted the 16S rRNA genes of bacteria, archaea, and cyanobacteria. Each primer set was conjugated to four distinct oligonucleotides that served as a unique identifier of the targeted mat type. The amplicons of each mat type were then pooled and sequenced with a GSFLX pyrosequencer and pre-processed using the MOTHRUR analysis pipeline [6]. At least 1500 sequences were recovered from each mat type. This was repeated for the bacteria, archaea and cyanobacteria communities totaling over 20,000 high-quality sequencing reads. Sequences were aligned with NAST in Greengenes [2] then were compared using a pairwise distance calculation and clustered into operational taxonomic units at a 3% distance. OTUs were then assigned to a phylogeny using the Greengenes classifier.

Diversity indices of the microbial communities in the thrombolitic mat types reveal an even distribution

of ecotypes within all four mat types. In all mat types Alphaproteobacteria were the dominant sub-phyla with each mat types rich in phototrophic purple non-sulfur bacteria such as *Rhodovulum* spp. as well as methanotrophs such as *Methylobacterium* spp.. The second dominant subphyla in the four mat types were the Gammaproteobacteria specifically the sulfide oxidizing bacterium *Achromatium* spp as well as other Thiotrichales. Although there was extensive overlap in the several of the major taxa there were distinct differences detected between the four community types. The most prominent differences were in the cyanobacteria community. The black and pink mat types were dominated by coccoid morphotype specifically the Pleurocapsales (e.g., *Xenococcus* spp; *Pleurocapsa* spp.) and the Chroococcales (e.g. *Cyanothece* spp.; *Synechococcus* spp.). The beige and button mat types, however, were rich in filamentous mat-building Oscillatoriales cyanobacteria such as *Leptolyngbya* spp.. The button mats were unique in that they contained additional Nostocales sequences with similarity to the heterocystous forming *Dichothrix* spp., which has been shown to be closely associated with the formation of calcified filaments within the button-like thrombolitic mats [5]. To assess the overall relationship between these community types at the genus- and species-level quantitative hierarchical clustering analysis of these four thrombolitic mats using MOTHRUR indicated that the pink and beige mat-types were most similar to each other while the black mat-type was the most different. These clustering patterns may reflect differences in the extent of sediment burial each mat experiences in the high-impact intertidal zone. Black mats are most often buried and exhibit the lowest structural relief of the four mat types, whereas the button mats exhibit relief that extend up to 40 cm in height.

Of the four mat types, previous studies have shown that the button-like mat type was the most biogeochemically productive [4]. Therefore, the button mat type was selected for functional gene metagenomic analysis through pyrosequencing (GSFLX). Although less than 5% of the button thrombolitic mat metagenome, which is estimated to be at least 3.7 Gb based on the aforementioned diversity analyses, has been sequenced. Recovered genes have provided critical insight into the major functional subsystems of the button thrombolitic mat metagenome in particular those pathways associated with carbonate mineralization.

For example, more than 10% of the recovered genes are associated with the production of extracellular polysaccharides (EPS) namely alginate, colanic acid, sialic acid metabolisms. The production of EPS materials is a critical component in the stabilization and development of thrombolitic mat communities. Genes associated with several key metabolisms known to promote the precipitation of carbonates were also recovered including oxygenic and anoxygenic photosynthesis, and dissimilatory sulfate reduction. However, genes were also recovered from metabolisms associated with carbonate dissolution such as fermentation and denitrification. Together these sequencing efforts are now elucidating the molecular pathways that underlie these critical biogeochemical processes in modern thrombolites.

In addition to energy metabolisms that are known to promote carbonate precipitation a few sequences were recovered that share sequence similarity to known carbonate mineralization genes. These genes include the *tetR* regulatory gene essential for extracellular carbonate mineralization in *Bacillus subtilis* [1] and fatty acid CoA ligase, which is known to be upregulated in biomineralization conditions in marine coccolithophorids [7]. By delineating the thrombolitic mat metagenome it may be possible to identify other pathways such as fatty acid metabolisms or cell-cell signaling that may be critical for thrombolitic mat accretion and development.

- [1] Barabesi, C., et al (2007) *J Bact* 189:228-235.
[2] DeSantis, T. Z., et al. (2006) *Nucleic Acids Res* 34:W394-399. [3] Kah, L. C., and J. Grotzinger (1992) *PALAIOS* 7:305-315. [4] Myshrall, K., et al. (2009) *Geobiology*: in review. [5] Planavsky, N., et al. (2009) *Geobiology* 7:566-576. [6] Schloss, P. D., et al. (2009) *Appl Environ Microbiol* 75:7537-41. [7] Wahlund, T. M., et al. (2004) *Micropaleontology* 50:145-155.