METAGENOMIC COMMUNITY PROFILING OF MODERN STROMATOLITES FROM THE SOUTHERN EXUMA CAYS, THE BAHAMAS.

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Stromatolites are laminated carbonate structures formed by the metabolic activities of lithifying microbial mats. To delineate the molecular processes associated with the formation of these structures 16S rRNA amplicon and metagenomic libraries were generated to characterize both the microbial and functional gene diversity within stromatolites collected from Little Darby Island located in the Southern Exuma Sound, The Bahamas. Additionally, we compared these data to publicly available lithifying and nonlithifying microbial mat libraries across different geographical locations to assess those shared and divergent pathways.

Sequences generated using the Illumin HiSeq platform were analyzed using MetaCV and MEGAN to characterize the stromatolite metabolic pathways and the relative bacterial contribution. The 16S rRNA data set was processed using QIIME and PICRUST for assessing the taxonomic composition and predicting metagenome functional content from marker genes. Results indicate that the stromatolite-forming microbial community from Little Darby is represented by 10 phyla with the Proteobacteria (50%) and Cyanobacteria (28.4%) as the most dominant. The Cyanobacteria phylum was composed by six classes and 10 orders, of which Chroococcales and Oscillatoriales were the most abundant. The most abundant taxon within the Little Darby Island metagenome, was associated with the family Oscillatoriophycideae (28%). Based on morphological analysis of the Little Darby Island microbialites this enriched Oscillatoriophycideae may represent the filamentous *Schizothrix* sp., an organism known to be critical for the trapping and binding of sediment in Bahamian stromatolites.

Metagenomic analysis revealed a total of 2,944 KEGG gene functions with the most abundant pathways related to nucleic acid metabolism, photosynthesis and oxidative phosphorylation. Two-component system associated functions were also enriched, suggesting a strong ability of stromatolite microorganisms to sense and potenitally respond to changes in different environmental conditions. Additionally, we found glyoxylate, dicarboxylate, nitrogen, methane, pyruvate and propanoate metabolisms to be higher compared to other functional gene categories.

We also assessed the dominant microbes contributing to photosynthesis, glucose and exopolysaccharide (EPS), nitrogen and sulfur metabolisms. All genes classified within the photosynthesis system were primarily associated with the bacterial order Chroococcales (Cyanobacteria), whereas within the EPS and nitrogen pathways the order Rhodobacteriales (Alphaproteobacteria) was dominant. Genes associated with sulfur metabolism corresponded to the Deltaproteobacteria and Gammaproteobacteria classes.

Lastly, we compared our 16S rRNA amplicon libraries with publicly available datasets of microbial mats derived from freshwater, marine and hypersaline envrionments across the globe for a total of 171 samples. Rarefaction curves showed that the lithifying microbial mats from Little Darby, The Bahamas to have the highest species richness of all samples examined. Beta diversity, as computed by Unweighted UniFrac, defined different clusters among samples belonging to the same environmental location and even when derived from different studies ($p = 0.001$, $R = 0.93$; ANOSIM). Also considering the salinity, we identified clusters that grouped samples from freshwater to hypersaline environments, respectively ($p = 0.001$, $R = 0.39$; ANOSIM). Meta-diversity comparisons to other microbial mats communities suggest that Bahamian stromatolites are highly conserved and share a core microbiome. Together, the results of this study will help to improve our understanding of the underlying metabolic processes and microbiome associated with biologically induced mineralization in modern stromatolites.