

MODERN FRESHWATER MICROBIALITES OF CUATRO CIÉNEGAS, MEXICO. I: METAGENOMIC AND STABLE ISOTOPIC ANALYSES TO ASSESS MICROBIAL COMMUNITY STRUCTURE AND FUNCTION. M. Breitbart¹, D. Hollander¹, A. Nitti¹, B. Van Mooy², J. Siefert³, V. Souza⁴, ¹University of South Florida; Saint Petersburg, FL, USA, mya@marine.usf.edu; ²Woods Hole Oceanographic Institution, Woods Hole, MA, USA; ³Rice University, Houston, TX, USA; ⁴Universidad Nacional Autonoma de Mexico, Mexico.

Ancient biologically-mediated sedimentary carbonate deposits, including stromatolites and other microbialites, can provide insight into conditions on early Earth. However, interpretation of the environmental and evolutionary significance of microbialites throughout the geologic record is dependent upon an understanding of the complex linkages between microbial community composition, metabolic gene expression, local chemical environments, and the incorporation of isotopic and molecular organic geochemical signals into carbonate minerals in modern microbialite systems.

Through the coordinated application of metagenomic and stable isotopic analyses, we have examined the microorganisms and chemical processes in the actively-accreting freshwater microbialites found in Rio Mesquites, Cuatro Ciénegas, Mexico. Examining the pathways present in the microbialite metagenomes coupled with performing stable isotope analyses is a powerful strategy for determining the interplay between microorganisms and chemical processes that influence carbonate precipitation.

The Cuatro Ciénegas Basin (CCB) is an extremely phosphorus-poor, nitrogen-rich environment in the Chihuahuan desert of Coahuila, Northern Mexico. Metagenomic sequencing was performed on an actively-accreting microbialite from Rio Mesquites to examine the composition and metabolic potential of the microbialite community. Analysis of the microbialite metagenome revealed a diverse, redox-dependent microbial community, consisting of Bacteria, Archaea, eukaryotes, and viruses. The vast majority (95%) of the identifiable sequences were from Bacteria, and these sequences were dominated by cyanobacteria (74% of the bacterial sequences). Genes involved in the light-dependent and light-independent reactions of photosynthesis were abundant in the microbialite metagenome. A wide variety of photopigments (including chlorophyll, allophycocyanin, phycocyanin, phycoerythrin, phycoerythrocyanin, and photoreceptor phytochromes) were identified, suggesting that the microbialite cyanobacteria can access a variety of wavelengths of light. Examination of the $\delta^{13}\text{C}$ in the carbonate organic matter system of Cuatro Ciénegas also supports the presence of aerobic phototrophy. The $\delta^{13}\text{C}$ of intracrystalline organic matter in the microbialite was -27‰ , which is consistent with the enzymatic

fractionation associated with oxygenic photosynthesis from a DIC source of $+4\text{‰}$.

If calcium carbonate was precipitating in equilibrium with the ambient waters, the predicted $\delta^{13}\text{C}$ of the calcium carbonate in the Rio Mesquites microbialite would be $+6.2\text{‰}$. However, the measured $\delta^{13}\text{C}$ value of the microbial carbonate was $+1.1\text{‰}$. The depleted $\delta^{13}\text{C}$ values of the microbialite carbonate can only be explained through the addition of a $\delta^{13}\text{C}$ -depleted source of carbon to the local DIC reservoir. Respiration of the ^{13}C -depleted photoautotrophic biomass by heterotrophic organisms would release ^{13}C -depleted CO_2 (-27‰) to the DIC reservoir, resulting in a localized negative shift in the ^{13}C -DIC. Based on mass balance calculations with only ambient water ($+4\text{‰}$) and remineralized organic matter (-27‰) as contributors to the local DIC reservoir, it is predicted that at least 20% of the carbon in the carbonate mineral matrix is derived from heterotrophic respiration. In support of this conclusion, numerous genes for heterotrophic processes (such as sulfate reduction and denitrification) were recovered from the microbialite metagenome. The abundance of sulfate in this system, as well as the genomic and isotopic evidence for sulfate reduction, suggests that sulfate reduction may be the dominant heterotrophic process influencing microbialite precipitation in Cuatro Ciénegas.

Overall, the microbialite metagenome was highly distinctive from other sequenced freshwater and marine communities in both taxa and function. Genes involved in phosphorus metabolism and regulation/cell signalling were significantly more abundant in the microbialite metagenome than in previously sequenced free-living aquatic microbial communities. The overrepresentation of genes involved in phosphorus metabolism (including metabolism of phosphate, polyphosphate, phosphonate, and alkylphosphonate) demonstrates that the microbes have adapted to unique low phosphorus waters of Cuatro Ciénegas. An additional adaptation to the low phosphorus environment was the presence of phospholipid alternatives, such as sulfolipids, which were confirmed by both metagenomic sequencing and analysis of the intact polar lipids.

The microbialite metagenomes contained a large number of genes involved in cell signalling, motility, the production of extracellular polymeric substances (EPS), and the formation of biofilms. EPS and the structural complexity of the microbial biofilm are like-

ly critical to the coupling of autotrophic and heterotrophic processes and the maintenance of a depleted local DIC pool. In addition to the spatial complexity of the biofilm, microbial activity is tightly controlled by sensory and regulatory systems (e.g., circadian clock genes), which allow for the coordination of metabolic processes and create conditions suitable for the precipitation of calcium carbonate. Together, the metagenomic and stable isotopic results presented here demonstrate the complexity of the biological community and chemical system of the Cuatro Ciénegas microbialites, prompting a more detailed spatial analysis that is presented in the companion abstract by Hollander et al.