Microbial Communities of Pavilion Lake Microbialites

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Introduction

Pavilion Lake, a freshwater body in British Columbia's Marble Canyon, is host to one of the largest concentrations of freshwater microbialites in the world. Microbialites are calcareous aggregates produced by organosedimentary processes by various classes of microbes. They differ from stromatolites, ubiquitous throughout the Precambrian fossil record, in that they are actively growing. Typically, microbialites are found in environments that preclude grazing metazoans from inhibiting their growth. However, Pavilion Lake is not a hostile environment for these creatures, yet microbialites thrive. DIC and DOC analysis firmly points to a biotic origin for these structures and these particular microbialites have been continuously growing for roughly 12,000 years (Laval et. al 2000, Brady et. al 2009).

Stromatolites contain the oldest signatures of life in the rock record, and as their living counterparts, microbialites offer a unique opportunity to more fully understand Earth's earliest life, as well as the unique characteristics that may be required for life to form on any planetary body. Based on microscopic observations, it was determined that various species of purple and green pigmented cyanobacteria are the most dominant phylotypes of the small nodular formations that comprise the most significant surface feature of the microbialites (Laval et al. 2000). Recent work has posited that these nodules are the focal point of carbonate precipitation and crucial to microbialite accretion mechanisms (Brady et al. 2010).

Here, we present a synthesis of molecular biological, microbiological, and geochemical experiments aimed at elucidating the interplay between microbial activity and microbialite formation. From these results, and continuing work, we can begin to understand the unique distribution of these structures both in Pavilion Lake, nearby Kelly Lake, and across the globe. In addition, these results can translate to a greater knowledge of early Earth fossil records and the development of life on this planet.

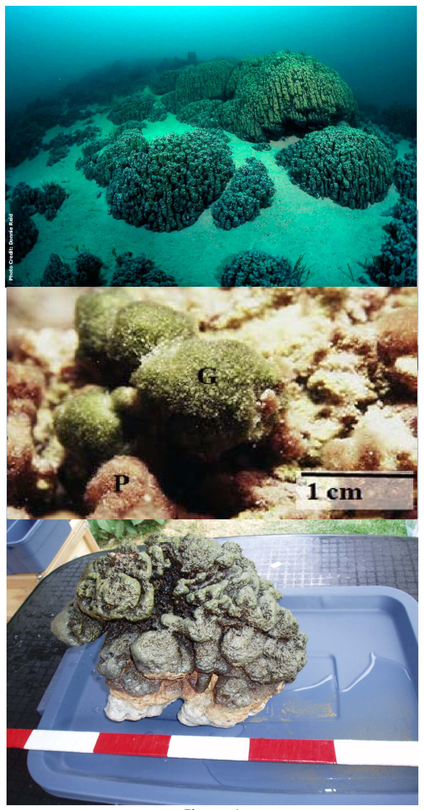

Figure 1
Photos of microbialite samples from Pavilion Lake, British Columbia showing in-situ growths (top), purple and green nodules (middle) and a cauliflower morphotype (bottom).
(Photo Credit: Allyson Brady, Donnie Reid)

Methods

Samples were selected for variation in depth, location within the lake, and morphology. Three samples of non-lithified microbial mat were analyzed to determine differences between hard-rock microbialites and non-lithifying communities. Microbialites were crushed to a coarse powder under sterile conditions. DNA extractions were performed using the *MoBIO Powersoil DNA* extraction kit (MoBIO Inc., Carlsbad, CA). For analysis of the cyanobacteria communities, PCR was performed with cyano-specific 16s rRNA primers; CYA359F and CYA781Ra/b. For analysis of total bacteria community, PCR was performed with 16s rRNA primers for general bacteria; 27F and 338R. PCR products were prepared for 454 sequencing per facility instructions using Library A adapters (Engencore: Columbia, SC, USA). Initial sequence analysis included BLAST (http://www.ncbi.nlm.nih.gov/blast/). OTU groupings were calculated using MOTHUR software. These groupings were used in statistical analysis between sites and depths using the Primer6 software (PrimerE Ltd., UK). T-RFLP analysis was done for all bacteria using Pointing lab standard methods. Isotope methods and analysis is explained in Brady et al. 2010.

Cyanobacteria species diversity of shallow and middle depth microbialites using Sanger sequencing of 16s rRNA with cyano-specific primers shows greater diversity on shallow structures than at depth.

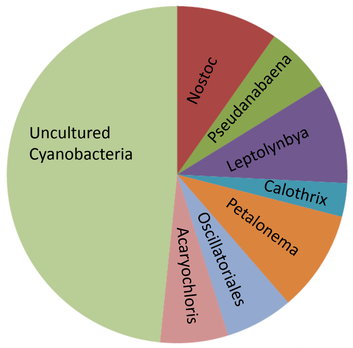

Figure 2a: Cyanobacteria species diversity of Three Poles 35 ft. microbialite as determined by highest sequence similarity (>95%) through BLAST analysis.

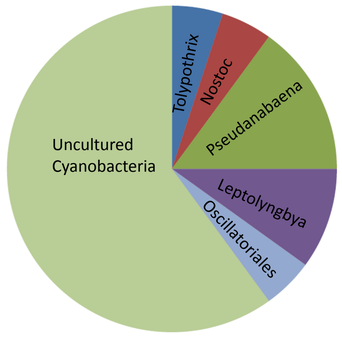

Figure 2b: Cyanobacteria species diversity of Willow Point 106 ft. microbialite as determined by highest sequence similarity (>95%) through BLAST analysis.

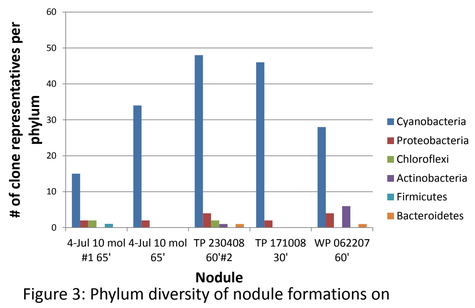

Figure 3: Phylum diversity of nodule formations on different microbialites throughout Pavilion Lake. Determined by BLAST analysis at >95% sequence similarity.

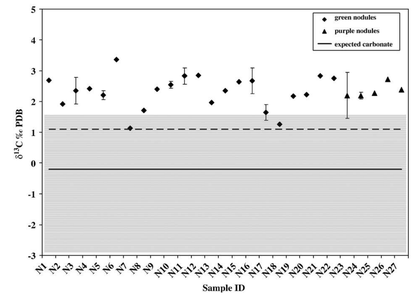

Figure 4: Relationship between carbonate δ13C values of individual green (◆) and purple (▲) nodules to the predicted carbonate δ13C values precipitated under abiotic conditions with no biological influence from the mean DIC δ13C value in Pavilion Lake. The shaded area illustrates the total range in predicted abiotic precipitation δ13C values based on all measured DIC values. The solid line represents the mean predicted carbonate δ13C values while the dashed line represents one standard deviation of the mean DIC values. Green and purple nodules carbonate δ13C are elevated above the range expected for abiotic precipitation. (From Brady et al. 2010)

Small nodular growths on the surface of microbialites, comprised predominantly of cyanobacteria, appear to be the nucleation sites of carbonate precipitation.

Targeted Sanger sequencing of cyanobacterial populations shows a significant difference between lithifying (microbialites) and non-lithifying (microbial mat) communities. Removing the mats, samples begin to group by whether they are above or below the chemocline (60 ft). All depths in feet. Groupings are hand drawn.

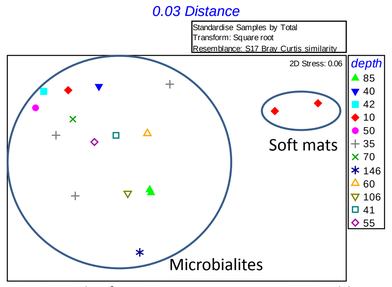

Figure 5: MDS plot from Sanger sequencing OTU table at 97% similarity including mat samples.

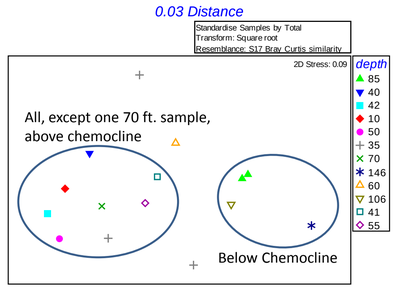

Figure 6: MDS plot from Sanger sequencing OTU table at 97% similarity without mat samples included.

454 pyrosequencing upholds the difference between lithifying and non-lithifying communities and grouping by depth breaks down.

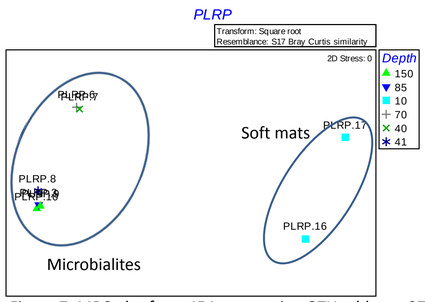

Figure 7: MDS plot from 454 sequencing OTU tables at 97% similarity with mat samples included (15 and 17)

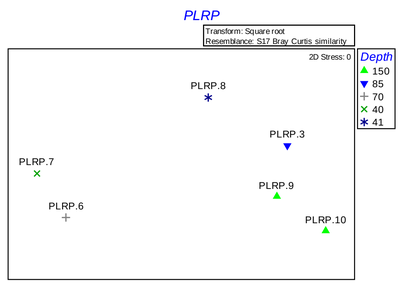

Figure 8: MDS plot from 454 sequencing OTU tables at 97% similarity without mat samples

References:

Brady, A.L. et al. 2009. Constraining carbon sources and growth rates of freshwater microbialites in Pavilion Lake using C-14 analysis. Geobiology. Vol 7. 544-555

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T-RFLP analysis of total bacterial community between sites and seasons

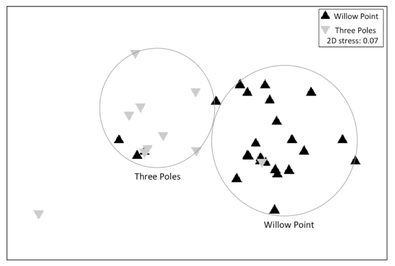

Figure 9: NMDS ordination of 16S rRNA TRFLP for Pavilion Lake. Samples shown are for all depths. There is no significant difference between depths, but a small yet significant difference between the 2 sites.

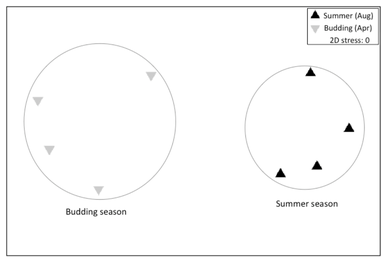

Figure 10: NMDS ordination of season based 16S rRNA TRFLP for Pavilion Lake. Samples from Three Poles only. A small yet significant difference exists.

Significant differences of microbial communities are seen on microbialites from different locations in Pavilion Lake, as well as between seasons

Results:

- Molecular and geochemical data point to cyanobacteria being the dominant drivers of microbialite accretion
- Trends of total microbial diversity with depth are not statistically robust. Thus, we cannot attribute changes in microbialite morphology with depth to changes in microbial community assemblage.
- Molecular evidence points to higher diversity than initial microscopy studies indicated.
- A significant difference was seen between soft mats and microbialite bacterial community assemblage.
- Location and growing season appear to be more significant drivers of microbial communities than depth.

Future Work:

In July 2011, the Pavilion Lake Research Project moved its operations to Kelly Lake, a smaller lake 12 km North of Pavilion Lake, where microbialite formation has been identified. Prolific sampling was conducted and continuing work will involve further deep pyrosequencing efforts and studies of carbonate deposition dynamics. Comparisons will be made between Kelly Lake and Pavilion Lake systems in order to further constrain evolutionary differences that may allow illustration of microbialite formation and a deeper understanding of life on early Earth.