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A Spatial and Temporal Analysis of Microbial Communities in Great Boiling Spring, Nevada, USA

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Objectives

1. To describe the spatial distribution of the bacteria and archaea inhabiting Great Boiling Spring (GBS).
2. To examine the temporal variability of the microbial community at the different sites throughout GBS.
3. To compare and contrast the water and sediment-bourne microbial communities of GBS.

Introduction

GBS is a large, circumneutral, long residence time geothermal spring located in the US Great Basin. It is mineralogically homegenous, with analyses showing major solutes of Na^+ and Cl^- and an active nitrogen cycle [1,2]. Twelve samples were taken from four different sediment sites and the bulk water of GBS on up to four different dates. Microbial community composition and diversity were assessed through the analysis of more than 300,000 16S rRNA gene pyrotags. To our knowledge, this is the most detailed study of the spatial and temporal variation in any geothermal spring. This study underscores the distinctness of water and sediment-borne communities and the importance of temperature in driving the spatial and temporal variation of microbial phylotypes throughout the source pool.

Methods

Eight sediment samples were collected using sterile coring devices and four water samples were collected using either normal or tangential flow filtration, as previously described. Sediment samples were collected from four sites at the edge of the hot spring, ranging in average temperature from 61.9 - 82.6 °C (Fig 1). Samples were stored on dry ice and transported to the laboratory, where DNA was extracted using a slightly modified version of the Joint Genome Institute's (JGI) CTAB protocol [3]. Extracted DNA was shipped on dry ice to the JGI, where DNA sequencing of the the V8 portion of the 16S rRNA gene was performed using the Roche 454 GS FLX Titanium System. The resulting reads were run through the PyroTagger pipeline [4], where they were filtered for quality, requiring a minimum length of 200 bp and Phred values of at least 27 for 90% of bases, and clustered at 97% sequence identity. Sequnces identified by PyroTagger as potential chimeras, along with additional low abundance sequences that did not align properly, were confirmed as chimeras by BLASTing in NCBI and discarded from the analysis. The Quantitative Insights Into Microbial Ecology pipeline [5] was then used to perform cluster and principal coordinate analyses (PCoA), utilizing the Morisita-Horn index and abundance-weighted and unweighted UniFrac distance metrics. The Morisita-Horn index is a similarity index that nearly independent sample size [6] and the UniFrac metric is a method of comparing microbial communities based on phylogentic information [7].

Results and Conclusions

Water and sediment-borne microbial communities were distinct with very little overlap, regardless of the sampling location or temperature (Fig 2). Water-borne communities were extremely uneven and were dominated by a single phylotype related to *Thermocrinis* in the *Aquificales*. Sediment-borne microbial communities grouped according to temperature and sampling location. Two locations, Site A (80-87°C) and Site B (79°C), were predominantly composed of the crenarchaeal class *Thermoprotei*, the novel archaeal lineage pSL4, and the novel bacterial lineage GAL35. Populations of the ammonia-oxidizing archaeon "*Candidatus* Nitrosocaldus yellowstonii" comprised 5-15% of all samples when Site A was cooler than normal (80°C) and at cooler sites throughout the spring (76-62°C). At cooler temperature sites (76-62°C), the phylum-level diversity and evenness were significantly higher, and bacteria made up a significantly higher percentage of the population. Cluster analysis results from weighted UniFrac and Morisita-Horn showed the water-borne samples clustering together and distinct from the sediment-borne samples, with jackknife values of 100% separating the water-borne clusters from the rest of the trees (Figs 3a,b). The result of the unweighted UniFrac cluster analysis had lower jackknife values overall and less well-defined clustering (Fig 3c) than the Weighted UniFrac and Morisita-Horn trees; this suggests that relative OTU abundance has a greater influence on the sample clusters in the weighted metrics (weighted UniFrac and Morisita-Horn) than the specific OTUs observed in each sample. Clusters of high temperature sample sites (0812.A/1002.B/1002.A and 0906.A/1007.A) were maintained in all three trees, with jackknife values of at least 70% at the main cluster node. This suggests that with or without abundance weighting, temperature is a driver of community composition. Weighted UniFrac and Morisita-Horn PCoA results showed clustering similar to the respective cluster analysis, with water-borne samples (Fig 4a,b) grouping tightly. Site type, water or sediment, was the major principal coordinate in both weighted UniFrac and Morisita-Horn, explaining 74.45 and 53.59% of variation between samples, respectively; temperature was responsible for much of the remaining variation, explaining 13.70 and 22.76% of weighted UniFrac and Morisita-Horn, respectively. This amounts to an explanation of 88.15 and 76.35% variation in the weighted UniFrac and Morisita-Horn analyses, respectively. The results of the unweighted UniFrac analysis (Fig 4c) showed a less defined grouping, with only 19.77% of variation explained by P1, temperature, and 15.80% by P2, site type, resulting in a total variation explained of 35.57%. This result is similar to the unweighted UniFrac cluster analysis, again suggesting that relative OTU abundance has a greater influence on the sample clusters in the weighted metrics than the specific OTUs observed in each sample.These results show that the water and sediment-borne communities of GBS are dominated by different organisms in different relative abundances, and that between sediment-borne communities, temperature has a significant influence on community composition.

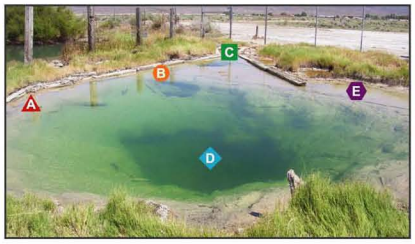

Figure 1 Photograph of GBS with sample sites, A-E, indicated. Average recorded temperatures (°C) at each site: A , 82.6, B, 79.2, C, 73.8, D, 81.0, and E, 61.9. Samples from site D are of the water-borne microbial community and samples from sites A, B, C, and E are sediment communities.

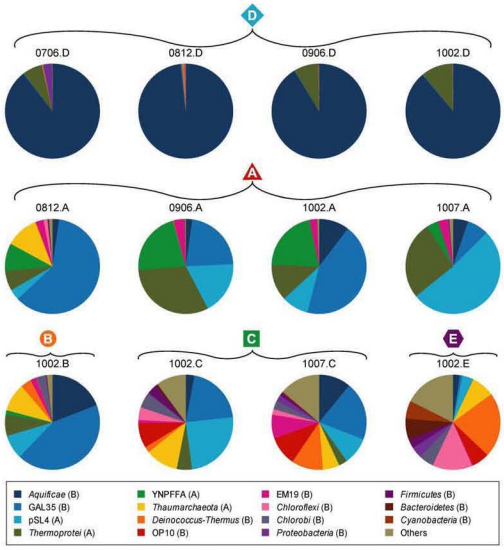

Figure 2 Pie charts describing community composition of four water and eight sediment samples, including 15 most abundant taxa of all 12 samples, with remaining taxa grouped as "Others". "(A)" or "(B)" after taxon name in pie chart legend designates archaeal or bacterial taxon, respectively. Sample names indicate date and site of sampling, formatted as YYMM.site (Ex. 0706.D, sampled June 2007 at site D).

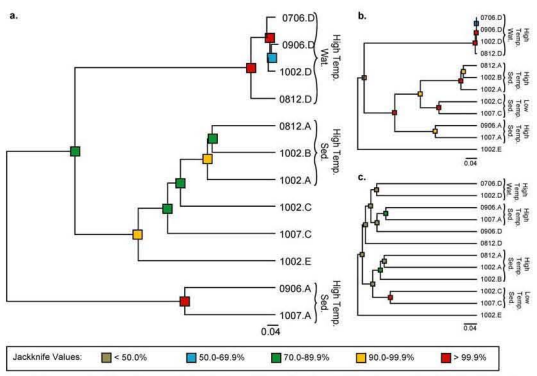

Figure 3 Trees resulting from cluster analysis using three different algorithms, (a) Abundance-weighted UniFrac (b) Morisita-Horn (c) Unweighted UniFrac. Jackknife value ranges are shown at each node. Clusters grouped by site type (water or sediment) or temperature (high or low) are indicated by a bracket. Wat., water sed., sediment.

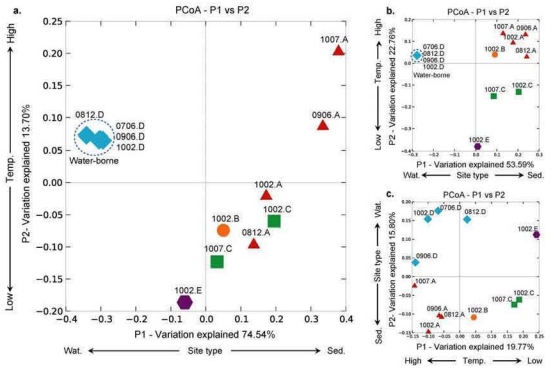

Figure 4 Results of PCoA using three different algorithms, (a) Weighted UniFrac (b) Morisita-Horn (c) Unweighted UniFrac. Interpretations of the environmental factors responsible for variation explained, either temperature or site type, by each coordinate, P1 or P2, are indicated. Clusters of water-borne samples are circled. Wat., water; sed., sediment; temp., temperature.

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Acknowledgements

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