

**THE 470 MEGABASE METAGENOME OF THE BISON POOL (YELLOWSTONE NATIONAL PARK) ALKALINE HOT SPRING OUTFLOW CHANNEL.** W. D. Swingley<sup>1</sup>, E. B. Alsop<sup>1</sup>, H. D. Falenski<sup>1</sup>, and J. Raymond<sup>1</sup>, <sup>1</sup>School of Natural Sciences, University of California – Merced, 5200 N. Lake Rd., Merced, CA 95343  
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**Introduction:** It has long been thought that life on Earth (~3.5 Ga) developed under extremely hostile conditions, following the cessation of planet-sterilizing asteroid and comet bombardment [1]. Phylogenetic data has suggested that the root of all life emerged from the hot, sulfur-rich environments present in this hostile ancient planet [2].

The diverse biogeochemical profiles along the Bison Pool outflow channel typify possible “extreme” conditions on extraterrestrial bodies. Understanding how microorganisms react to and affect elemental changes in their environment is crucial to identifying extraterrestrial targets for potential life signs. Bison Pool offers a unique opportunity to unravel this interplay between organism and environment. Synergistic research efforts in geochemistry, biology, and informatics have laid the groundwork for a systems-level

understanding of Bison Pool and given us the the opportunity to generate more than 470 megabases of metagenomic information to correlate with over 80 chemical and geological parameters.

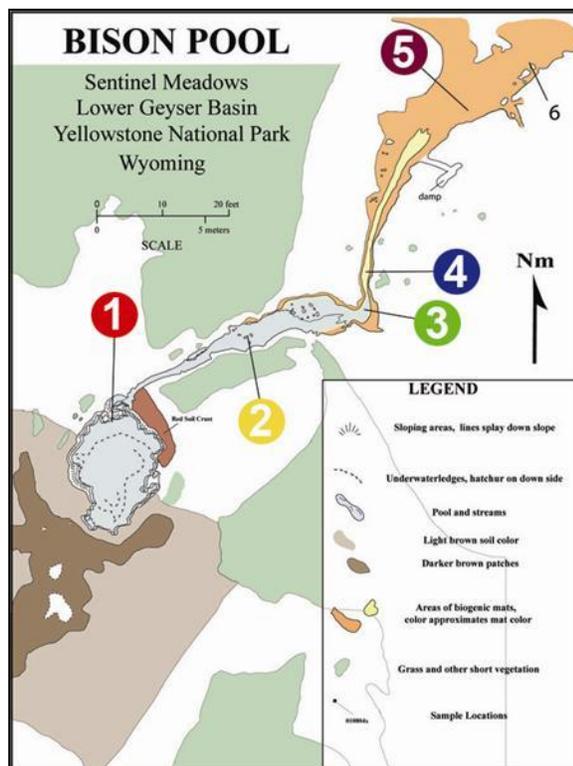
Bison Pool is an alkaline hot spring in the Sentinel Meadow (Lower Geyser Basin) of Yellowstone National Park (Fig. 1). The relatively simple (compared to other Yellowstone hot springs) microbial community structure at Bison Pool has made this site a target for research efforts coordinating geochemical data and genomic data[3].

Recent metagenome sequencing at the Bison Pool hot spring has produced over 470 megabases of sequence data along with nearly 2500 separate 16S rDNA clones, providing a taxonomic basis for the analysis of metagenomic data. The Bison Pool metagenome incorporates five separate sample environments spread along a temperature gradient in the pool’s outflow channel (Fig. 1).

The Bison Pool outflow channel acts as an ~11m gradient of not only descending temperature, but increasing dissolved oxygen, which affects the redox state and solubility of many biologically important elements, such as iron, manganese, and cobalt [4]. The dissolved oxygen levels, and, by proxy, oxidation state of elements correlates directly to the advent of oxygenic photosynthesis at ~67 ° C. This barrier marks the upper temperature limit for thermophilic cyanobacteria that not only saturate their immediate environment with oxygen, but also fix elemental nitrogen from the atmosphere into biologically available sources.

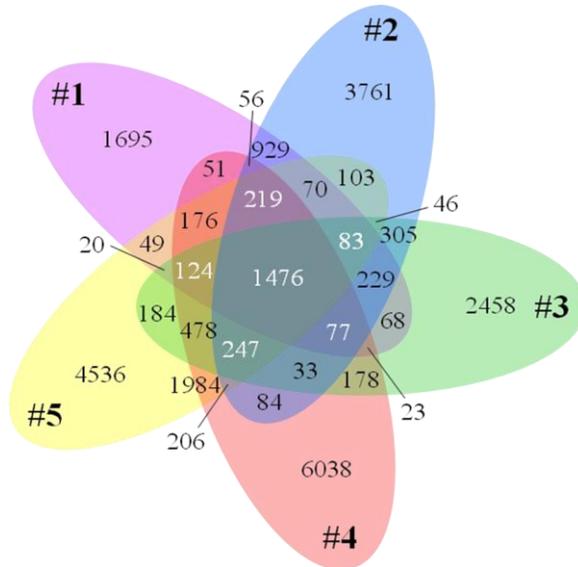
We have completed the annotation of the Bison Pool metagenome using BLAST-based Markov clustering. The Bison Pool metabolomes encapsulate the diverse communities ranging from the anaerobe-rich pool 1 to the phototroph-rich pool 5. This analysis has revealed a remarkable level of protein family conservation across the >36°C temperature gradient, with nearly 1500 families present in all sites (Fig. 2). We have investigated the evolutionary changes that allow these enzymes to function across this large temperature gradient.

The lowest temperature Bison Pool sites (4 & 5) share a striking degree of homology, sharing many metabolic pathways, such as the nitrogen and carbon-dioxide fixation, that do not function beyond ~68°C. The two hottest sites (1 & 2) also share a large number of homologous protein families specific to the anaerobe-rich pool 1 to the phototroph-rich pool 5.



**Figure 1.** A schematic of the Bison Pool alkaline hot spring. Numbers indicate locations of community samples, starting at the source (1=92.8°C) and proceeding downstream (2=79.6°C, 3=68.2°C, 4=65.6 °C, and 5=56.2 °C). Sites 1-3 are chemotrophic, sites 4 and 5 are phototroph-dominated.

**Figure 2.** Venn diagram representing the overlap in Markov protein families found at the five Bison Pool sites.



robes and microaerophiles that inhabit these extreme temperatures. Site 3, just beyond the temperature limit of photosynthesis and nitrogen-fixation, appears to be both the most diverse and most hostile environment, where no one species can dominate under the severe nitrogen limitation.

The Bison Pool metagenomes represent a surprising diversity of bacterial and archaeal species. Understanding how these species evolved to fit their precise hydrothermal niche will allow us to better understand the evolutionary changes that have occurred during Earth's transition from a hot and anaerobic to cool and aerobic environment.

**References:** [1] Davis W. L. and McKay C. P. (1996) *Orig. Life Evol. Biosp.*, 26, 61-73. [2] Woese C. R. (1987) *Microbiol. Rev.*, 51, 221-271. [3] Meyer-Dombard D. R. et al. (2006) *Geochim. Cosmochim. Acta*, 70, A418. [4] Zerkle A. L. (2005) *Am. J. Sci.*, 305, 467-502.