

## Geochemical Microenvironments in Hydrothermal Ecosystems

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Hydrothermal ecosystems remain poorly characterized due to their inherently complex geochemical and biological processes. In particular, little is known about the dependence of thermophilic microbial communities on the geochemical environment, or the effect that these communities have on the geochemistry. To facilitate the study of thermophilic communities in hydrothermal ecosystems, we have employed methods from a triad of disciplines; geochemistry, microbiology, and molecular biology.

Field work done in the Greater Obsidian Pool Area of Yellowstone National Park, USA, in the summers of 1998 and 1999, indicates extreme geochemical diversity in this location. The thermal features in this approximately 100 m<sup>2</sup> area display temperature ranges of 35 to 92°C, with an accompanying pH range of 1.9 to 6.7. Large geochemical gradients were measured as well. For example, within the Greater Obsidian Pool Area, we found the conductivity to range from 670 - 1530  $\mu$ S, nitrate to range from 0.68 - 6.26 ppm, sulfate from 23.85 - 1970 ppm, and chloride from 4.8 - 412.5 ppm. The stable isotope composition of these hot spring waters is also diverse, with  $\delta^{18}\text{O}$  values ranging from -6.32 to -18.53, indicating the possibility of multiple sources of parent fluids.

The unique geologic and geochemical setting of the Greater Obsidian Pool Area provides a plethora of microbial habitats which cater to thermophilic micro-organisms with a wide variety of metabolisms. The complexity of metabolic functions within the microbial communities in these hot springs is unknown because, in general, cultures of thermophilic organisms at the Greater Obsidian Pool Area have not been obtained. Each hot spring in the Obsidian Pool Area is unique physically and chemically, and provides many possible "microenvironments" for microbial growth. For example, due to disequilibrium, the chemistry of the main pool in this area may be appealing to nitrate reducers, ammonia oxidizers, sulfate reducers, or organisms which oxidize elemental sulfur or

magnetite, and a complex combination of metabolic reactions may be utilized by the microbial community in this pool. In addition, microbial community structure may fluctuate within a pool depending on microenvironment dynamics. Thermophiles using oxidative metabolic reactions may prevail in regions of shallow water, while deeper waters with a copious supply of reduced volcanic gases may support organisms utilizing reductive metabolic reactions.

Two recent studies done in this location have revealed genetic information of 32 novel species of Archaea (Barns *et al.*, 1996), and 54 novel species of Bacteria (Hugenholtz *et al.*, 1998), covering the full range of thermophilic life on the prokaryotic branches of the universal phylogenetic tree. While these results have provided a catalog of genetic information for Obsidian Pool, the metabolic characteristics of these organisms are not known. This connecting link between thermophilic organisms and their geochemical environment can only be examined if a culture of the organisms can be studied in the laboratory. Using geochemically derived growth media, we have obtained 16 actively growing cultures from the Greater Obsidian Pool Area. In addition, we have isolated a novel, anaerobic, heterotrophic, thermophilic Bacterium, belonging to the genus *Fervidobacterium* from this area. Preliminary phylogenetic analysis shows this Bacterium to be different from any of the 54 species discovered by Hugenholtz *et al.* (1998). These physical representatives of microbial communities in the Greater Obsidian Pool Area, along with our geochemical data, will allow investigation of the microenvironments that support these organisms and shed light on the complexity of these biogeochemical systems.

Barns SM, Delwiche CF, Palmer JD, Pace NR, *Proc. Natl. Acad. Sci. USA*, **93**, 9188-9193, (1996).

Hugenholtz P, Pitulle C, Hershberger KL, Pace NR, *J. Bacteriology*, **180**, 366-376, (1998).