General Meeting
of the
NASA Astrobiology Institute

Current Earth Environments As Analogues for Extraterrestrial Environments

Biogeochemical Processes in Microbial Ecosystems

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The hierarchical organization of microbial ecosystems determines process rates that shape Earth's environment, create the biomarker sedimentary and atmospheric signatures of life, and define the stage upon which major evolutionary events occurred. In order to understand how microorganisms have shaped the global environment of Earth and, potentially, other worlds, we must develop an experimental paradigm that links biogeochemical processes with ever-changing temporal and spatial distributions of microbial populations and their metabolic properties**.**

Photosynthetic microbial mats offer an opportunity to define holistic functionality at the millimeter scale. At the same time, their biogeochemistry contributes to environmental processes on a planetary scale. These mats are possibly direct descendents of the most ancient biological communities; communities in which oxygenic photosynthesis might have been invented. Mats provide one of the best natural systems to study how microbial populations associate to control dynamic biogeochemical gradients. These are self-sustaining, complete ecosystems in which light energy absorbed over a diel (24 hour) cycle drives the synthesis of spatially-organized, diverse biomass. Tightly-coupled microorganisms in the mat have specialized metabolisms that catalyze transformations of carbon, nitrogen, sulfur, and a host of other elements.

Light sustains oxygenic photosynthesis, which in turn provides energy, organic photosynthates and oxygen to the community. Due to both absorption and scattering phenomena, the incident light changes with depth in the mat, both in intensity and spectral composition. Motile photosynthetic organisms optimize their position with respect to the resultant light gradient; some biota even harvest light in the infrared spectral range. When oxygenic photosynthesis ceases at night, the upper layers of the mat become highly reduced and sulfidic. Counteracting gradients of oxygen and sulfide shape the chemical environment and provide daily-contrasting microenvironments separated on a scale of a few millimeters. Radiation hazards (UV, etc.) as well as oxygen and sulfide toxicity elicit motility and other physiological responses. This combination of benefits and hazards of light, oxygen and sulfide promotes the allocation of the various essential mat processes between light and dark periods and to various depths in the mat.

While photosynthetic bacteria dominate the biomass and productivity of the mat, many aspects of the ecosystem's emergent behavior may ultimately depend on the associated nonphotosynthetic populations, including the anaerobes. These nonphotosynthetic processes create the ultimate biological filter on chemical, isotopic, and geologic biomarkers passing into the fossil record. Transformations of photosynthetic productivity by the microbial community may contribute diagnostic "biosignature" gases that could alert us to species that could serve as search targets for remote spectroscopic life detection efforts (e.g., Terrestrial Planet Finder). To understand the overall structure and function of mat communities, it is thus critical to determine the nature and extent of interactions between phototrophic and nonphotosynthetic, including anaerobic, microorganisms.

The organismal and functional complexity of mats, coupled with the highly proximal and ordered spatial arrangement of microorganisms, offers the potential for a staggering number of interactions. At minimum, the end-products of each group affects the other in both positive and negative respects: cyanobacteria generate organic matter (potential substrates) but also oxygen (a toxin with respect to many anaerobes). Anaerobic activity recycles nutrients to the phototrophic community but also generates potentially toxic sulfide. This interaction creates the microenvironmental landscape upon which microbial strategies developed in order to cope with the daily oscillation between extremes of eutrophy and toxicity.

The potential for a more substantial coupling arises through cyanobacterial production of hydrogen and small organic acids. Bacterial production of small nitrogen and sulfur compounds is also important. These compounds fuel a flow of energy and electrons in anaerobic microbial ecosystems and thus represent a potential basis for interactions between groups of microorganisms. For example, interspecies transfer of hydrogen facilitates many well-studied anaerobic consortia. In these cases, hydrogen represents not only an agent of electron transfer but also an important thermodynamic control with the potential to alter significantly the metabolic function of either partner. Virtually every member of the anaerobic microbial community is subject to such effects, so that participation by cyanobacteria in the cycling of hydrogen and organic acids could directly and dramatically affect biogeochemical function and community composition. Similarly, consumption by anaerobes of these end-products might provide an important feedback on fermentation and nitrogen fixation by cyanobacteria at the level of both enzyme and gene regulation.

Coordinated observations of population distribution, abundance, and activity for an entire community would make fundamental questions in ecology accessible. Perhaps the most fundamental of these questions addresses those factors that sustain the remarkable diversity of microorganisms now being revealed by molecular techniques.

Ecogenomics: Ensemble Analysis of Gene Expression in Microbial Communities

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The first organisms on Earth were microbes, and for more than 3.5 billion years, these creatures of untold diversity have dominated every corner of our biosphere. The evolution and continued survival of all multi-cellular forms including plants, animals and fungi would not be possible without single-cell organisms. They control key processes in geochemical cycling, biodegradation and in the protection of entire ecosystems from environmental insults. Process oriented studies in which the microbial world is treated as a "black box" alert us to feedback loops between biogeochemical gradients and structured microbial populations. Yet, there are no comprehensive descriptions of underlying biochemical and genetic mechanisms that govern these processes. The goal of **Ecogeonimcs** is to define the relationship between microbial diversity, complex gene expression patterns, and biogeochemical processes that shape planetary environments. To achieve this goal we envisage five linked experimental programs that will focus upon the hypersaline cyanobacterial mats of Guerrero Negro, Baja California, Mexico. 1) We will characterize biogeochemical patterns in the microbial mats with special emphasis upon gradient location and shape. 2) We will employ molecular techniques to develop a quantitative assessment of microbial population structure in this microbial ecosystem. 3) We will use DNA microarrays to measure gene expression patterns for genes of known function at different locations in the environment. 4) We will explore responses to transient and periodical environmental perturbations imposed by diel cycles. Finally, based upon the results of these empirical measurements, 5) we will model feedback loops between microbial gene expression and formation of biogeochemical gradients that shape planetary evolution.

This is a cross institute collaboration with active participation by NAI teams from NASA AMES Research Center, Arizona State University, the University of Colorado, the Marine Biological laboratory at Woods Hole and the University of Washington in Seattle. Little molecular work beyond microbial population profiles has been published for the hypersaline cyanobacterial mats of Guerrero Negro, Baja California, Mexico, however the AMES research team has had considerable experience sampling and measuring biogeochemical parameters. At this early stage, we have no new molecular data to present because the first field trip occurred March, 2001. Using a collaborative sampling scheme, detailed molecular based measurements of microbial population structures in response to diel cycling will be collected with simultaneous measurement of biogeochemical parameters. Using representative cultures from the field site, we will begin the required sequencing and construction of DNA microarrays that will be used to monitor changing patterns of gene expression. During the 2001 Astrobiology Institute meeting we will summarize the salient features of the field site and describe our general experimental strategies for charactering population structure and gene expression patterns of its resident microbial communities.

Sulfur / Carbonate Springs and Life in Glacial Ice

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Introduction: Ice in the near subsurface of Mars apparently discharges liquid water on occasion [1]. Cold-tolerant microorganisms are known to exist within terrestrial glacial ice [2], and may be brought to the surface as a result of melting events. We are investigating a set of springs that deposit sulfur and carbonate minerals, as well as evidence of microbial life, on the surface of a glacier in the Canadian arctic.

Field Observations: The springs are located at 81°01'N, 81°35'W, near the northwest coast of Ellesmere Island. The sampling area is characterized by widespread glaciers and deep fiords. Outcrops in the sampling area include the Upper Carboniferous to Lower Permian Nansen formation (limestone, minor sandstone, siltstone and shale) and the Upper Permian Trold Fiord Formation (siltstone, sandstone, minor bioclastic limestone, conglomerate and chert) [3]. Permafrost with depths of 400 to 600 m has been documented on nearby Axel Heiberg Island [4]. The climate, as monitored at the Eureka meteorological station on Ellesmere Island, includes cold, dry winters and cool summers. The mean annual air temperature is -19.7°C (-36.1°C in January; +5.4°C in July), and seasonal extremes of -55°C and +20°C are not uncommon [4].

Samples: Sterile samples of spring water and associated solid deposits were collected from several locations during July, 2000. The springs are located on the surface of a glacier several hundred meters thick. All of the sites are typically characterized by large accumulations of yellow and white, or only white, minerals. A strong smell of H_2S was apparent during sampling. There is evidence for both active and past water discharge at these sites.

Water. Water temperatures are low (1° to 2°C), but higher than surface melt water (0.2°C). The spring waters have pH values of approximately 9 to 9.8, distinctly different from glacial melt water streams and pools without sulfur that have pH values of around 5.2. Total dissolved solids in the spring water range from approximately 200 to 300 mg/l, as compared to <1 mg/l for melt water elsewhere on the ice.

Solid Deposits. The solid samples were air dried and ground in an agate mortar and pestle for powder X-ray diffraction (XRD) analysis. The XRD patterns of all samples are nearly identical, and include peaks for abundant sulfur and calcite. Minor gypsum was identified following dissolution extraction.

Dissolved / Suspended Solids. One water sample was passed thru a 0.2 μm filter by vacuum filtration. The filter was air dried and a portion was chromium-coated and mounted for analysis by a field emission scanning electron microscope (FE-SEM). A second water sample was centrifuged, and the solids were mounted and coated for FE-SEM analysis. Elemental abundances were determined using an energy-dispersive X-ray spectrometer operated in a windowless configuration, allowing detection of elements as light as carbon.

The FE-SEM analyses of solids filtered from the water detected numerous sub-spherical sulfur particles, generally 1 to 2 μm in diameter. A second population of 1 μm spheres are characterized by myriad radiating spikes. These spheres consist of Ca, P and O. The tentative mineral identification of apatite could not be confirmed by XRD, due to peak overlap.

Biofilm and Cells (?). The sulfur and apatite (?) particles are partially enmeshed in a carbon-rich webbing fractions of a micrometer thick. This material matches the morphology and composition of the extracellular polymeric substance (EPS) produced by many microorganisms. In nature EPS envelops microbial cells and detrital particles in a three dimensional, water-rich structure known as a biofilm [5]. Upon drying and exposure to the FE-SEM vacuum, EPS characteristically dehydrates and shrinks to a web-like structure. The FE-SEM images also show micrometer-scale, carbon-rich spheroids, which may be microbial cells. Further examination will be required to confirm the presence of microbes in these samples.

Discussion: *Hydrology*. Relative to more temperate locales, springs are rare in the Canadian arctic. A site on Axel Heiberg Island contains perennial springs in permafrost that form travertine deposits [4]. Also, seasonal "spring" discharges have been observed near the toes of glaciers at other localities. In these cases discharge occurs for only a matter of days and there are no associated precipitates.

Meltwater within glaciers moves through complex under-ice plumbing systems. Ice melted at the surface can move into the plumbing system through vertical cracks and pipes. In addition, ice can be melted at the base of the glacier by geothermal heat.

Water discharge onto glaciers occurs because the under-ice plumbing is all connected. As the melting season advances, the hydraulic head increases as the melt line advances up glacier. Surface discharges are fed by ice-enclosed streams, in which the water sometimes moves at very high speeds. In some cases the water coming out of the springs carries a high load of solids because it has run at or close to the bed.

Mineralogy. These springs may be expressions of a low-grade hydrothermal system (currently undetected) beneath Ellesmere Island. Hot spring water passing through hundreds of meters of glacial ice would be strongly cooled, but might still maintain a temperature slightly above freezing, as in the present case.

Sulfur-rich hot springs that precipitate calcite are common in Yellowstone, the Valle Grande, and thermal areas of Italy [6]. At Yellowstone the rising sulfur-rich water becomes saturated with $CaCO_3$ as it passes through a limestone horizon. Bedrock exposed closest to the Canadian springs contains Carboniferous and Permian limestone [3]. A reverse fault ~1 km south of the springs has abundant sulfide mineralization. This, and thick evaporite deposits in the underlying Otto Fiord Formation, are the likely sulfur sources.

Microbiology. FE-SEM examination of filtered and centrifuged material revealed extensive EPS and possible microbial cells. Microorganisms were also reported in the spring water discharging from permafrost on Axel Heiberg Island [4]. In both cases, further investigation will be required to determine if the microorganisms are living within the spring plumbing systems or only at the points of discharge.

Bacteria can survive subfreezing (and even cryogenic) temperatures. Viable spore-forming bacteria (Bacillus and Actinomycetes species) have been recovered from glacial ice [2]. If cold-tolerant microorganisms exist in glacial meltwater they will be brought to the surface where meltwater discharges.

Implications: The glacial springs of the Canadian arctic are useful terrestrial analogs to the channels and seeps issuing from beneath frozen strata on Mars. These glacial springs demonstrate that mineral-rich water can move through, and discharge from, solid ice. Liquid water, even at near-freezing temperatures, can support microbial life and bring evidence of that life to the surface.

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Bioinformatics, Organic Chemistry, and Paleontology. Building a Comprehensive Model for Recent Life on Earth

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One of the great challenges of Astrobiology is to seamlessly integrate concepts from Natural History with those from Physical Science, using technology from both, as well as concepts and technology from information sciences and engineering. Disciplines from Natural History include *inter alia* geology, paleontology, and ecology; disciplines from Physical Science include chemistry, physics, and molecular biology.

In the past, this integration has required collaborations between many different laboratories representing the different disciplines. For the future, however, this scientific/technological scope must be captured by single individuals. These individuals will move easily from organic synthesis to the field to cloning DNA to computer programming, generating the kind of insights that come from a single mind joining disjunct facts. These individuals will drive Astrobiology as a field in a way that will elude even the best organized collaborations.

The key to such integration is the natural progression that makes it easier, over time, to do everything. This means that it is easier for an individual to do more things, both in a training program and in a career. This progression is easy to see. Airplanes make it easier to do paleontology. Computers make it easier to do computation. Kits makes it easier to do molecular biology. Nuclear magnetic resonance, Fourier transform ion cyclotron mass spectrometry, and capillary electrophoresis make it easier to do organic synthesis.

This talk/poster will focus on two bioinformatics tools developed in our laboratories that make it easier to exploit genomic sequence data to build a broadly comprehensive model for the history of life on Earth, "Darwin" and "The Master Catalog".

Darwin is a bioinformatics workbench developed by the Benner and Gonnet groups in the late 1980's, in anticipation of the flood of genomic sequence data. It was intended to help organic chemists, who understand reactivity in the organic molecules that genomic sequence data represent, to use genomic data to do organic chemistry.

The Master Catalog is a database that comprehensively organizes genomic and non-genomic sequences using the "natural organization" outlined over a decade ago using Darwin. It captures perhaps 90% of the known sequence diversity in the biosphere via ca. 25000 families of protein modules. Each module is represented by a multiple sequence alignment, an evolutionary tree, reconstructed ancestral sequences throughout the tree, and a variety of tools for dating events interpreting the "function" (and change in function) within the trees. It therefore makes it very easy for the chemist to join the

structures of the organic molecules in the genomic database with the paleontological record.

The Master Catalog and Darwin solve two classical problems in bioinformatics: rapid search and detection of distant homologs. This makes it possible to address a range of new problems in bioinformatics, including the comprehensive unification of the record of life on Earth as told by the paleontological/geological records with that told by the molecular record. It can be used to generate experimentally testable hypotheses for most protein families and biological functions. Those that will be discussed include:

(a) The emergence of new reproductive strategies and their associated molecular biology during the global warming in the Miocene, 25 million years ago.

(b) The evolution of advanced neurological function and its associated molecular biology in primates in response to the Oligocene paleoclimatological trauma 38 MYA.

(c) The creation of new adaptive strategies and their associated enzymology in snake venoms in response to continental drift and changing faunal food sources over the past 70 million years.

(d) The evolution of fungal metabolism in response to the emergence of flowering/fruiting plants as an important component of the terrean flora ca, 80 MYA.

(e) The development and recruitment of new higher order function within the cytokine/receptor/signal transduction families in response to geological episodes influencing vertebrates over the past 350 million years.

(f) Historical episodes for the creation of new biological function in nematodes over the past 150 million years.

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Methane-Consuming Microbial Consortia Identified and Studied Using a Novel Combination of Fluorescent *in-situ* Hybridization and Ion Microprobe $\delta^{13}C$ Analysis

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Despite geochemical and experimental evidence that methane is oxidized under anaerobic conditions (e.g., Alperin et al., 1988; Hoehler et al., 1994), the precise means by which methane is oxidized anaerobically is unknown, and the responsible organism or consortium of organisms has yet to be isolated and grown in culture. Recently, Hinrichs et al. (1999) found archaeal lipids that were highly depleted in ^{13}C in sediments from the Eel River Methane Seep off the coast of California, suggesting an archaeal prokaryote was consuming methane under anoxic conditions. In this investigation, Hinrichs et al. also conducted parallel studies of rRNA genes from the location, revealing several groups of previously unknown archaeal lineages. Hinrichs et al. suggested that these novel archaeal groups are responsible for anaerobic oxidation of methane. Additional circumstantial evidence, linking these novel microbial groups to the process of anaerobic methane oxidation, was subsequently provided by fluorescent *in-situ* hybridization (FISH) studies of methane seep environments by Boetius et al. (2000) and Orphan et al. (in press). The FISH technique revealed microbial aggregates consisting of an archaeal core surrounded by a shell of sulfate-reducing bacteria (SRB). Boetius et al. (2000) and Orphan et al. (in press) have suggested that these aggregates containing Archaea and SRB are responsible for anaerobic methane oxidation (producing CO_2 and H_2) driven by the scavenging of hydrogen by the SRB. However, no direct evidence has linked the process of methane oxidation to these aggregates.

We utilized a combination of FISH and ion microprobe analysis (House, 2000), to clearly establish that the cells of these microbial consortia predominantly consist of ^{13}C-depleted methane-derived carbon. Environmental samples from the Eel River Methane Seep were fixed and then deposited on clean glass slides. The samples were then treated with two different phylogenetic stains – one targeting an archaeal rRNA sequence identified by Hinrichs et al. and one targeting SRB. Aggregates containing the archaeal core surrounded by the SRB shell were located by their florescence - and the carbon isotopic composition of the identified target cells was determined using a method modified from that developed previously for the analysis of Precambrian fossils (House, 2000). For comparison, we also studied cells that did not stain for the archaeal rRNA of interest, as well as bacterial and archaeal cells cultivated in the laboratory. The results show that the cell aggregates that contain the archaeal rRNA signature for the species suspected of being responsible for anaerobic methane oxidation are highly depleted in ^{13}C (to $\delta^{13}C$ values of –97.3±2.2), whereas all of the other cells investigated are not. This high depletion in ^{13}C proves that these archaeal species do consume methane. This new technique may be of importance to Astrobiology as it allows for the direct examination of the physiologies of yet uncultivated microorganisms.

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Composition of Hydrothermal Vent Microbial Communities as Revealed by Analyses of Signature Lipids, Stable Carbon Isotopes & Aquificales Cultures

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Extremely thermophilic microbial communities associated with the siliceous vent walls and outflow channel of Octopus Spring, Yellowstone National Park, have been examined for lipid biomarker and carbon isotopic signatures. These data were compared with that obtained from representatives of three Aquificales genera. *Thermocrinis ruber*, *Thermocrinis* sp. HI, *Hydrogenobacter thermophilus*, *Aquifex pyrophilus* and *Aquifex aeolicus* all contained phospholipids composed not only of the usual ester-linked fatty acids, but also ether-linked alkyl moieties. The fatty acids of all cultured organisms were dominated by very distinct pattern of *n*-$C_{20:1}$ and *cy*-C_{21} compounds. The alkyl glycerol ethers were present primarily as $C_{18:0}$ monoethers with the exception of the *Aquifex* spp. in which dialkyl glycerol ethers with a boarder carbon-number distribution were also present. These Aquificales biomarker lipids were the major constituents in the lipid extracts of the Octopus Spring microbial samples. Two natural samples, a microbial biofilm growing in association with deposition of amorphous silica on the vent walls at 92°C, and the well-known 'pink-streamer community' (PSC), siliceous filaments of a microbial consortia growing in the outflow channel at 87°C were analyzed. Both the biofilm and PSC samples contained mono- and dialkyl glycerol ethers with a prevalence of C_{18} and C_{20} alkyls. Phospholipid fatty acids were comprised of both the characteristic

Aquificales *n*-$C_{20:1}$ and *cy*-C_{21}, and in addition, a series of *iso*-branched fatty acids from *i*-$C_{15:0}$ to *i*-$C_{21:0}$, with *i*-$C_{17:0}$ dominant in the PSC and *i*-$C_{19:0}$ in the biofilm, suggesting the presence of two major bacterial groups. Bacteriohopanepolyols were absent and the minute quantities of archaeol detected showed that Archaea were only minor constituents.

Carbon isotopic features of the PSC yielded information about community structure and likely physiology. Biomass was ^{13}C-depleted (10.9‰) relative to available CO_2 from the source water inorganic carbon pool with lipids further depleted by 6.3‰ relative to biomass. The C_{20-21} Aquificales fatty acids of the PSC were somewhat heavier (average $\Delta\delta^{13}C_{CO2}$= –18.4‰) than the *iso*-branched fatty acids (average $\Delta\delta^{13}C_{CO2}$= –22.6‰). The carbon isotopic signatures of lipid biomarkers were also explored using a pure culture, *T. ruber*, previously isolated from the PSC. Cells grown on CO_2 with O_2-H_2 were only slightly depleted (3.3‰) relative to the C-source while cells grown on formate with O_2 showed a much higher fractionation (19.7‰), possibly the result of a metabolic branch point involving the assimilation of C-formate to biomass and the dissimilation to CO_2 associated with energy production. *T. ruber* lipids were slightly heavier than biomass (+1.3‰) whether cells were grown using CO_2 or formate. Fatty acids from CO_2 grown *T. ruber* cells were also slightly heavier (average = +2.1‰) than biomass. The relatively depleted PSC C_{20-21} fatty acids suggest that any associated *Thermocrinis* biomass would also be similarly depleted and much too light to be explained by growth on CO_2. The C-fractionations determined with the pure culture suggest that growth of *Thermocrinis* in the PSC is more likely to occur on formate, presumably generated by geothermal activity.

This study points to the value of the analysis of the structural and isotopic composition of lipid biomarkers both in pure culture studies, and in establishing community structure and physiology, as a complement to genomic profiles of microbial diversity. This is especially so when the members of the microbial community are novel and difficult to cultivate in the laboratory.

Application of Fe Isotopes to the Search for Life and Habitable Planets

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The relatively new field of Fe isotope geochemistry can make important contributions to tracing the geochemical cycling of Fe, which bears on issues such as metabolic processing of Fe, surface redox conditions, and development of planetary atmospheres and biospheres. It appears that Fe isotope fractionation in nature and the lab spans about 4 per mil (‰) in $^{56}Fe/^{54}Fe$, and although this range is small, our new analytical methods produce a precision of ±0.05 ‰ on sample sizes as small as 100 ng (10^{-7} g); this now provides us with a sufficient "signal-to-noise" ratio to make this isotope system useful. We review our work in three areas: 1) the terrestrial and lunar rock record, 2) experiments on inorganic fractionation, and 3) experiments involving biological processing of Fe.

One of the most remarkable features that has come out of our new high-precision work is the high degree of isotopic homogeneity of most "lithologic" sources of Fe, such as continental or oceanic igneous and metamorphic rocks or clastic sediments that have not undergone significant organic diagenesis. This result suggests that at least the suspended load flux of Fe from the continents to the oceans has a constant Fe isotope composition, indicating that clastic sedimentation, in the absence of organic and/or redox processes, appears to produce little or no Fe isotope fractionation in the solids. Moreover, low-temperature or arid environments, where chemical weathering is minimal, seems to produce no Fe isotope fractionation; this suggests that environments such as those on the surface of Mars will produce no Fe isotope fractionations that might be confused as a "biosignature". Finally, the relative constancy in isotopic compositions of "lithologic" Fe may significantly restrict the uncertainty in "starting compositions" we must account for when tracing biochemical cycling as compared to other stable isotope systems.

In contrast, essentially all of the variations in Fe isotope compositions seen on Earth lie in chemical sediments, including Fe-Mn nodules and crusts and Banded Iron Formations (BIFs). It is notable that these large variations are preserved in weakly metamorphosed Archean BIFs, as well as greenschist- to amphibolite-facies Proterozoic BIFs, suggesting that Fe isotope anomalies may be preserved during at least moderately high grades of metamorphism. In detail, we see that magnetite- and hematite-rich layers in BIFs define the highest δ^{56}Fe values, whereas siderite- and pyrite-rich layers define the lowest δ^{56}Fe values. These results immediately raise the question: are these isotopic variations due to inorganic (equilibrium) isotopic fractionations, such as is well known for light stable isotopes (e.g., C and O) or are they related to ancient biological activity. Regardless of the ultimate "inorganic vs. organic" origin of the Fe isotope variations in chemical sediments, these results strongly indicate that Fe isotope variations are only produced in systems where minerals were precipitated from low-temperature aqueous solutions, strongly suggesting that *if any Fe isotope variations are found on a planetary body, they must have been produced in the presence of liquid water.*

Our connection between the rock record and inferring the Fe isotope compositions of ancient fluids lies in experimental determination of mineral-fluid isotope fractionation factors. Such experiments are also essential for recognizing biological or "vital" effects. Distinction between equilibrium and kinetic isotope fractionation in laboratory experiments is critical, and it is quite easy to produce significant isotope fractionations in the lab, particularly kinetic effects, but these would seem to have limited application to nature. Rigorous demonstration of isotopic equilibrium in mineral-fluid systems at low temperature is quite difficult, but we have successfully measured the *equilibrium* Fe isotope fractionation between hematite and Fe(III) at 100°C, and there appears to be essentially no isotopic fractionation. In contrast, the *equilibrium* $[Fe^{III}(H_2O)_6]^{3+}$ - $[Fe^{II}(H_2O)_6]^{2+}$ Fe isotope fractionation is +2.7 ‰ for $^{56}Fe/^{54}Fe$ in dilute aqueous solutions. Combining our speciation and mineral-fluid experiments suggests that oxides may reflect the isotopic compositions of the fluids from which they precipitated, and that the isotopic distinction between ferric and ferrous minerals we see in BIFs may in part reflect differences in Fe speciation in the ancient oceans.

Fe-reducing bacteria produce a +1.3 ‰ fractionation in $^{56}Fe/^{54}Fe$ between ferric substrate and Fe(II), which, overall, likely reflects a kinetic isotope or "vital" effect. Our current focus in on the exact pathways by which biologically-produced Fe reduction occurs, and our experimental work on speciation suggests that if Fe(III) is transported to the cell, the exact nature of the ligand may have a strong effect on the net isotopic fractionation of the process. Overall, our experimental data may provide an explanation as to why ferric oxides precipitated in the modern oceans (low δ^{56}Fe values) are so different from those precipitated in Archean and Proterozoic BIFs (high δ^{56}Fe values). Such contrasts may reflect the fact that essentially all of the Fe in the modern oceans exists as Fe(III), shifting the burden of Fe mobilization entirely onto organisms, such as Fe-reducing bacteria that process Fe at the sediment-water interface. Determining how Fe isotope fractionation may reflect a "biological component" during evolution of the biosphere must account for temporal variations that may occur in the mass balance of different Fe reservoirs.

Although we have only begun to understand the principles that govern Fe isotope variations in nature, it is now clear that Fe isotope geochemistry has an important role to play in development of biosignatures as applied to astrobiology.

Pigments and Other Biomolecules in Extreme Antarctic Microbial Habitats: Analogues for Evaluating Raman Spectroscopic Evidence of Preserved or Relict Life on Mars?

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The unequivocal detection of biomolecules amongst extraneous organic materials on or near the surface of Mars is a primary goal for astrobiology. The characteristics of any biomolecules on Mars cannot be assumed to be identical to those on Earth, despite the common carbon-based nature of organic compounds in the Solar System as evidenced by carbonaceous chondrites and spectra of interplanetary dust [1]. However, the evolutionary pressure of environmental constraints on early Mars such as UVB and UVC radiation, high and low temperatures, desiccation and hypersalinity would have required protective strategies to promote the origin, survival and evolution of any microbial life [2]. Chemo-lithotrophic growth may have originated near the surface of early Mars [3], and heterotrophic microbial growth on organic compounds derived from meteorites, may have evolved on the surface. However, the most efficient known source for metabolism available in the Solar System is light from the Sun which drives photosynthesis. If it evolved this would have required pigments to capture photons and screen UV radiation. Pigmentation would also be advantageous for exposed heterotrophs.

Missions to Mars are dependent on the coincidence of many geomorphological and hydrological for optimal chances of encountering any relict biomolecules. Target compounds must therefore be unequivocally identified as biogenic. They must be distinguishable from organic compounds from meteorites [1]. Although fundamental for the exploration process [4], the mere detection of dispersed organic material on Mars is not unequivocal evidence of former microbial life, because of this extraneous input. A dense biofilm concentrated by a steep environmental gradient, such as a photosynthetic microbial mat (analogous to a cyanobacterial stromatolite) would be an optimal source of biomolecules for unequivocal analysis. Primitive phototrophs could have evolved by trapping solar photons with infra-red-absorbing pigments such as ancient rhodopsins [5]. Sophisticated assemblies of bacteriorhodopsin and chlorophyll could have evolved later [6] in organisms analogous to primitive bacteria like *Rhodopseudomonas* and *Chloroflexus* [7] or even cyanobacteria. Photosynthetic organisms would need protective pigments such as carotenoids and scytonemin to absorb harmful UV radiation [8].

These biomolecules can be characterized by laser Raman spectroscopy, which is a non-intrusive technique based on the scattering of laser light at shifted wavelengths dependent on the vibrational transitions of components of the target [9]. Both the inorganic and organic compounds in the target give Raman spectral bands to reveal components of the habitat, such iron-doped quartz, and functional organic moieties e.g. bio-weathering calcium oxalate and UV-absorbing quinone rings [10]. Every compound has a unique Raman spectral fingerprint depending on its component parts. Prior identification of the target compounds is not necessary, which is a major asset for a remote Mars lander.

Bands of key molecules can be compared with an ever-increasing database of Raman spectra for terrestrial materials from analogous habitats in Antarctica [11]. **A**nalysis of potential biomolecules *in situ* in their natural state within their mineral substrata is a major benefit. Pigments often have functional components in common, such as the UV-absorbing quinone ring of parietin (from lichens) but also different UV-absorptive features as in scytonemin and calycin with their distinctive Raman spectra [9, 10, 11,].

Carotenoids are common in photosynthetic microbes exposed to UV radiation, ranging from primitive bacteria (e.g. *Rhodobacter*) to more evolved cyanobacteria [12]. Pigments can be detected in the fossil record of oil-bearing shales up to 3.5 Gya, e.g. porphyrins derived from chlorophyll [13] and isoprenoids from carotenoids [14]. Raman spectroscopy is especially valuable as a descriptor rather than a mere detector. It would be eminently suitable for the sideways scanning of a drilled profile in Martian regolith beneath the oxidized surface zone, within which most biomolecules would have been degraded by peroxides. It could detect transitions through any former stromatolites in lacustrine sediments (c.f. Gusev Crater and other paleolakes [15]). A prototype miniature confocal-microscope / Raman-spectrometer (CMARS) has been developed at Montana State University for a future Mars lander mission and will be evaluated in Antarctica [16]. A field version will be tested on stromatolites from ice-covered hypersaline lakes and nearby paleolake sediments in the McMurdo Dry Valleys region [17].

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Iron Geomicrobiology of the Tinto River

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Sulfur metabolism has been considered a key element on geomicrobiology probably as a consequence of its redox potential. Although iron is considered and extremely important element for life, its role has been mainly associated to structural enzymatic activities. From the geomicrobiological characterization of the Tinto River (Iberian Pyritic Belt), a 90 km acidic river (mean pH 2.3) holding a high level of prokaryotic and eukaryotic diversity, an earth living system under the iron control is emerging. Iron, in addition of its important structural role in biochemistry, can be used not only as an energy source, but also as an electron acceptor for anaerobic respiration, is responsible for the maintenance of a constant pH in the water table (probably needed to sustain the high level of microbial diversity), and can protect cell systems from ultraviolet radiation. A geomicrobiological model of this habitat considering most of the geological, physical, chemical and biological variables will be presented and its astrobiological implications discussed.

Imaging and Geochemistry of Black Smoker Chimneys Using Three-Dimensional Synchrotron X-Ray Computed Tomography

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Hydrothermal system black smoker chimney structures are critical to geologists, biologists, and fluid flow engineers for understanding where biology could occur due to mineralogy, geochemistry, and fluid flow dynamics. Studies of this extreme environment must be conducted at microscales (on the order of 10^{-4} to 10^{-9} m) on structurally preserved samples because chemical, thermal, mineral, and biological gradients occur at these scales. Understanding of microorganism ecology in hydrothermal systems is important for evaluating this setting as a possible origin of life environment. Three-dimensional data sets that combine physical structure and chemical composition are needed to reconstruct the microenvironments that support microbial colonies in black smoker chimneys.

Synchrotron X-ray tomography images of black smoker chimney samples were collected on inner and outer portions of chimneys from the Juan de Fuca Ridge and 9° N East Pacific Rise. Ten samples were cut into 0.5-3.0mm rectangles or cubes and mounted with modeling clay onto the sample holder. Data was collected on GeoSoilEnviroCARS (a synchrotron-based research facility) Sector 13 bending magnet beamline at the Advanced Photon Source with a Si(220) channel cut monochromator tuned to 40 keV incident energy. A phosphorescent screen downstream of the sample generated visible light that was imaged with a Mitutoyo microscope onto a 12-bit CCD camera. The readout was binned by a factor of two in each direction. Pixel sizes of 21.6μm and 7.8μm were used to collect data. Analysis of data was performed using IDL software and routines written by GSECARS on an SGI Origins computer. Elemental mapping and point analyses by

electron microprobe were done on two-dimensional cross-sections of the samples. Bulk analysis, x-ray diffraction, and sulfur isotope ratios of pyrites were used to supplement tomography and microprobe results. Mineralogical data were then extrapolated through the three-dimensional tomography images.

Mineralogy was classified based on reflectivity of minerals in the visible light region represented by pixel values of 0 (no reflectivity) to 255 (high reflectivity). Samples from the inner part of the chimneys were composed mainly of iron and zinc sulfide. Sulfur isotope ratios of pyrite grains showed little variation within and between grains and suggest that sulfides formed from high temperature hydrothermal fluids (~180 to ~255°C). Electron microprobe and tomography images show pores, ranging from 20 μm to 500 μm, were lined with amorphous silica (~60 to ~172°C), a result of lower temperature fluids flowing through the chimney. Minor amounts of barite (~6 to ~60°C) occurred around some of the pore spaces due to mixing of hydrothermal fluids with seawater at lower temperatures. Outer chimney samples composed of amorphous silica and barite result from mixing of seawater and hydrothermal fluid at lower temperatures. Minor amounts of iron sulfide surrounded by amorphous silica are present.

Tomographic reconstructions of chimney volumes provide a three-dimensional microscale image of the silica- and barite-lined pores and distribution of sulfide minerals from which the correlation of mineralogy, fluid composition, and temperature can be traced along the flow path. From this data set we can determine thermal and chemical conditions present in specific areas of the chimney. Future studies will include *in situ* staining of microbes that will allow us to determine the location of microbial colonies in these extreme environments and their relationship to mineral substrates and fluid flow paths.

Hyperthermophilic Microbial Communities in Silica-Depositing Yellowstone Hot Springs Exhibit More Morphological and Sequence Diversity than Previously Detected

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A picture of the kinds of hyperthermophilic microorganisms that inhabit slightly alkaline near-boiling silica depositing hot springs is beginning to emerge. We have reported previously (Blank et al., 1999, GSA Annual Meeting Abstracts, **31**(7)) that the microbial diversity as measured by small subunit ribosomal DNA (SSU rDNA) techniques in various springs in Yellowstone National Park is rather variable: some contain only one or two taxa whereas others contain up to six taxa. All taxa in these springs branch deeply in the bacterial domain of life near the last common ancestor, as do all hyperthermophilic bacteria. No archaeal taxa were found in these springs using PCR, despite repeated attempts to find them. All springs studied contain the lineage corresponding to EM17, now designated as *Thermocrinis ruber*, a hydrogen oxidizer belonging to the Aquificales (Huber et al., 1998, Appl. Environ. Microbiol. **64**:3576) isolated from the pink filaments of Octopus Spring (White Creek Area, Lower Geyser Basin). Communities associated with spicular geyserite at the air-water interface of Octopus Spring and Queen's Laundry Pool (Sentinel Meadows, Lower Geyser Basin) contain a slightly higher number of taxa than communities in the subaqueous environment in both springs. This is attributed to a slightly lower temperature in the splash zone where spicular geyserite forms. In addition, several of the taxa seen on the spicular geyserite are many of the same taxa found in the cooler subaqueous regime.

In situ hybridization analyses were performed using fluorescent RNA probes complementary to the rRNA gene from each taxa in these springs. These probes were hybridized against cells attached to glass microscope slides that were incubated in the springs for 3-5 days. Such hybridizations show that *Thermocrinis*-EM17 in the Octopus

Spring source pool (89-92°C) has several distinct morphologies. One morphological type is that of long rods of variable length with regularly-spaced septa along the filament. Another is that of very long filaments with a holdfast secreted at one end of the filament and only occasional septa. Yet another has a morphology of short, fatter rods of variable length. Hybridizations with the same probe showed that *Thermocrinis*-EM17 from Boulder Spring (Sentinel Meadows, 92-95°C) were short, thin rods with bright terminations. *Thermocrinis*-EM17 cells from Eclipse Geyser (White Creek Area, 89-93°C) are short rods, while in nearby Spindle Spring (92-94°C) they have both the long-filament-with-holdfast and short-fatter-rod morphology. This variation in morphology clearly does not correlate with diversity on the rDNA level, since the SSU rDNAs from *Thermocrinis*-EM17 from all of these springs are identical or nearly identical (99.60% identical on average with 25,375 bp sequenced).

Variation in morphology was also observed at the submicroscopic scale with the use of a scanning electron microscope (SEM). The pink filamentous organisms in the Octopus Spring outflow channel (85-88°C) are long filaments with regularly spaced septa which have a polysaccharide layer with parallel ridges along the length of the filament. This morphology was not observed in the adjacent source pool, although both contain identical or nearly identical *Thermocrinis*-EM17 sequences. These variations in morphology could be due to a number of different factors: variation in geochemistry, the presence of unique species or sub-species of *Thermocrinis* in these springs, or phenotypic plasticity (due perhaps to differences in hydrodynamics or the age/maturity of the cells in the community).

In order to investigate this morphological variation further, an examination of the internal transcribed spacer (ITS) region was done on the *Thermocrinis*-EM17 clade in these springs (the ITS region lies between the large and small subunit rDNA genes and evolves at a much higher rate). Preliminary results show that despite there being a very limited amount of sequence diversity on the level of the SSU rDNA, there is a much larger amount of sequence diversity on the level of the ITS region. In addition, this increased level of sequence diversity on the ITS level appears to correlate with the observed distribution of morphologies. So far, at least four different classes of ITS sequences within the *Thermocrinis*-EM17 lineage have been found in Queen's Laundry, Octopus, and Boulder Springs. The ITS sequence from Boulder Spring is unique from that in any other spring (belonging to cluster 4), correlating to the unique morphology found in this spring by fluorescence *in situ* hybridization. The pink filamentous streamers in the Octopus Spring outflow channel have two other ITS sequence classes (clusters 1 and 3). The Octopus Spring source pool has at least one other ITS sequence class (cluster 2), while the main pool of Octopus Spring (84˚C) contains cluster 1, and the sediment just below the photosynthetic mats in Octopus Spring (71°C) has clusters 2 and 3.

Further work is currently being done to elucidate the extent and cause of this morphological variation in the *Thermocrinis*-EM17 clade in these and other springs in Yellowstone.

Flagellate Growth and Survival Under Conditions Potentially Encountered at Deep Sea Hydrothermal Vents

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Eighteen strains of flagellated protists, representing 9 species from 6 taxonomic orders, were isolated and cultured from four deep-sea hydrothermal vents. Many of the vent isolates are ubiquitous members of marine, freshwater, and terrestrial ecosystems worldwide, suggesting a global distribution of these flagellate species. This discovery advanced the hypothesis that ubiquity in distribution patterns among heterotrophic flagellates implies high tolerance and/or adaptability to a wide range of environmental conditions. Experiments under vent conditions of high pressure and high concentrations of metals and sulfide showed that some of these species are very tolerant to extreme environmental conditions.

Three isolates of deep-sea flagellates were grown in culture at 1-300 atm to measure their growth response to increasing hydrostatic pressure. The growth rates of two vent flagellates, *Caecitellus parvulus* and *Rhynchomonas nasuta*, were compared to the growth rates of shallow-water strains of the same species. Deep-sea isolates of *C. parvulus* and *R. nasuta* had a higher rate of growth at higher pressures than did their shallow-water counterparts. Vent strains of *C. parvulus* and *R. nasuta* were capable of growth at pressures corresponding to their respective depths of collection, indicating that these species could be metabolically active at these depths. However, *C. parvulus* and *R.*

nasuta encysted at pressures greater than their depth of collection. The choanoflagellate isolate was observed to encyst at pressures greater than 50 atm.

The survival rates of three species of deep-sea hydrothermal vent flagellates were measured after exposure to chemical conditions potentially encountered in vent environments. The survival rates, measured as viability through time of *Caecitellus parvulus*, *Cafeteria* sp. and *Rhynchomonas nasuta* were determined and compared to shallow-water strains of the same species after exposure to increasing concentrations of sulfide or the metals Cu, Fe, Mn and Zn. Responses were variable but in all cases these flagellates showed very high tolerance to extreme conditions. *Cafeteria* spp. were remarkable in that both strains showed 100% viability after a 24 h exposure to 30 mM sulfide under anoxic conditions. By contrast, the highest naturally-occurring sulfide concentrations ever measured are only 18-20 mM. There was little effect from metals at concentrations up to 10^{-3} M total metal, but a sharp decrease in viability occurred between 10^{-3} M and 10^{-2} M total metal, due either to a rapid increase in the availability of free metal ions or colloid formation or both. This study is consistent with other previously reported studies that indicate these flagellate species are present and capable of being active members of the microbial food webs at deep-sea vents.

Carbonate Biogenic Structures in Storr's Lake, Bahamas

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Storr's Lake, an inland hypersaline lake on San Salvador Island, Bahamas, contains calcium carbonate-rich lithified mats of filamentous microorganisms, diatoms, associated photosynthetic and chemotrophic bacteria, and trapped sediment (1). In addition, 16S rRNA analysis indicates the presence of five sulfur-reducing genera of bacteria.

These microbes are potential modern-day analogs to some ancient stromatolitic structures. The goals of this study are to identify unique compositional and biogenic features, possibly correlating some of these with some of the sulfate-reducing bacteria.

Our field emission scanning electron microscope (FE-SEM) indicates a range of microbial life forms on the fractured stromatolite surfaces. Spheroidal features are the most common, with four distinct populations, characterized by their highly uniform intrapopulation sizes: large (mean size 5.5 μm), medium (mean size 2.0 μm) and tiny (mean size 0.13 μm). The surface textures range from smooth and taut to wrinkled and shrunken. The large spheres (Fig. 1) and medium spheres populations (Fig. 2) are isolated from each other and the other two smaller populations. Most of the large spheres have uniform surface indentations. Most of the medium spheres are clustered together in aggregates of three or four. The small and tiny spheres are closely associated with each other. They are also commonly embedded in biofilm. The biofilm, alternately viscous

and brittle according to degree of mineralization, is composed of thick filaments and web-like film.

Diatoms and long, hollow tube-shapes that may be cyanobacteria sheaths are also present (Figs. 3,4). These structures are plentiful, but not as common as spheres.

The EDS analysis conducted are both inconsistent and inconclusive. However, the predominant cations detected in the large and medium-sized spheres are calcium. Some of the large and medium-sized spheres, in addition to calcium, are enriched with magnesium. The tiny spheres are similar in composition, although some also contain silicon. Phosphorous, an important component of biological structures such as DNA, is not present.

The large, medium, and small spheres may represent the mineralized (or fossilized) remains of coccoid microbes, including a sulfur-reducing bacteria, *Desulfococcus. Desulfococcus* is one of the microbes identified by 16S rRNA. The size and shape of the bacteria can be significantly altered during fossilization, therefore all of these may represent a single genus or species or they may represent several different genera and species (2). The smallest spheres may represent biogenically mediated precipitates (3). They could be nanobacteria, or they may represent solid, abiotic precipitates (4). It is unlikely that they are wholly inorganic due to their intimate association with the small spheres and biofilm (3). We suggest that all features described here are likely biogenic or biogenically influenced. Further chemical analysis may provide additional insights into the origin of the tiny spheres.

Water on the Martian surface may have formed subtidal pools that are similar to Storr's Lake. Stromatolites, which are essentially bacterial colonies on an enormous scale, could be the first step in life's mass aggregation in any environment where bacteria-like organisms live.

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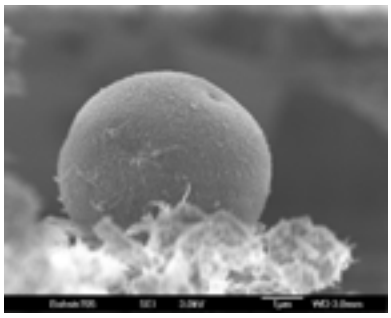

Figure 1. Large sphere (5.5µm) with elevated levels of Ca enriched with Mg.

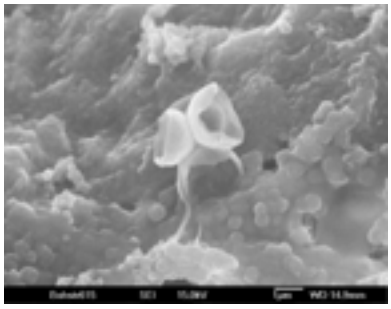

Figure 2. Medium-sized sphere with lysed cells.

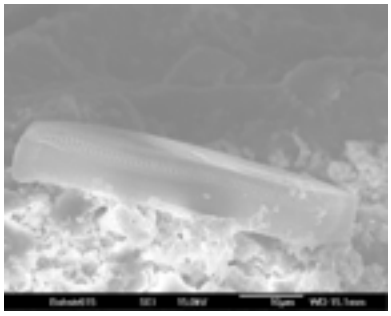

Figure 3. Diatom.

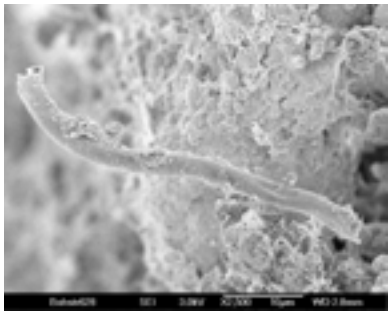

Figure 4. Cyanobacterial sheath.

Protection Ways Against Extreme Solar Ultraviolet Radiation: A Preliminary Study

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Solar UV-B radiation is the more harmful radiation affecting the biological processes occurring in the Earth's ecosystems, and especially it is responsible for the increase of DNA damage in the living organisms [1]. In this sense, UV levels on the Earth's surface can extremely change depending on atmospheric conditions.

A preliminary study of possible protection ways, natural as well as anthropogenic, in order to scream this radiation, or part of this radiation, is performed. Several forms of protection ways are considered: 1) a water layer, 2) a dense particulate layer of natural or anthropogenic origin, and 3) the mixed case of a aquatic media where are dissolved particles (possibly metal ions). Solar UV radiation reaching the surface, as well as its later propagation through the aquatic media is simulated with a "two-stream" radiative transfer model. Input parameters as ozone content, solar zenith angle, aerosol concentration, etc., are varied for modeling the extreme UV radiation conditions, and the description of aquatic conditions is also simulated [2].

This study is also extrapolated to other planets where the solar UV radiation conditions are considered highly extremes, and it has been supposed that these forms of protection could have existed in these planets in the past.

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Molecular Basis of Spectral Tuning in Opsins

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One of the most common organism-environment interactions is light perception. The visual system of animals is a well studied light perception example. Animal vision exhibits a range of sensitivities to different wavelengths of light, and visual sensitivity in different colors is due principally to differences in the amino acid sequences of opsins, which are the G-protein coupled receptors.

We are using opsins as a model system to study organism-environment interactions and genotype-phenotype relationships. Our work consists of several parts, including laboratory work on odonate (dragonflies and damselflies) opsins, and computer analyses of opsins from a wide range of organisms. It is this later aspect we describe here. The specific research questions include the following.

- How much of the variance in the wavelength of light absorbed by opsins can be explained by primary amino acid sequence alone?
- What amino acid positions are important in determining differences in the spectrum of light absorbed by opsins?
- What biochemical characteristics of amino acids have the best relationship to light absorbing properties of opsins?

We used decision tree induction to analyze the relationship between amino acid sequence and light absorption in opsins. Decision trees have been used for a variety of problems including whether a space shuttle pilot should use the autolander or land manually. For the present problem the data were 107 opsin sequences from vertebrates for which corresponding wavelength of maximum absorption (λmax) were available. We generated a regression tree, which is a binary tree that maximizes the difference between high and low values of λmax. Analyses were done using the rpart library for the statistical package R.

The results of the preliminary investigation are outstanding; a single partition based on any of four amino acid positions explained 70.9% of the variance in λmax. Overall the tree model explained 95.2% of the variance in λmax by using four amino acid positions.

Therefore we are able to distinguish the phenotypically relevant genetic differences from among the hundreds of polymorphic amino acid positions.

Evidence of Novel Eukaryotes in Guaymas Basin Hydrothermal Vent Sediments: Life at the Extremes

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The phylogenetic composition of eukaryotic communities in hydrothermal sediments of the Guaymas Basin (Gulf of California, Mexico) was surveyed by cloning and sequencing of PCR-amplified, partial 18S rRNA genes from environmental DNA. Cores with a clear and reproducible temperature profile were sliced and subsampled. We analyzed 18S rRNA genes from the top 3 cm of cores and from the sediment-seawater interface, with temperature profiles ranging from 4-65 ºC. Many sequences fall into a wide range of known eukaryotic taxa, including fungi, animals, diatoms, stramenopiles, alveolates, dinoflagellates, apicomplexa, and ciliates. Of particular interest are several groups of deep-branching eukaryotic sequences with no known close relatives in available SSU rRNA databases. This suggests that the Guaymas Basin hydrothermal sediments provide a habitat for novel eukaryotic microorganisms that may provide important clues to early eukaryotic evolution.

The eukaryotes of the Guaymas sediments are placed in context by the bacterial and archaeal communities that share this environment, providing clues about its chemical characteristics (Presentation by Teske and coworkers, this meeting). The prokaryotic community includes close relatives of novel archaea that may be anaerobic methanotrophs; sulfate-reducing and sulfur-oxidizing bacteria of the delta, gamma-, and epsilon-Proteobacterial subdivisions; and bacteria of the GNS, OP11 and OP8 phyla. Many of these Guaymas relatives are found in geothermal springs, or at sites contaminated with petroleum hydrocarbons, including toxic, aromatic and chlorinated compounds (Hugenholtz et al. 1998; Dojka et al. 1998). Clones with clear thermophilic affiliation are present, but do not appear to dominate the community. Thus, the prokaryotic community of the upper Guaymas sediment layers indicates a methane-rich, sulfidic, anaerobic, temperate habitat with natural hydrocarbon contamination. Some cultured hydrothermal vent eukaryotes, including *Cafeteria*, *Caecitellus*, and *Rhynchomonas* spp., tolerate high sulfide and metal concentrations (Atkins et al. 2001). Most likely, the uncultured deeply-branching eukaryotic lineages in the Guaymas Basin

sediments are specifically adapted to this hydrothermal vent environment, since they have not been detected anywhere else in marine or terrestrial environments.

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Morphological and Metabolic Aspects of Neutrophilic, Lithotrophic Fe-Oxidizing from Deep-Ocean Hydrothermal Vents in the Pacific Ocean

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Ferrous iron is a common and often abundant substituent of hydrothermal vent fluids; however there has been limited research on the microbes involved in oxidation of iron at deep-sea hydrothermal vents. We have investigated several hydrothermal vent sites at the summit of the Loihi Seamount (depth, 1100 m), which is part of the Hawaiian archipelago. Hydrothermal vent fluid at Loihi ranges in temperature from 10°C to 165°C and contains up to 50 µM Fe(II); however there is very little H_2S present. As a result, most of the vents have abundant microbial mats that are heavily encrusted with Fe-oxides. Observation by light microscopy revealed the mats were composed of three morphotypes of hydrous ferric oxides (HFOs). These were: I*) 'Leptothrix ochracea'*-type, hollow tubular sheaths with diameters of 1 - 3 µm; II) twisted or irregular thin filaments, diameter 1 -2 µm, and III) amorphous particulate oxides. Forms I and II can be directly attributed to the action of Fe-oxidizing bacteria; form III oxides may be formed biologically or abiologically. We quantified the morphotypes of oxides present at different vents at Loihi and found that at least 60% of the oxides at some sites were of form I or II, indicating that the bulk of iron deposition was biologically mediated. Using cultural techniques we demonstrated that Fe-oxidizing bacteria were present at all the vent sites we tested, and were often abundant with numbers of up to 10^7 cell/cc of mat. All the enrichments for lithotrophic, microaerophilic Fe-oxidizers done at 12°C or 25° C were successful; enrichments at 60°C or above did not yield microaerobic Fe-oxidizers.

We have also isolated two strains of lithotrophic Fe-oxidizing bacteria from Loihi, strains PV-1 and JV-1, and will report on their growth characteristics. In terms of morphology, the strain PV-1 is especially interesting, since it formed a twisted, filamentous oxide that closely resembled the form II morphotype that was a common constituent of the in-situ mats. Preliminary studies done growing this strain in a bioreactor with Fe(II), indicated that it accounted for up to 80% of the Fe oxidation, compared to either abiological or poisoned controls. It appeared that the filamentous oxides formed by PV-1 are precipitated upon an organic matrix that is presumably secreted by this organism. Together these results indicate that lithotrophic Fe-oxidizers are common at the Loihi site, and contribute significantly to Fe-oxidation. Furthermore, the signature morphologies these organisms form are interesting in light of paleontological work showing similar structures associated with ancient hydrothermal vent habitats on Earth. This raises the possibility that these morphotypes could be useful biomarkers for demonstrating the existence of iron-oxidizing bacteria on other planets.

Microbes That Follow the Water

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Water is arguably Nature´s most central molecule, and the most indispensable ingredient for active life. While some organisms can tolerate periods of severe desiccation (anhydrobiosis), the presence of liquid water is always required, at least temporarily, in order to guarantee metabolic activity and survival. This is the simple, compelling *sine qua non* of astrobiology: *to search for life, follow the water.*

Biological desert crusts are excellent microbial communities in which adaptations to life under a regime of alternating anhydrobiosis and hydration can be studied. Driven by the photosynthetic activity of terrestrial cyanobacteria and microalgae, these topsoil formations occur worldwide in soils where plant growth is restricted. There is also evidence that they might have been the only terrestrial communities on Earth before the advent of higher plants (quite late in Earth´s history). During most of the time, the biota are in a desiccated, metabolically dormant state, but when water precipitation events occur, the organisms rapidly hydrate, start respiration within seconds from initial wetting, photosynthesis within several minutes, and nitrogen fixation within tens of minutes. Areal biogeochemical exchange rates then can match or exceed those of permanently wet mesotrophic lakes. Some of the cyanobacteria in crusts are "shade types", presenting saturation of photosynthesis at low irradiance, high cellular pigment concentrations and an absence of sunscreen secondary metabolites. These shade types develop one to several mm deep in the immediate soil subsurface, where light intensities are significantly lower that those at the surface. During rainy, overcast periods, incident light intensities are usually low, and motile desert soil cyanobacteria migrate towards the surface, resulting in an apparent soil surface greening. Photosensory responses, universal in photosynthetic organisms, are thought to be responsible for such small-scale vertical migration, because this greening can be prevented by exposure to high light intensities.

But in our experiments we observed that if the soils were allowed to dry under unchanged illumination, the greening stopped and eventually reversed, with cyanobacterial filaments apparently moving back into the soil following a retreating desiccation front. We could induce such upward and downward migrations repeatedly by recurrent wetting and drying manipulations, and additional experimentation showed that a cellular photosensory mechanism or merely a surface-tension effect could not be the basis for this

phenomenon. The *cyanobacteria displayed hydrotaxis*, a term previously used to describe the behaviour of large animals seeking drinking holes during drought periods; cyanobacteria, as astrobiologists, strove to follow the water. An interpretation of the selective advantage of such hydrotactic behavior for the cyanobacteria is that, by overwhelming their natural tendency to seek optimal illumination, it allows them to reach future environmental refugia, and prevents them from being trapped in a desiccated state and unable to move in high-exposure, near surface environments, in anticipation of a looming drought.

The requirements for light in cyanobacteria, which restricts them to the lit areas of topsoils, made the direct observation of hydrotaxis possible. But if indeed this capacity is widespread among prokaryotes, it may be a crucial and overlooked physiological attribute that contributes significantly to the survival, dispersal and extent of bacterial populations in terrestrial environments at large. Soil and deep-subsurface bacterial populations may not only be sedentary populations awaiting episodes of water availability or passively being transported along with ground waters, but may constitute an inherent part of a moving water pocket, able to actively follow it and to exploit its available resources. In the same manner, bacterial populations, if they exist, may follow available water pockets in extraterrestrrial soil environments such as those of Mars, where water itself , if still present, appears to have become a very localized resource.

GEOPULSE: Gene Expression Observations for Planetary Life Study

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The Biogeochemistry at a Hydrothermal Vent Setting:

A further understanding of hyperthermophiles is pertinent to astrobiology because the origin of life may have occurred in a hydrothermal setting, hyperthermophiles may be similar to the last common ancestor (Wachtershauser, 1990; Pace, 1997), and chemolithoautotrophic communities may reside in the subsurface of Mars or under the ice of Europa (McCollum, 1999). In order to detect life in these planetary environments, the biogeochemistry of hydrothermal systems must be understood in greater detail. In order to understand the biogeochemistry of hydrothermal vent systems, a number of geochemical conditions will be investigated during growth of *Pyrobaculum aerophilum* and *Pyrobaculum islandicum*. Interactions of these microbes with minerals, metals, and various organic substrates under various growth conditions will be evaluated, coupled with gene expression studies to pinpoint specific genes involved in the biogeochemical processes.

Pyrobaculum aerophilum, a marine hyperthermophile with an optimal growth temperature of 100^{o}C, can be grown under range of conditions from microaerophilic to strongly anaerobic and with a variety of electron donor-acceptor pairs, whereas *Pyrobaculum islandicum* is from a terrestrial hot spring. Metal uptake and isotopic fractionation experiments will monitor gene expression involved in these processes by P*yrobaculum islandicum* under anaerobic conditions, and by *Pyrobaculum aerophilum* under aerobic and anaerobic conditions forming a link between genomics and geochemistry of a hydrothermal vent settings.

Anaerobic Methane Oxidation:
Anaerobic methane oxidation is well observed in various field studies and may play an important role in global methane cycling from the Archaean to the present. Initial studies suggest a strong link between methanogens and sulfate-reducing bacteria in this process. Here we are attempting to document anaerobic methane oxidation mediated by methanogen and sulfate-reducer consortia in a laboratory setting.

Initial lab efforts have focused on establishing pure anaerobic methanogen and sulfate-reducing cultures. Five methanogen species have been grown completely chemoautotrophically over a temperature range from 30° to 85°C. Three sulfate-reducing species have been anaerobically cultured over a temperature range of 30° to 80°C. These cultures were grown under a carbon dioxide and hydrogen atmosphere.

Initial attempts at consortia formation with cultured species was performed by combining samples of actively growing methanogens and sulfate-reducers in a fresh media under a methane, carbon dioxide, and nitrogen atmosphere. Isotopically enriched (^{13}C) methane is used as a marker in the study. Future analysis will also focus on the isotopic composition of methanogen extracts from the consortia. Metabolic uptake of labeled methane by methanogens should result in isotopic enrichment in archeols extracted from methanogen membranes. Further, fluorescence *in-situ* hybridization (FISH) staining may be used to selectively stain methanogens and sulfate-reducers in a consortium, allowing for the exploration of the spatial and physical relationship between the two species if consortia formation is observed. Finally, analysis of methanogen gene expression under low hydrogen conditions will help identify genes related to this process.

Visible/Near-infrared and Thermal Infrared Field Spectroscopy Of Modern And Ancient Calcareous Tufa Deposits

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Introduction: Calcareous tufa deposits on Earth form through precipitation of $CaCO_3$ when calcium-bearing spring waters come in contact with carbonate in saline or alkaline lake waters. Modern to Pleistocene-age deposits are observed at Mono Lake, CA [1] whereas tufa deposits near Searles Lake, CA are 10^4-10^5 years old [2]. Field observations of tufas from both regions using visible/near-infrared (VNIR) and thermal infrared (TIR) spectrometers were undertaken to determine if tufa spectral signatures varied with age. Understanding such temporal variability in carbonate mineralogy will be relevant to the spectral search for carbonates on Mars [3].

Methods. We acquired VNIR and TIR spectra of a variety of tufa deposits at both sites. At Mono Lake tufa vary in color from white/gray to dark brown. In some spires cream-colored coatings conceal darker interiors. At Searles Lake tufa mounds are grayer in color but also exhibit white and brown color variations between interior and exterior surfaces. VNIR spectra were acquired from 400-2500 nm with a FieldSpecFR™ (ASD) fiberoptic spectrometer using a portable light source [4]. TIR spectra were acquired from 715-1250 cm^{-1} (8-14 μm) with a Designs and Prototypes μFTIR field spectrometer [4-5].

Results. *Visible/near-infrared.* Typical VNIR spectra for different tufas at Mono and Searles Lakes are compared in **Figure 1** to laboratory spectra of aragonite and calcite [cf. 6,7]. Absorption bands near 1450 nm and 1935 nm are associated with water while those near 2340 nm and >2500 nm result from carbonates. Gaffey [7] showed that these and other subtle bands could be used to discriminate calcite, aragonite, and dolomite. Based partly on these methods, preliminary analyses of these VNIR spectra suggest that the Mono Lake white tufa may be more aragonitic and the brown tufa more calcitic. The sandy tufa (carbonate-cemented sand spires [1]) may also contain other evaporite minerals [cf. 8] or minor dolomite plus sands derived from nearby rhyolites.

At Searles Lake the spectrum of the interior of a tufa tower exhibits a sharper 2340 nm band than the tufa exterior. This is likely related to more crystalline (less weathered) carbonate in the interior tufa. The "interior-2" spectrum is from a lower interior portion

of the tufa tower. It exhibits shifts in the dominant bands to shorter wavelengths like those in the sandy tufa spectrum at Mono Lake. The "cauliform surface" spectrum is from a white, bulbous surface located on the interior of another tufa tower. It lacks a dominant 2340 nm band but has several narrow, deep absorptions and other more subtle bands suggestive of sulfate, chloride, and/or other carbonate evaporite minerals (gaylussite, pirssonite [8,9]). The South Exposure tufa mounds at Searles Lake are the oldest observed. They exhibit spectral features not observed in younger deposits (e.g., a small band near 2214 nm) that are most suggestive of trona [2,8].

Thermal infrared. **Figure 2** shows typical TIR emission spectra for tufa mounds at Mono and Searles Lakes compared to laboratory spectra of calcite and aragonite powders. The characteristic calcite absorption centered near 886 cm^{-1} (11.29 μm) matches the brown tufa well, whereas the 875 cm^{-1} (11.43 μm) aragonite band matches the white tufa. At Searles Lake both tufa types exhibit absorptions that fall between the characteristic aragonite/calcite bands. The exterior tufa has a slightly more aragonitic-like absorption than the internal tufa, although this may result from weathering and/or mixing of aolian transported dusts.

Conclusions. Field spectroscopy of tufas can discern differences between dominant carbonate types and detect the existence of secondary minerals. Mono Lake tufa deposits exhibit absorptions characteristic mainly of carbonates (calcite, aragonite, possibly dolomite). Carbonate types are not as well discriminated at Searles Lake, but VNIR evidence for additional evaporite minerals is prevalent [8]. Higher humidity at Mono Lake could result in greater adsorbed water on samples and subdue some of the sharp VNIR bands otherwise observed in Searles Lake tufa. If this is not the case, the observed spectral variations may reflect real differences in surface mineralogy between these tufa deposits. This could be related to variations in geochemistry during carbonate precipitation [2,9] or subsequent weathering differences between both regions.

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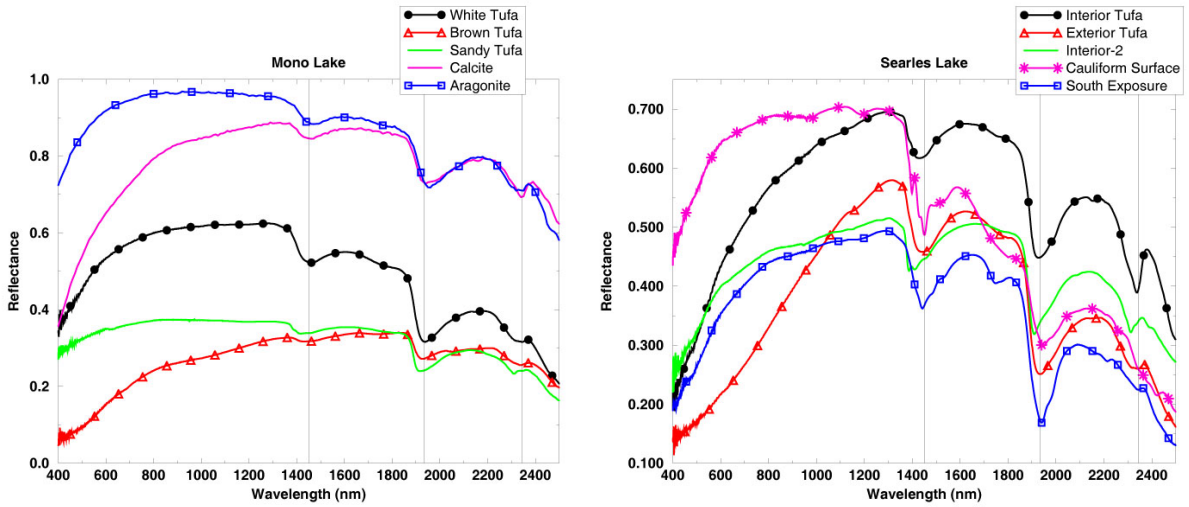

Figure 1. Visible/near-infrared spectra of tufa deposits: (*left*) Mono Lake white, brown, and calcareous sandy tufa deposits compared to aragonite and calcite spectra; (*right*) Searles Lake interior vs. exterior (weathered) tufa spires.

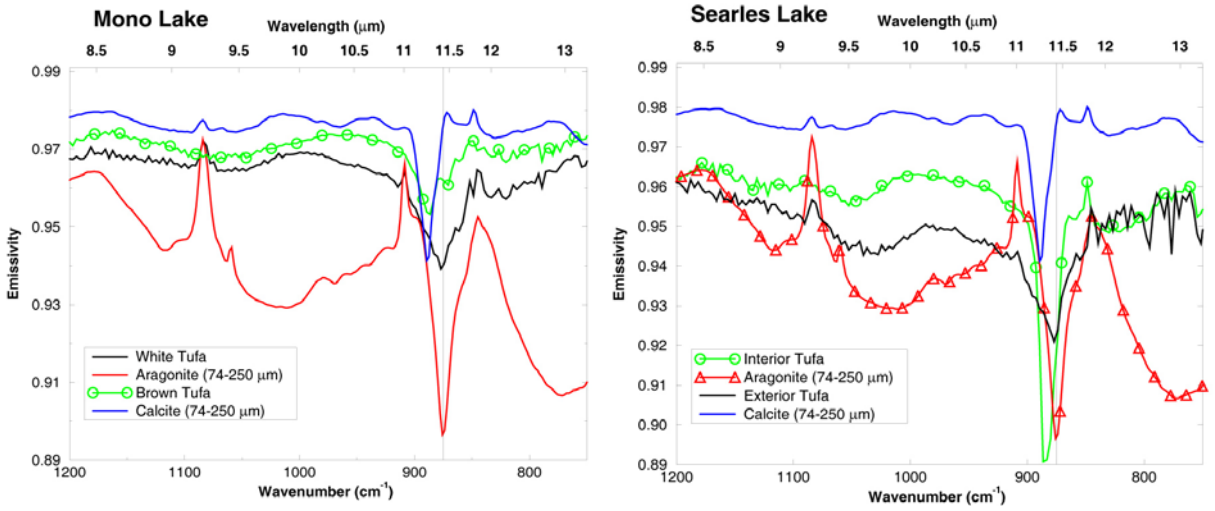

Figure 2. Thermal infrared spectra of tufa deposits: (*left*) Mono Lake white and brown tufa compared to inverted biconical reflectance spectra of aragonite and calcite [9]; (*right*) Searles Lake interior vs. exterior (weathered) tufa surfaces.

The Purification and Characterization of Superoxide Dismutase from *Chloroflexus aurantiacus* and the Effects of UV Radiation on the Activity of SOD and Catalase in Hydrothermal Mats of Yellowstone National Park

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Chloroflexus aurantiacus is a thermotolerant anoxygenic green phototrophic bacterium that is prominent in alkaline hot springs at temperatures between 52 and 60°C. This species often grows in the hyperoxic environment beneath cyanobacterial mats at higher temperatures up to 70 - 72ºC. *Cf. aurantiacus* is an evolutionarily important organism since it is in the earliest branch of the eubacteria that are capable of photosynthesis and many of its characteristics can be found in other diverse groups of phototrophic bacteria.

The mechanism by which *Cf. aurantiacus* deals with oxidative stress is currently being investigated. The metalloenzymes superoxide dismutase and catalase are proposed to play key roles in protecting cells from oxidative damage. Superoxide dismutase enzymes (SOD's) catalyze the conversion of superoxide anion radicals to hydrogen peroxide and molecular oxygen while catalase converts hydrogen peroxide to molecular oxygen and water. SOD's and catalases are found in nearly all aerobic and obligate anaerobic organisms, with the enzymes differing primarily in their metal prosthetic group.

Lab experiments at Arizona State University focus on characterizing the antioxidant enzymes superoxide dismutase and catalase and their responses to oxidative stress. Future experiments in the field at the hot springs of Yellowstone National Park will focus on the changes in the level of these enzymes during the day in response to oxidants and to the different types of ultraviolet radiation.

Detection and Enumeration of Microbial Life in the Perennially Ice Covered Lake of the McMurdo DryValleys

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The McMurdo Dry Valleys of Antarctica (160°-164°E, 76°20'-78°20'S) represent the most Martian-like analogs on Earth. The extreme cold and arid conditions in this region have influenced the evolution of terrestrial, aquatic and ice ecosystems that may provide models of past Martian ecosystems. A unique feature of the Dry Valleys is the perennially ice covered lakes. These lakes can be generally characterized as having no vertical mixing and being highly stratified in regard to supersaturation of gases (O_2, NO_2, H_2S), strong salinity gradients (5-300 ‰), temperature (-5-25°C), organic and inorganic constituents and nutrients. Also, sunlight penetration to the water column is reduced by >98% by the overlying ice cover.

In spite of these harsh conditions, the lakes in the Dry Valleys support very active microbial ecosystems. We investigated the prevalence of and interaction between bacteria and viruses at several depths within Lake Bonney (east and west lobes), Lake Fryxell, Lake Hoare and Lake Vanda. Samples were collected from depths within, above and below a region of elevated microbial activity based on chlorophyll-*a* concentrations. Collected samples were processed to determine the number of bacteria and viruses and the percentage of the bacterial population that was lysogenic (i.e., was host to a dormant virus). All samples were labeled with the nucleic acid stain SYBR Gold and counted using epifluorescent microscopy. Our data indicate that the bacterial, viral and lysogen concentrations are similar to those found in open oceanic waters in more temperate climates. Bacteria, viruses and lysogens were recovered from waters with very high salinities (>200‰), low temperatures (-5°C) and supersaturated gas concentrations.

We have demonstrated that bacteria and viruses can survive and persist in aquatic environments that may model pockets of liquid water in the areas of the Martian ice caps and below the cryosphere. The use of fluorescent labeled molecular probes and detection methods provides an easy, dependable and rapid method to detect microbial life in samples collected from extreme environments.

Mass-Independent Fractionation of Oxygen Isotopes in Earth's Atmosphere

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Nearly all oxygen-bearing geological materials exhibit normal, mass-dependent fractionation of oxygen isotopes, such that the fractionation of ^{18}O is approximately twice the fractionation of ^{17}O. Recent measurements of mass-independently fractionated (MIF) oxygen isotopes in Miocene sulfate deposits (Bao et al, 2000) demonstrate the capability of sediments to record the presence of MIF in the atmosphere. In Earth's present atmosphere MIF derives almost entirely from the photochemical production of ozone (Thiemens and Heidenreich, 1983). Oxidation of SO_2 to sulfate occurs in the lower atmosphere by reaction with O_3 and O_3 products in aerosols. Thus, observation of MIF of oxygen isotopes in barites (for example) might serve as a marker for ozone chemistry throughout Earth history.

Our understanding of MIF in Earth's modern atmosphere is far from complete. Because O_3 is a photochemically active molecule, its MIF signature can be imparted to other atmospheric molecules. Using a photochemical equilibrium model I have computed the MIF expected for various molecules of interest due solely to interactions with ozone. The model predicts substantial MIF in HNO_3 and smaller MIF in H_2O_2. Evidence for the latter has been obtained recently for hydrogen peroxide extracted from rainwater samples (Savarino and Thiemens, 1999). Additionally, the model predicts that H_2O produced photochemically in the stratosphere will exhibit MIF, in contrast to tropospheric water, which is mass-dependently fractionated during evaporation and condensation.

The photochemical model presented here represents a first step towards understanding oxygen isotope chemistry in the atmosphere. The model suffers from many poorly known rate coefficients, and most likely from missing oxygen exchange reactions. Once these reaction rates have been better determined, such models may be applied to Earth's atmosphere during earlier epochs and to the atmosphere of Mars.

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Archaeal and Bacterial Diversity in a Moderately Acidic Thermal Spring

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We have characterized microbial communities in a thermal spring (~70^0C, pH 4) in Yellowstone National Park in order to relate the dominant microbial metabolic potentials (inferred from phylogenetic data) to the changing geochemistry of the spring in August 1999 and June 2000. The high temperature and chemolithotrophic energy sources within this spring may be analogous to the conditions during early life on Earth and possibly Mars, making it an excellent environment to discern interactions between evolving biological and geological parameters. Sediment and water samples taken from the collecting pool and run off channels were subject to chemical analysis, direct DNA extraction, PCR amplification with universal Archaeal and Bacterial primers, cloning, RFLP screening, and sequencing of the 16S rRNA genes. During the first sampling event the spring had more reducing conditions, while the spring had more oxidizing conditions during the second sampling event. There were also significant differences in the concentrations of minor ions between the sampling events. Phylogenetic analysis has revealed a diverse community mainly belonging to the sulfur-dependent thermoacidophilic Crenarchaeota branch of Archaea, which are apparently responding to the geochemical changes. A majority of the 16S rRNA genes we analyzed are clustering in two distinct groups within Crenarchaeota with no close relatives. Several clones containing Bacterial inserts were collected; these mainly consisted of close phylogenetic relatives of *Hydrogenobacter* and *Bacillus subtilis*. Cluster analysis of RFLP ribotype frequencies grouped by sampling events, suggesting a correlation between the microbial community and the changing geochemistry. The phylogenetic positions of these ribotypes suggest that the biogeochemical reactions mediated by the microorganisms from which they are derived remain consistent with the geochemistry of the spring environment.

Cytosolic pH Maintenance in Eukaryotic Acidophiles

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The waters of the Rio Tinto in Southwestern Spain are kept between pH 1.7 – 2.5 by chemolithotrophic bacteria and an ambient iron buffer content around 0.5 mg/ml and as high as 20 mg/ml. The acidic conditions dissolve large amounts of heavy metals like Cu, As, Zn, Ni, and Ag that are nearly insoluble at neutral pH. These harsh conditions are not suitable for multicellular life forms but the Tinto does support an abundant unicellular, eukaryotic population (see poster by Linda Amaral Zettler, Eukaryotic Diversity in an Acidic, Metal-Rich Environment: Spain's Tinto River). We are currently studying a euglenid and chlamydomonad species from the Tinto that have been successfully cultured at pH ~ 2. With the fluorescent pH indicator BCECF we have found that both species maintain a neutral pH cytosol above 6.5. Along with a slightly negative membrane potential there is a near 10^5-fold electrochemical transmembrane H^+ gradient. Neither species is an obligate acidophile; both grow at similar rates at pH 7 as at pH 2. Using weak acids to lower the cytosolic pH we find that the chlamydomonad can quickly neutralize the cytosolic pH after a brief cytosolic acidification. However, if the cytosolic pH drops to around 6.2, cells cannot recover cytosolic neutrality. From these data we suggest that cytosolic neutrality is necessary in order to maintain the transmembrane H^+ gradient. Presumably cytosolic acidification damages proteins necessary to maintain cellular function and yet the extracellular portions of membrane proteins do not appear to be affected by the extremely low extracellular pH. We are characterizing the active mechanisms by which these cells maintain the cytosolic neutrality by screening a battery of H^+ transporter inhibitors. Preliminary work shows that low concentrations of Diethylstilbestrol, a H^+-ATPase inhibitor, lead to cytosolic acidification. Our working hypothesis is that these species protect their cytoplasm by maintaining a neutral pH environment, minimally, by actively transporting H^+ out of the cell via H^+ transporters. Additionally barriers to H^+ influx, at the level of the plasma membrane, may help to

maintain cytosolic neutrality. However, we postulate that the surface proteins, exposed to the low extracellular pH, have been modified in order to function under these harsh conditions.

Hydrothermal Habitats in Astrobiology

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The search for life beyond Earth depends on accurate predictions about the environments and organisms that we can expect to find, using Earth ecosystems as templates. Hydrothermal systems on Mars and Europa are possible havens for life because they can supply the requisite water, energy and sources of carbon. The geologic history of Mars indicates that the planet, like early Earth, may have been wet and warm (9). Extensive volcanic activity (13) and water on and below the surface (4) would have inevitably led to the presence of hydrothermal systems (12, 6). The inhospitable nature of the surface of Mars and the possibility of more amenable habitats in the subsurface require serious consideration of ecosystems and energy sources which are not dependant on sunlight as potential oases for life. Hydrothermal systems driven by tidal heating on Europa could also result in potential habitable zones of liquid water in the subsurface environments far beneath the frozen crust (11, 5), and well beyond the reach of sunlight.

Organisms living independent of sunlight require the availability of other energy sources to survive. The chemotrophic organisms in hydrothermal systems on Earth are an excellent example of the intricate relationship between biological and geochemical processes. These thermophilic organisms thrive on the chemical energy and mineral precipitates created by water/rock reactions and the cooling or mixing of hydrothermal fluids, processes that often result in chemical systems out of equilibrium (10). A model ecosystem for Mars or Europa, one necessitating operation independent of sunlight, would require the consideration of chemosynthetic life as candidate occupants. It is logical to look first to the hydrothermal environments on Earth as working templates for astrobiological models.

One ideal place to study chemotrophic thermophiles is Yellowstone National Park, U.S.A. The hot springs, mudpots, and geysers in Yellowstone have yielded a host of cultured thermophilic organisms from both the Bacterial and Archaeal domains. However, these organisms are seldom considered in the context of their geochemical

environments. Extreme ecosystems on Earth can only be useful as templates for astrobiological ecosystems if the environments are characterized along with the organisms that inhabit them.

In an effort to characterize thermophilic organisms in the context of their geochemical environments, we have chosen the Greater Obsidian Pool Area (GOPA), Yellowstone National Park, as our study area. One major hydrothermal feature at GOPA, Obsidian Pool, is already well-known for its great diversity of thermophilic organisms determined by culture independent studies (1, 2, 8). These studies revealed 86 separate 16S rRNA signatures, few of which are represented by pure cultures (3, 7). GOPA is a thermal area covering 1 km^2 that is composed of 7 "major" hot spring features and a great number of equally interesting "minor" features and channels. We have completed extensive analyses of geochemical samples taken in the field in 1999 and 2000 from hydrothermal environments in this area. These springs vary widely in fluid chemistry and physical characteristics, providing a plethora of microbial habitats in one small study area. For example, within the thermophilic environments, pH ranges from 2.1-7.9, and temperature ranges from 70-89 ºC.

In conjunction with defining the geochemistry at GOPA, we are also culturing thermophiles from these environments so that organisms representative of these thermophilic communities can be studied in the laboratory. Unlike most traditional growth studies, we used aqueous growth media based on our geochemical data, so culturing conditions realistically represent the natural environment. Using this method, it is possible to design growth media for specific sample locations. Also, the most energetically favorable chemotrophic reactions were calculated for many sample areas, and nutrients were added to the media to target specific metabolic pathways. To date, we have obtained 38 mixed cultures from GOPA using our location-specific media, and two thermophilic Bacteria have been isolated from these cultures using a serial dilution method. We are currently characterizing these Bacteria for publication, and efforts are underway to isolate more organisms from our mixed cultures. Our success with culturing thermophiles from these environments reflects the importance of accurately representing the geochemical environment in culturing studies. Because these isolates were obtained in the context of their natural environment, we are able to link them directly to the geochemical processes which govern these hydrothermal ecosystems.

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Potential Evaporite Biomarkers from the Dead Sea

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The Dead Sea is located on the northern branch of the African-Levant Rift systems. The rift system, according to one model, was formed by a series of strike slip faults, initially forming approximately 2 million years ago (1). The Dead Sea is an evaporite basin that receives freshwater from springs and from the Jordan River. The Dead Sea is different from other evaporite basins, such as the Great Salt Lake, in that it possesses high concentrations of magnesium and has an average pH of 6.1. The dominant cation in the Great Salt Lake is sodium, and the pH is 7.7. Calcium concentrations are also higher in the Dead Sea than in the Great Salt Lake (2). Both basins are similar in that the dominant anion is chlorine and the salinity levels are approximately 200‰. Other common cations that have been identified from the waters of the Dead Sea and the Great Salt Lake include sodium and potassium (1).

A variety of Archea, Bacteria, and a single genus of a green algal, *Dunaliella,* has been described from the Dead Sea (3). Earlier studies concentrated on micobial identification and analysis of their unique physiology that allows them to survive in this type of extreme environment. Potential microbial fossilization processes, microbial fossils, and the metallic ions associated with fossilization have not been studied thoroughly. The present study is restricted to identifying probable microbial morphologies and associated metallic ions.

XRD analysis indicates the presence of halite, quartz, and orthoclase feldspar. In addition to these minerals, other workers have reported potassium chloride, magnesium bromide, magnesium chloride, calcium chloride, and calcium sulfate (3,4). Halite, calcium sulfate, and orthoclase were examined in this report for the presence of microbes, microbially induced deposits or microbial alteration.

Neither the gypsum nor the orthoclase surfaces possesses any obvious indications of microbial life or fossilization. The sand-sized orthoclase particles are weathered with

extensive fan-shaped mineral deposits. (Fig. 1). The gypsum deposits are associated with halite minerals and also exhibit extensive weathering.

Halite minerals represent the only substrates that have probable rod-shaped microbial structures with long, filamentous, apical extensions. EDS analysis of the putative microbes indicates elevated calcium levels that are enriched with magnesium.

The rod-shaped structures exhibit possible fossilization stages (Fig. 2,3). Rhombohedral-shaped minerals of magnesium-enriched calcium carbonate are deposited on the microbial surfaces, and eventually coat the entire microbial surface. The sodium chloride continues to crystallize on nearby halite surface and even crystallizes on the fossilized microbial remains.

The putative fossils are found exclusively on halite surfaces, and all contained elevated levels of calcium magnesium cations. Both of these metallic cations are associated with microbial activity and fossilization (6,7). Their morphological diversity is low in comparison with the reported living Dead Sea microbial population. If we examine the fossil record for multicellular organisms, fossilization rates are lower for soft-bodied organisms than for those possessing hard parts, i.e. shells, bones. For example, smaller, single celled organisms would have a smaller chance of fossilization; their fossilized shapes could be mistaken for abiotic products. Another consideration is that dead organisms in the water column are probably utilized as a food source by other microbes before fossilization processes are completed. This may be an important consideration as we attempt to model and interpret ancient microbial environments either on Earth or on Mars.

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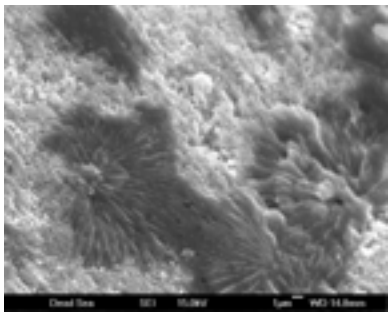

Figure 1. Fan-shaped mineral deposits on weathered orthoclase. Elevated levels of Mg are present.

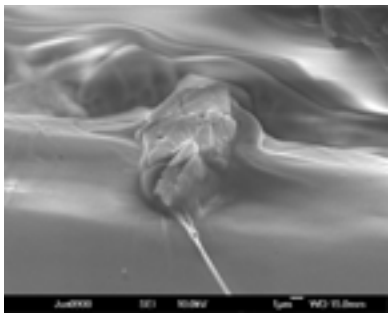

Figure 2. Rod-shaped (?)microbes on halite surfaces. Structures possess elevated Ca enriched Mg.

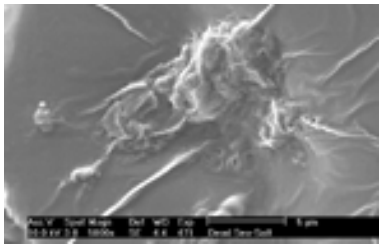

Figure 3. (?)Microbial fossilized surface. Structures possess elevated Ca enriched with Mg.

Amino Acid Analyses of Acid Hydrolysates in Desert Varnish

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There has long been a debate as to whether rock varnish deposits are microbially mediated or are deposited by inorganic processes. Varnished rocks are found throughout the world primarily in arid and semi-arid regions. The varnish coats are typically up to 200 μm thick and are composed of clays and alternating layers enriched in manganese and iron oxides. The individual layers range in thickness from 1 μm to >10 μm and may continue laterally for more than a 100μm. Overlapping botryoidal structures are visible in thin section and scanning electron micrographs. The coatings also include small amounts of organic mater and detrital grains. Amino-acid hydrolysates offer a means of assessing the organic composition of rock varnish collected from the Sonoran Desert, near Phoenix, AZ. Chromatographic analyses of hydrolysates from powdered samples of rock varnish suggest that the interior of rock varnish is relatively enriched in amino acids and specifically in d-alanine and glutamic acid. Peptidoglycan (murein) is the main structural component of gram-positive bacterial cell walls. The d-enantiomer of alanine and glutamic acid are specific to peptidoglycan and are consequently an indicator for the presence of bacteria. D-alanine is also found in teichoic acid which is only found in gram-positive bacteria. Several researchers have cultured bacteria from the surface of rock varnish and most have been gram-positive, suggesting that gram-positive bacteria are intimately associated with varnish coatings and may play a role in the formation of varnish coatings.

Visible-Near Infrared Spectroscopy of Siliceous Sinter, Yellowstone National Park - Search for Organic Signatures

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Widespread volcanism and fluvial activity during the Noachian and Hesperian periods of Mars suggest that volcanic hydrothermal systems, having surface expressions similar to those in Yellowstone National Park, Iceland or New Zealand may have occurred. The apparent absence of widespread carbonates on Mars suggests such martian hydrothermal systems would be dominated by silica. Given that hyperthermophiles are among the most primitive terrestrial organisms [1], it has been suggested that hyperthermophiles might have developed on Mars as well [2]. Therefore, an examination of terrestrial hydrothermal silica deposits, to determine the extent to which they preserve detectable evidence of the associated hyperthermophile organisms, is relevant to Mars exploration.

Several studies have examined siliceous and carbonate sinter for biologic indicators. Macroscopic fossils are rare, but SEM images reveal a diversity of features due to biologic activity [3]. Since spectral data - visible to near infrared reflectance (VNIR) and thermal emission - will be key to site selection and analysis, we have examined siliceous sinter in Yellowstone National Park to determine the extent to which biotic signatures are preserved. We have also collected VNIR data of the hyperthermophile organisms to characterize their spectral properties (see accompanying abstract).

VNIR data of sinter at Octopus Spring, Mushroom Spring, and several geysers in the Old Faithful area were collected using an ASD Field SpecFr spectrometer under both natural and artificial illumination. Octopus and Mushroom Springs are alkaline with extensive hyperthermophile mats dominated by *Thermocrinis ruber* at high temperature [4], *Synechococcus lividus-Chloroflexus aurantiacus* at T<73-74°C and *Mastigocladus laminosus* at T< 63-64°C [5]. Visible hyperthermophile populations are absent at Spa, Spiteful and Bulger Geysers and these therefore serve as a control of the sinter spectra.
Spectra for Spa, Spiteful and Bulger (Fig. 1) geyserite is typical of amorphous silica (e.g., opal and chalcedony); reflectance rises from the visible into the near-infrared with the steepest slopes at <600 nm. Maximum reflectance occurs at 1000-1250 nm beyond which reflectance decreases. Major absorptions at 1415, 1910 and 2275 nm and minor absorptions at ~970 and 1160 nm are due to bound water. Other significant absorptions are not noted.

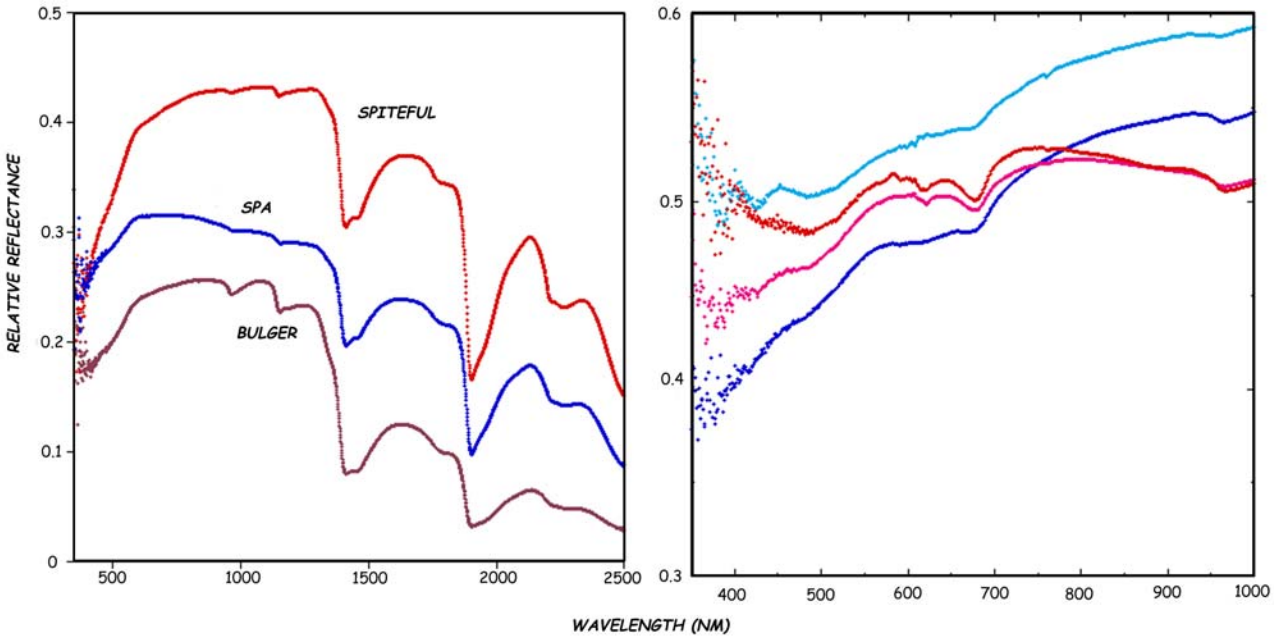

Fig. 1. Average spectra of geyserite from Spiteful, Spa and Bulger Geyser cones.

Fig 2. Spectra for sinter deposits at Octopus (blue) and Mushroom (red) Springs.

Spectra for sinter deposits at Mushroom and Octopus Springs, however, display several minor, but significant visible wavelength absorptions that are unrelated to silica. Fig. 2 illustrates spectra in the 350 -1000 nm range for sinter from Mushroom and Octopus Springs. The prominent absorption at 680 nm is due to chlorophyll *a*. Additional minor absorptions occur at ~590, 610, 620 (all may be phycobiliproteins) and at ~760 nm. These absorptions may reflect organics and occur at positions similar to absorptions of the hyperthermophile organisms. These data suggest that signatures of biotic processes can be detected in siliceous sinter deposited in association with hyperthermophile organisms.

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Visible-Near Infrared Spectroscopy of Hyperthermophile Organisms, Yellowstone National Park

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Hyperthermophile organisms have been suggested to lie at the base of the tree of life and may represent the earliest organisms on the Earth. By analogy, hyperthermophilic organisms may have developed on Mars [1]. From an exploration standpoint, the question is whether such organisms (living or dead and entombed in sinter) could be detected in spectral data. We collected Visible-Near Infrared Reflectance (VNIR) data at springs in Yellowstone National Park to characterize the reflectance spectra of living hyperthermophile organisms. Data for Octopus Spring and Nymph Creek are presented here. *In situ* (350-2500 nm) data were collected using an ASD Field SpecFr spectrometer under both natural and artificial illumination. Octopus Spring is alkaline (pH 8.0, T 88°C) with temperature-dependent biotic mats lining the effluent channels [2]. Pink filamentous *Thermocrinis ruber* occurs at high temperature [3], green *Synechococcus lividus*-*Chloroflexus aurantiacus* at T< 73-74°C and greenish-orange *Mastigocladus laminosus* at T< 63-64°C. Nymph Creek is acidic (pH 2.9, T 60°C) and the primary organism is *Cyanidium caldarium* [4].

Despite being acquired through several cm of water, the data indicate the algae and bacteria exhibit unique spectral features. Figure 1a shows spectra for *Cyanidium caldarium* from Nymph Creek. It has a green peak at 542 nm, an otherwise flat visible spectra, a "red edge" at 710 nm and an absorption at 878 nm. *Mastigocladus laminosus*, from Octopus Spring, (Fig. 1b) has maxima at 590 and 648 nm, chlorophyll *a* absorption (675 nm), and absorptions at 740 (bacteriochlorophyll *c*), 798, and 874 nm. Spectra of extracted *Synechococcus* and *Chloroflexu*s are in Fig. 2a and *in situ* spectra of the mat in Fig. 2b. *Synechococcus lividus* has a chlorophyll *a* absorption, red edge at 765 nm and absorptions at 716, 798 and 877 nm. For the in situ observations, the red color of the Chloroflexus is subdued as it lies below the *Synechococcus*. The general character of the spectra are similar to that obtained from *in vivo* studies by [5], although those data were collected with lower spectral resolution.

These data indicate that each hyperthermophile organism has unique spectral characteristics and these can be used to separate the organism in the field with *in situ* data. This provides a basis for quick evaluation of such environments in Yellowstone (and elsewhere) and a basis for understanding how such organisms (most likely in fossil form) might be recognized on Mars. The next step will be the analysis of sinter deposits to assess the extent that incorporated biota [5] retain their spectral signature.

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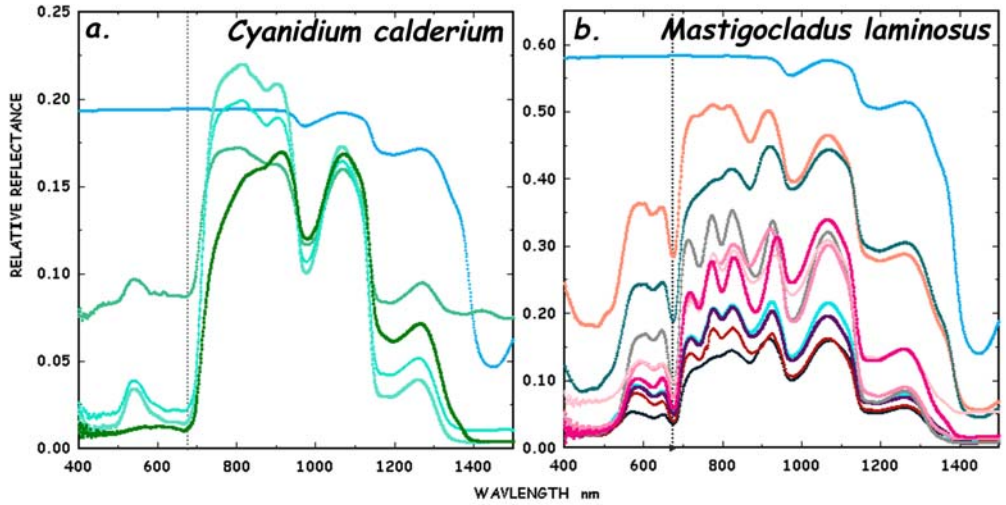

Figure 1a. *Cyanidium caldarium* - Nymph Creek. b. *Mastigocladus laminosus* - Octopus Spring. Aqua blue line is water spectra Vertical dashed line denotes Chl *a* absorption.

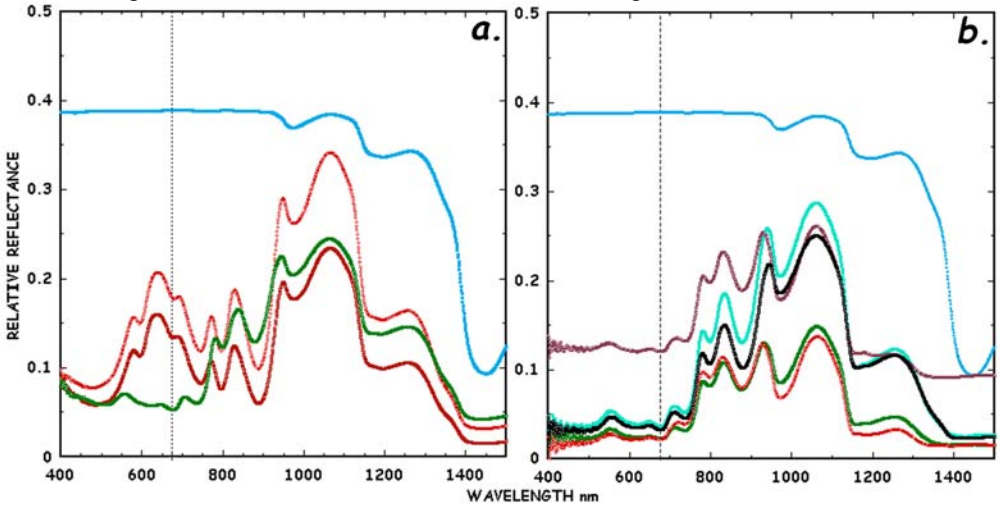

Figure 2a. Extracted *Synechococcus* - green lines; *Chloroflexus* - red lines. (b) *In situ Synechococcus-Chloroflexus* mat. Vertical dash and aqua blue lines as above.

How Cyanobacterial Distributions Reveal Flow and Irradiance Conditions of Photosynthetic Biofilm Formation

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Microbial life on Earth is enormously abundant at sediment-water interfaces. The fossil record in fact contains abundant evidence of the preservation of life on such surfaces. It is therefore critical to our interpretation of early Earth history, and potentially to history of life on other planets, to be able to recognize life forms at these interfaces. On Earth this life often occurs as organized structures of microbes and their extracellular exudates known as biofilms. When such biofilms occur in areas receiving sunlight photosynthetic biofilms are the dominant form in natural ecosystems due to selective advantage inherent in their ability to utilize solar energy.

Cyanobacteria are the dominant phototrophic microbes in most modern and ancient photosynthetic biofilms, microbial mats and stromatolites. Due to their long (3.5 billion year) evolutionary history, this group has extensively diversified resulting in an enormous array of morphologies and physiological abilities. This enormous diversity and specialization results in very specific selection for a particular cyanobacterium in each available photosynthetic niche. Furthermore these organisms can alter their spatial orientation, cell morphology, pigmentation and associations with heterotrophic organisms in order to fine tune their optimization to a given micro-niche. These adaptations can be detected, and if adequate knowledge of the interaction between environmental conditions and organism response is available, the detectable organism response can be used to infer the environmental conditions causing that response.

This presentation will detail two specific examples which illustrate this point. Light and water are essential to photosynthesis in cyanobacteria and these organisms have specific detectable behavioural responses to these parameters. We will present cyanobacterial responses to quantified flow and irradiance to demonstrate the interpretative power of distribution and orientation information.

This study presents new results, but many such examples are already found in the literature. However this information exists in such a wide variety of journals, spanning decades of research that the utility of the vast storehouse of information is limited, not by the ability of cyanobacteria to respond in recognizable ways to environmental stimuli, but by our ability to compile and use this information. Recent advances in information technology will soon allow us to overcome these difficulties and utilize the detailed responses of cyanobacteria to environmental microniches as powerful records of the interaction between the biosphere and lithosphere.

Use of the $\delta^{13}C$ Associated With Amino Acid Biosynthesis as a Proxy for Examining the Flow of Carbon Through Biological Systems

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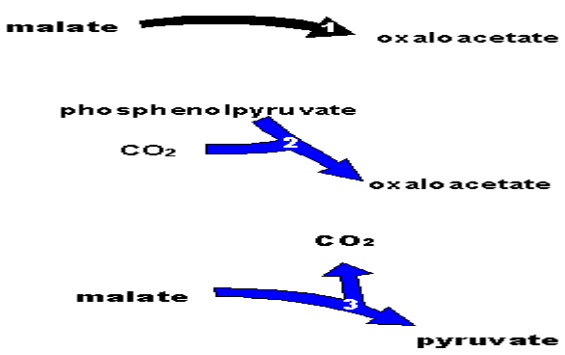
By examining the $\delta^{13}C$ associated with specific amino acids it is possible to follow the flow of carbon through the intermediary metabolism of various microbes. We have examined how the pathways associated with methanogenesis, methylotrophy, and heterotrophy effect the $\delta^{13}C$ of specific amino acids. Furthermore our work indicates that key amino acids are proxies for essential intermediates in the central metabolism of microorganisms. By using aspartic acid, glutamic acid and alanine as proxies for essential central metabolic intermediates (oxaloacetate, 2-oxoglutarate and pyruvate) we are able to examine the flow of carbon through various metabolic pathways. For example, every cell must have a pool of oxaloacetate and pyruvate to grow. Our work indicates that $\Delta(\delta^{13}C_{\mathbf{ASP}} - \delta^{13}C_{\mathbf{ALA}})$ (Table 1) provides a powerful indicator of how these two pools are replenished. The action of PEP carboxylase in *Escherichia coli* grown anaerobically on pyruvate, *Shewanella oneidensis* MR1 grown with nitrate and fumurate as electron acceptors led to an average Δ = -2.39 ± 0.20 ppm. This clearly indicates that in these bacteria during anaerobiosis oxaloacetate is formed by the carboxylation of pyruvate (or PEP). In contrast, methylotrophs and *E. coli* growing on acetate yields a Δ = 2.67 ± 1.04 ppm. In methanogens the difference in Δ is even more dramatic (see Table 1). In the case of methylotrophy, methanogenesis and *E. coli* growing on acetate, pyruvate is formed from oxaloacetate (or malate) through a wide range of enzymes (see Figure 1). The next step will be to use our observations on pure microbial cultures to examine the flow of carbon in complex biological communities.

Table 1

Organism	$\Delta(\delta^{13}C$**asp**$-\delta^{13}C$**ala**$)$
Malate dehydrogenase(oxaloacetate is formed from malate)	
***Escherichia coli* (aerobically with pyruvate)**	0.56
Malate synthase (pyruvate is formed from oxaloacetate via malate)	
***E. coli* (aerobically with acetate)**	2.45
***Methylobacterium extorquens* AM1(Methane)**	3.8
***Methylobacterium extorquens* AM1(Methanol)**	1.75
Acetyl CoA pathway (pyruvate is formed from oxaloacetate)	
Methanobacterium (CO2 +H2)	5.03
Methanosarcina (acetate)	6.64
PEP carboxylase (oxaloacetate is formed from phosphoenolpyruvate(or pyruvate))	
E. coli (anaerobically with pyruvate)	-2.57
Shewanella oneidensis (anaerobically w/ lactate and fumarate)	-2.44
Shewanella oneidensis (anaerobically w/ lactate and nitrate)	-2.17

Figure 1



Pyruvate aerobic $\Delta\delta^{13}C_{asp}$ = 0.56
(complete TCA; (1)malate dehydrogenase)

anaerobic $\Delta\delta^{13}C_{asp}$ = -2.57
(incomplete TCA; (2)PEP carboxylase)

Acetate $\Delta\delta^{13}C_{asp}$= 2.45
(glyoxlate shunt; pyruvate is formed from malate through the (3)malic enzyme)

Observations on Microbial Metabolism at Extreme Pressures!

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One of the largest hurdles in the area of microbial ecology is the inability to study a wide range of physical and chemical gradients that have an impact on the growth and viability of living organisms. For example, little is known about the effect that the phase changes associated with hydrothermal conditions has on metabolic processes. We are developing techniques for monitoring metabolic responses at extreme conditions. Here we present some of the preliminary results towards the quantification of in-situ interrogation of metabolism of microbes at high pressures and temperatures. These results show that metabolic activity remains viable under surprisingly extreme physical and chemical conditions. These techniques and instrumentation are aimed at developing a collaborative facility for testing the viability of life in various terrestrial and non-terrestrial environments.

Molecular Survey of Microbial Diversity in Hypersaline Ecosystems

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The hypersaline microbial mats of Guerrero Negro, Baja California Sur, are unique ecosystems that have long been studied for their unique chemistry. Correlations of biological and chemical information are significantly restricted, however, because there is not a comprehensive survey of the specific kinds of organisms that comprise these hypersaline mats. The overarching goal of this work is to describe and understand the organismal composition, structure and physiology of a selected hypersaline microbial ecosystem using ribosomal 16S RNA gene sequences. The traditional reliance on pure culture techniques to describe microbiota is circumvented by this molecular approach. It is estimated that > 99% of naturally occurring microorganisms are not cultured using standard techniques.

As participants in the Ecogenomics focus group within NASA's Astrobiology Institute we are conducting a comprehensive survey to determine the phylotypes and relative abundance of organisms present in these hypersaline microbial mats. A description of the microbiota that occurs in this environment through their molecular phylotypes will provide us with an understanding of microbial complexity and put in place a biological context in which to understand the chemical processes of these ecosystems. Organisms of these kinds might be expected to be found in other hypersaline environments.

Representatives of all three domains of life, Bacteria, Archaea, and Eucarya will be characterized in several layers of the mats, based upon determined chemistry. We are currently studying representative samples obtained from *ex situ* Guerrero Negro microbial mats maintained at NASA Ames in a greenhouse, as well as samples recently obtained on site. We intend to explore several selected sites (4-6) at relatively high resolution, approximately 1000 cloned rRNA genes per site, to gain a comprehensive view of the communities.

Information from this molecular analysis will be crucial for further definition and delineation of the chemistries that occur within the mats. This work will advance substantially our understanding of the kinds and activities or organisms that comprise hypersaline microbial mats, and open new avenues for future investigations.

Determining Biosignatures by Complexity Analysis in Antarctic Cryptoendolithic Communities

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One of the most difficult problems of life detection is that of identifying biosignatures across a wide range of scales using multiple co-registered probes. The technique should be of equal utility across a wide range of search spaces from remote sensors probing volumes of space or planetary surfaces, visual eye or camera searches across the surface of a rock in Antartica, low resolution microscopic scanning of a rock or a space craft in situ, or high resolution electron microscope and computerized tomography scanning of geobiological samples.

We describe here an approach to this problem which derives in large part from past work done in the area of astrophysics - namely the analysis of complexity in galactic signals by data compression methods. This approach is a radically new one for geobiology and astrobiology, and allows us to assess the complexity (and thus potential biogenicity) of an object being examined. This is done by considering the information within pixels of an image (regardless the sensor used to gather the information) as an energetic system capable of description in terms of classical thermodynamics. The image data space is searched by an algorithm that judges complexity via data compression (e.g., the more compressible it is, the less complex, and vice versa) and maximum entropy as originally outlined by Shannon. At present we are implementing methods to utilize images from multiple sensors gathering different kinds of information (e.g., visible gray-scale data, color analyses, UV fluorescence, chemical information, etc). We present here preliminary data from deep UV fluorescence and ESEM images from a layered cryptoendolithic community of an Antarctic rock.

Deep UV Native Fluorescence Imaging of Antarctic Cryptoendolithic Communities

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An interdisciplinary team at the Jet Propulsion Laboratory Center for Life Detection has embarked on a project to provide *in situ* chemical and morphological characterization of Antarctic cryptoendolithic microbial communities. We present here *in situ* deep ultraviolet (UV) native fluorescence and environmental scanning electron microscopy images transiting 8.5 mm into a sandstone sample from the Antarctic Dry Valleys. The deep ultraviolet imaging system employs 224.3, 248.6, and 325 nm lasers to elicit differential fluorescence and resonance Raman responses from biomolecules and minerals. The 224.3 and 248.6 nm lasers elicit a fluorescence response from the aromatic amino and nucleic acids. Excitation at 325 nm may elicit activity from a variety of biomolecules, but is more likely to elicit mineral fluorescence. The resultant fluorescence images provide *in situ* chemical and morphological maps of microorganisms and the associated organic matrix. Visible broadband reflectance images provide orientation against the mineral background. Environmental scanning electron micrographs provided detailed morphological information.

The technique has made possible the construction of detailed fluorescent maps extending from the surface of an Antarctic sandstone sample to a depth of 8.5 mm. The images detect no evidence of microbial life in the superficial 0.2 mm crustal layer. The black lichen component between 0.3 and 0.5 mm deep absorbs all wavelengths of both laser and broadband illumination. Filamentous deep ultraviolet native fluorescent activity dominates in the white layer between 0.6 mm and 5.0 mm from the surface. These filamentous forms are fungi that continue into the red (iron-rich) region of the sample extending from 5.0 to 8.5 mm. Using differential image subtraction techniques it is possible to identify fungal nuclei. The ultraviolet response is markedly attenuated in this region, apparently from the absorption of ultraviolet light by iron-rich particles coating the filaments. Below 8.5 mm the filamentous morphology of the upper layers gives way to punctate 1-2 micron particles evidencing fluorescent activity following excitation at both deep ultraviolet wavelengths.

Novel Archaea in Guaymas Basin Hydrothermal Vent Sediments: Evidence for Anaerobic Methanotrophy

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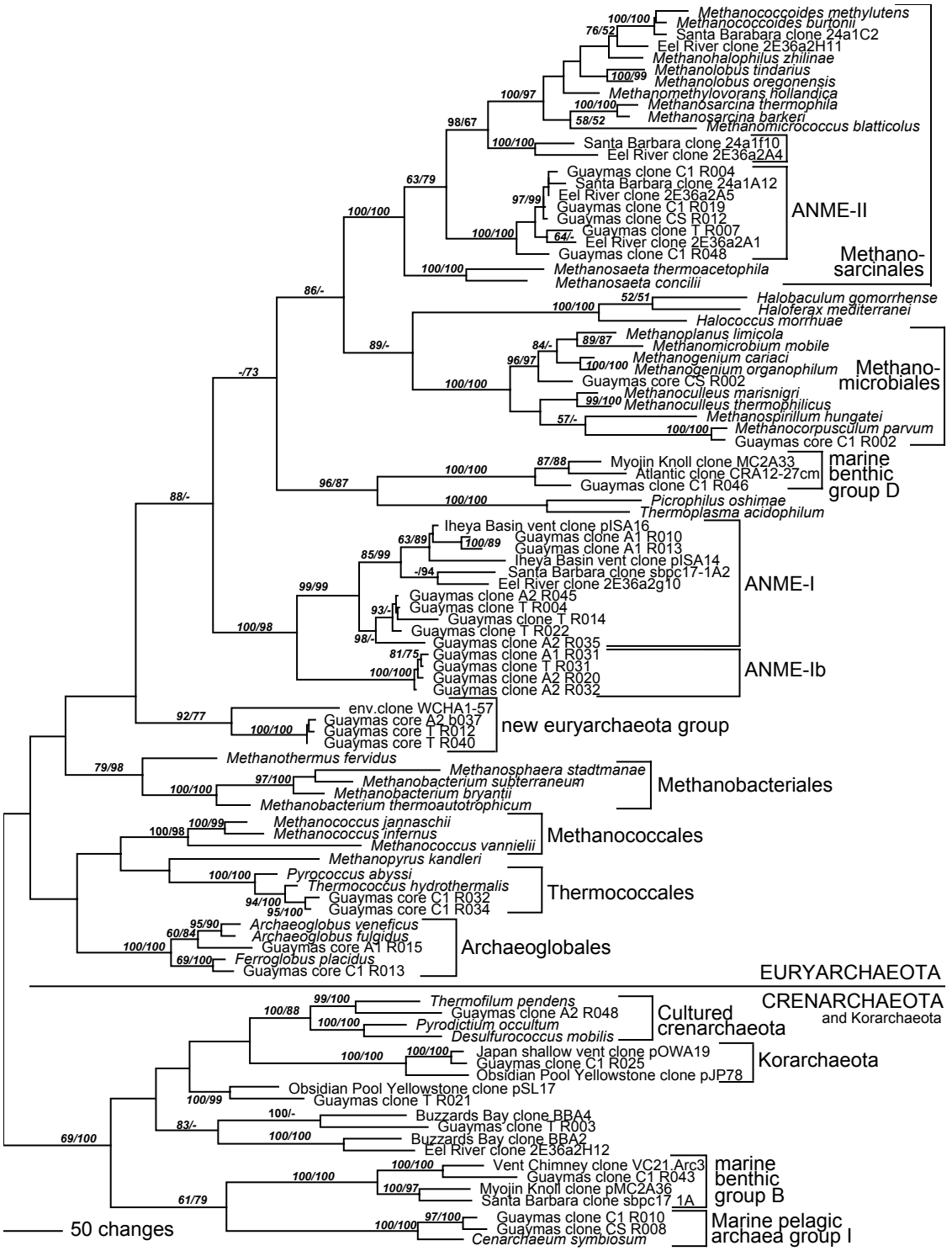
Microbial communities in hydrothermal sediments of the Guaymas Basin (Gulf of California, Mexico) were analyzed by cloning and sequencing of PCR-amplified 16S rRNA genes from environmental DNA, and by stable carbon isotope analysis of archaeal and bacterial lipids extracted from the same samples. In this way, the phylogenetic composition of the microbial community can be correlated to carbon assimilation pathways that are reflected in the isotopic composition of diagnostic lipids. The Guaymas sediments contained a major lineage of methanogen-related, uncultured archaea (ANME-1 and 1b cluster), and of uncultured members of the Methanosarcinales, the acetoclastic and methyl-disproportionating methanogens (ANME-2 cluster). Sequences of the euryarchaeotal ANME-1, ANME-1b and ANME-2 clusters dominated the archaeal 16S rDNA clone libraries of the Guaymas Basin; crenarchaeotal sequences were rarely found. The sequence profiles were congruent with lipid profiles. Predominant archaeal lipids in the Guaymas Basin sediments included archaeol, diagnostic for non-thermophilic euryarchaota, and sn-2-hydroxyarchaeol, diagnostic for members of the Methanosarcinales. These lipids were extremely ^{13}C depleted ($\partial^{13}C$ = - 80 to - 60 ‰), indicating that they originated from anaerobic methanotrophic archaea that presumably oxidized and assimilated ^{13}C-depleted methane, which is abundant in Guaymas Basin vent fluids. Archaeal populations with this lipid and sequence signature are widespread in cold methane seeps and anoxic marine sediments (for example Eel River Basin, Santa Barbara Basin), indicating that they are of general biogeochemical relevance.

The Guaymas Basin sediments harbored highly diverse bacterial populations, including gamma, delta, and epsilon-Proteobacteria, green non-sulfur bacteria, and members of the uncultured candidate subdivision OP11. Bacteria can consume hydrogen and acetate, the reaction products of archaeal methane oxidation, and thus make this process thermodynamically feasible; delta-Proteobacterial sulfate reducers are good candidates. With the exception of sulfur-oxidizing gamma and epsilon Proteobacteria, the bacterial community in the Guaymas sediments is anaerobic. Related bacterial species were found at other vent sites, terrestrial hot springs such as Obsidian Pool in Yellowstone, in anoxic marine sediments, hydrocarbon seeps and contaminated oil spill sites. Most likely, bacteria and

archaea together constitute an anaerobic, methane-cycling microbial consortium in the Guaymas Basin sediments, and provide a model system for microbial carbon cycling in anoxic extraterrestrial habitats and subsurface environments.



16S rRNA distance tree of Guaymas Basin Archaea, with distance and parsimony bootstrap values of 1000 replicates. The ANME-1, ANME-1b and ANME-2 clusters contain 75-85% of all 16S rDNA clones and are represented by selected sequences only.

"*Archaean Park*": A Research Project on Interaction Between Sub-Vent Biosphere and Geo-Environment

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A multi-disciplinary research project on sub-vent biosphere *Archaean Park* started in 2000 as a five-year program of Special Coordination Fund of the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan. The key research gear of the project is a tethered submarine drilling rig (BMS; Benthic Multicoring System) of Metal Mining Agency of Japan to do direct sampling of micro-organisms, rock cores and fluids from seafloor hydrothermal sites. The drilled holes will be approached by ROV and manned submersible to install various long-term monitoring and in-situ measurement instruments to delineate the system response to the geo-environmental change in temperature, fluid flow, and chemistry of the fluid. Extensive acoustic, seismic and electromagneic surveys will be conducted to map and explore the lateral extent and the structure of the biosphere beneath the vents.

Two target sites are located at western and eastern Pacific; that is, Suiyo Seamount caldera (D=1380m, Tmax=317°C) in Izu-Bonin Arc and the NOAA's NeMO Observatory site at Axial Seamount Caldera (D=1590m, Tmax=330°C) in the Juan de Fuca (JdF) Ridge. This is because the generation, evolution, and dispersion of the sub-vent biosphere will be best described if we compare long-lived mid-ocean ridge system with short-lived (<1Ma), sporadic hydrothermal system developed in independent edifices of volcanic arc.

A hydrothermal system is the "window" of the mostly unknown fluid flow system within the oceanic crust and mantle. The deeper portion of the system is likely to be hot and

strongly-reducing due to hydrogen, methane and hydrogen sulfide gases dissolved in the fluid. Such environment is analogous to the site for the origin and early evolution of life in Archaean age, since most of Domain *Bacteria* and Domain *Archaea* near the root of the Universal Tree are hyper-thermophilic. Direct drilling may lead us to find *Our Common Ancestor* which may have survived in least evolved form in such an environment *Archaean Park*. This is also a site to explore the temperature limit for the survival of micro-organisms. We can approach such environment within the depth limit (20 m) of the BMS from seafloor in high-temperature vent areas.

The upper part of the circulation system will become oxidizing due to invasion of oxygenated seawater. Supply of free oxygen to the metabolic cycle of micro-organisms such as sulfur-oxidizing and methane-oxidizing bacteria will greatly enhance their growth rate. Therefore, exploration of the oxidation/reduction front is essential to estimate the total biomass which is produced by chemo-synthesis of endogenic energy and nutrients from the solid earth. We noticed through long-term seafloor monitoring at southern East Pacific Rise (EPR) that the earth and/or ocean tides act as pump to move fluid back and forth which will consequently modify the redox front from a plane to a wide zone within the oceanic crust (Urabe et al., 1999). The tide-induced fluctuation, together with more critical influence from geologic perturbation such as change in magmatic degassing and local stress shift will significantly affect the nature and life span of the sub-vent biosphere.

On the other hand, most significant influence from sub-vent biosphere to the earth is the supply of chemo-synthetic biomass to the ocean through hydrothermal plumes. Dominance of *Archaea* in Juan de Fuca Ridge (Moyer et al., 1998) shows clear contrast to its absence (and dominance of *Bacteria*) in vent fluids and hydrothermal plumes at southern East Pacific Rise system (Maruyama et al., 1998). This may be due to the difference in the mode of fluid discharge in these two areas; that is frequent event plumes at JdF (Baker, 1994) vs. chronic venting at EPR (Urabe et al., 1995). Such hypotheses should be tested through direct drilling into active vent sites of different settings.

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A Structural and Molecular Approach for the Study Biomarkers

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Investigation of the nucleation and growth of crystals in both abiotic and biotic systems is critical to seemingly diverse disciplines of geology, biology, environmental science, and astrobiology. While there are abundant studies devoted to the determination of the structure and composition of inorganic crystals, as well as to the development of thermodynamic and kinetic models, it is only recently that research efforts have been directed towards understanding mineralization in biological systems (*i.e.,* biomineralization). Biomineralization refers to the processes by which living organisms form inorganic solids.

Studies of the processes of biomineralization under low temperature aqueous conditions have focused primarily on magnetite forming bacteria and shell forming marine organisms. Many of the biological building materials consist of inorganic minerals (calcium carbonate, calcium phosphate, silica or iron oxide) intricately combined with organic polymers (like proteins). More recently, efforts have been undertaken to explore the nature of biological activities in ancient rocks. In the absence of well-preserved microorganisms or genetic material required for the polmerase chain reaction (PCR) method in molecular phylogenetic studies, using biominerals as biomarkers offers an alternative approach for the recognition of biogenic activity in both terrestrial and extraterretrial environments.

The primary driving force in biomineralization is the interaction between organic and inorganic phases. Thus, the investigation of the ultrastructure and the nature of reactions at the molecular level occurring at the interface between inorganic and organic phases is essential to understanding the processes leading to the nucleation and growth of crystals. It is recognized that crystal surfaces can serve as the substrate for the organization of organic molecules that lead to the formation of polymers and other complex organic

molecules, and in discussions of the origins of life, is referred to as organic synthesis on mineral surfaces. Furthermore, it is suggested that the interaction between mineral surfaces and simple organic molecules resulted in the formation of amino acids, RNA, and perhaps other more complex molecules such as proteins. On the other hand, in natural systems, it is recognized that functional groups on cell walls or membranes of microorganisms serve as sites of nucleation and crystallization.

The precise replication of biominerals with controlled structure, morphology, size and texture is not confined to higher organisms as it also occurs in primitive prokaryotic cells such as magnetotactic bacteria and cyanobacteria. This suggests that the principal strategies of biomineralization were established early on in the evolutionary history of organisms. It is critical, therefore, to search for common mechanisms within diverse biological systems. One such common factor is the capability for organization and self-assembly. Organic macromolecules such as proteins and lipids can aggregate and polymerize forming membranes or extracellular matrix. At the organic-inorganic interface, several factors such as lattice geometry, polarity, stereochemistry and topography may act in concert to control nucleation and growth of crystals. Although several models have been proposed that discuss the significance of these factors for biomineralization, no comprehensive experimental data are available. In contrast to crystallization in exclusively inorganic systems, the kinetics of reaction and structural relationships between organic and inorganic phases in biominerals or biomimetic material is poorly understood. For example, it is not clear if the concept of epitactic growth (geometrical matching of unit cells at the interface of a secondary crystal growing on a primary crystal) applies to organic-inorganic systems. In contrast to inorganic templates that often have a smooth and rigid surface that promotes epitactic growth, biological substrates are usually rough and result in a large degree of mismatch. It is apparent that factors controlling the reaction at the crystal-matrix interface are strongly dependent upon the nature of the substrate. Therefore, characterization of the assembled organic surface and surface structure of the inorganic phase is crucial to understanding the processes of biomineralization.

The focus of our research is the investigation of the processes leading to the nucleation and growth of crystals on both natural and synthetic systems through an interdisciplinary approach that integrates molecular biology, morphology and mineralogy using advanced preparation and analytical techniques. We have studied run-products, particularly magnetite, siderite and other carbonates, that resulted from extracellular biomineralization by extremophiles isolated from a variety of extreme environments ranging from permafrost to hydrothermal vent systems. The results of this study are critical to recognizing biomarkers in terrestrial and extraterrestrial environments.

Photoendolithic Ecosystems: Molecular Diversity and Structure

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The endolithic environment, comprised of the pore-spaces in rock, is a ubiquitous microbial habitat on Earth, and may harbor life elsewhere in the universe. Endolithic environments are a haven for life in some of the most severe hot and cold deserts on Earth, including the Mojave Desert of North America and the Ross Desert in Antarctica. Analogous endolithic environments must occur on Mars today, and may contain evidence of past or present life. Understanding the biological and physical dynamics of endolithic ecosystems on Earth informs the search for life on other bodies in the solar system. Photosynthetically driven microbial communities form biofilm-like photoendolithic ecosystems in the upper millimeters of rock, wherever it is exposed to sunlight and even trace amounts of water. Despite a global distribution, little is known about these ecosystems on Earth.

This study examines the microbial composition and community structure of photoendolithic ecosystems inhabiting a variety of rock-types in the central Rocky Mountains of North America. A specific aim of this research is to test the hypothesis that rock-type plays a significant role in determining the microbial composition and community structure of photoendolithic ecosystems. A general principle in microbial ecology, that communities are driven by the chemical and physical characteristics of their environment, is predicted to influence photoendolithic community composition by determining the availability of nutrients along with other physico-chemical variables.

Among the rock types examined in this study are sandstone, limestone, basalt, and granite. 16S and 18S ribosomal RNA sequence-based phylogenetic analysis of sandstone and limestone communities reveals a pattern in which phylogenetically related, but unique microbial constituents form similar kinds of communities in different types of rock. Attempts have been made to control for other physical characteristics thought to influence photoendolithic communities, including aspect, elevation, water availability, photosynthetically active radiation, and temperature. Preliminary results of this study support the hypothesis that rock-types significantly influence the microbial composition of photoendolithic communities.

The Changing Geochemical Environment of a Thermal Spring May Provide Clues to Environmental Conditions and Microbial Evolution on Mars

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The chemistry of a thermal spring in Yellowstone National Park has been monitored since June 1996. This spring was one of four springs examined in a previous study [1, 2]. Thermal springs were selected for these studies because they are modern analogs to environmental conditions on early Earth when life evolved. Any life that evolved on other planetary bodies may have developed under similar conditions. Therefore, understanding how the geochemistry in a thermal system changes and how those changes affect the microbial populations is vital to understanding how life evolved on Earth and perhaps on other planetary bodies. Herein, we present data on the changing geochemical environment of a thermal spring in Yellowstone National Park. McHale and Kieft present data on the microbial populations for the different geochemical environments in the pool.

The Roadside Springs in Yellowstone National Park were first studied in 1996 as part of a study on hydrogen peroxide cycling in thermal waters [1, 2]. Subsequent observations indicated that the spring geochemistry was changing in the iron-rich spring. In June 1996, the pool floor was covered with a thick layer of white sediments. Subsequent analysis of these sediments indicated high concentrations of arsenic, barium, chromium, strontium, and silica. Iron and sulfur were also present. The white sediments continued to dominate the system until late-1999 or early-2000. When the spring was revisited in June 2000, a thin layer of red sediments covered much of the pool floor. By October 2000, the red sediments covered the pool floor except around the vent where outgassing occurred. These red sediments contain lower concentrations of aluminum, barium, chromium, and strontium but higher concentrations of arsenic and iron than the white sediments.

These observed changes in pool sediments are the result of changing oxidation conditions within the pool (i.e. the pool changed from a reducing environment to an oxidizing environment). The changing oxidation conditions may reflect changes in the source water for the spring. An increase in the amount of shallow groundwater mixing with the deeper, thermal water may produce more oxidizing conditions. Alternatively, less shallow groundwater mixing with the deeper, thermal water may produce more reducing

conditions. These changes in the amount of shallow groundwater mixing with the deeper, thermal water could be due to wetter or drier climatic periods, respectively.

Changing water chemistry supports the theory of an increased amount of shallow groundwater mixing with deeper, thermal water. From June 1996 to June 2000, the average mid-day temperature dropped from 78.3°C to 68.5°C. This drop in temperature is suggestive of more cooler water mixing with the thermal water. Additionally, an increase in water pH (3.37 to 4.00) during this time is indicative of an increase in the amount of shallow groundwater mixing with the deeper water. Finally, the concentrations of most metal ions decreased during the 4-year period reaching minimum concentrations in October 2000. The primary source for dissolved metals is leaching from the rock by the hot, acidic thermal waters. Therefore, an increase in shallow groundwater in the source water would result in decreased concentrations of dissolved metals.

The changing geochemistry of this thermal spring suggests that thermal springs are dynamic environments. It is likely that thermal springs on Mars would also be dynamic environments where the spring geochemistry is influenced by changing groundwater levels and chemistry. Therefore, it is possible that cycling between oxidizing and reducing conditions may have occurred in thermal springs on Mars.

Large outflow channels on Mars suggest that Mars once supported water on its surface [3]. Furthermore, the chaotic terrain at the head of the outflow channels suggests that water discharge in these channels would have been episodic [4]. These discharges would have been similar to the floods from Lake Missoula in Eastern Washington. However, the water for the Martian floods probably came from an underground aquifer rather than a surface lake. The massive discharge from these channels would have drained the regional subsurface aquifer. This would have resulted in less shallow water to mix with the deeper, thermal waters that formed springs in the area. Therefore, the regional thermal springs would have been a reducing environment. As the aquifer recharged, these thermal springs would become more oxidizing as the influence from the subsurface aquifer increased. The time scale for this cycling would have been several thousands of years rather than several years as seen on Earth. Therefore, each event horizon would have been considerably thicker than the horizons seen in this thermal spring.

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Eukaryotic Diversity in an Acidic, Metal-Rich Environment: Spain's Tinto River

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With the discovery that life can thrive under extreme conditions have come microbial studies devoted to the exploration of the diversity of life present in different extreme environments. Such studies rarely focus on eukaryotic microbes. As a result, the list of prokaryotic organisms capable of growing at extreme temperatures, pH, or ionic strengths has expanded enormously, giving the impression that only prokaryotes are capable of adapting to harsh conditions. Spain's Tinto River, a high metal, acidic environment with a pH range of 1.7 - 2.5, exhibits an impressive level of biodiversity, which spans not only the prokaryotic domains (Bacteria and Archaea), but also the Eukaryotic domain of life.

The Tinto River gets its name from the red color of its waters made so by the high concentrations of ferric iron dissolved in the water. Equally impressive are the shades of brown, green and ochre along the river's edge which result from algal blooms contributing as much as 60% of the biomass in some cases. The Tinto River is a unique extreme system in that, at least in some regions, it is an environment dominated by eukaryotes. We present the results of a full-length, small-subunit ribosomal RNA based molecular characterization of several sampling stations along the Tinto River, including samples taken at the source of the river. Many of our clones showed high similarity to taxa reported in previous studies employing light-microscopical techniques. These included the highly conspicuous members of the Euglenozoa (euglenids), Chlorophyta (chlamydomonad and chlorella-like relatives), as well as the Bacillariophyta (diatoms).

However, our results also revealed a diversity of other protists not previously identified with conventional technologies. These included several members of the stramenopiles (chrysophytes, bicosoecids) and the alveolates (ciliates, dinoflagellate-relatives). In addition there were several representative cercomonad relatives, one representative heterolobosean amoeba-relative, and a few clones that did not reveal high similarities with any known taxa. Other members of the Viridiplantae were represented by streptophyte and charophyte relatives. Our study also revealed a diversity of fungi somewhat different than those previously identified in the river using traditional methods. The majority of our clones belonged to the Ascomycota but there were a few representatives from the Zygomycota. We summarize our results in a phylogenetic analysis.

The high level of eukaryotic diversity found associated with the low pH and high metal concentrations proves that adaptation to extreme conditions is much more widespread than originally expected.

Eukaryotic Diversity in Alkaline Lakes of the Sandhills Region of Nebraska

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Extreme alkaline areas are less frequently encountered in nature than extremely acidic ones. By definition, environments having pH values greater than 10.0 are considered extremely alkaline. In the United States, the lakes of the Sandhills Region of western Nebraska represent some of the most alkaline features available for investigation. Such alkaline areas are often characterized by poor drainage and high mineralization (sodium > potassium > magnesium > calcium) created by leaching and evaporation. Given the rarity of alkaline environments, one might expect to find unique assemblages of organisms inhabiting such locations. Studies focussing on microbial diversity of these environments, certainly eukaryotic microbial diversity, are lacking.

In order to assess the eukaryotic diversity at alkaline extremes, we collected sediment samples in several alkaline lakes in the Alkali Lakes region (the Sheridan and Garden Counties) of western Nebraska. These lakes are compositionally diverse and range in salinity from fresh to brine. Several of the lakes sampled were unnamed– most were located in the Crescent Lake National Wildlife Refuge. We used a rDNA-based approach and screened rDNA clone libraries to assess diversity.

Perhaps the most surprising aspect of the eukaryotic diversity in the lakes we examined was that a large part of this diversity was not microbial. Our preliminary rDNA data analyses show a large metazoan component to many of the alkaline communities. These included such groups as the annelids, platyhelminths, nematodes, gastrotrichs, insects, ostracods, copepods, priapulids, rotifers, and sponges. Members of the Viridiplantae and Fungi were also present. Chlorophytes, streptophytes, and embryophytes were represented in BLAST hits for the Viridiplantae while fungi included chytrids, ascomycetes, and basidiomycetes. A diverse array of protists was also found. These included members or relatives of the alveolates (such as ciliates, dinoflagellates, apicomplexans), as well as representatives of the stramenopiles – diatoms, oomycetes, chysophytes, and labyrinthulids. Others groups detected included euglenids, amoeboid protists, glaucocystophytes, choanoflagellates, and cercomonads. Additional sequencing and phylogenetic analyses will determine the novelty of these clones.