Microbial Ecology and Energetics in Yellowstone Hot Springs

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John Spear holds a large glass slide used as a growth surface for microbiota in a Hayden Valley-region hot spring (76°C). The slide is clear when first inserted into the hot spring, hung with fishing line. After three days, the slide is heavily colonized with microbiota—the darkly colored, thick biofilm that can be seen covering the slide. The material is collected with a sterile razor blade and used for the kind of culturing, microscopy, and molecular microbial analyses described in this article.

ANY ECOSYSTEM on Earth is comprised of all living things (plants, animals, etc.) and non-living things (rocks, soil, water, etc.) in a given geographic area. That area can be on the scale of a landscape, such as the Greater Yellowstone Ecosystem (GYE, multiple states in size), to the local area around a single hot spring, to the area within some minute distance between bacteria. There is a constant exchange of materials between the living and non-living components in any of these ecosystems. One of the most important of these exchanged materials is energy. When we walk around the GYE, the energy source for the richness of life we can see is apparent: photosynthesis, the capture of light energy from the sun into usable form. This capture is made possible by the fixation of the sun's photons into useful chemical energy by plant cell chloroplasts.

The living components of an ecosystem are composed of a blend of organisms we now know to occupy three domains of life. In the first of these three domains, members of *Eucarya* make up most of the world we see, including plants, animals, and fungi. Members of the other two domains, *Bacteria* and *Archaea,* are all microbial, and perform many ecosystem services, such as primary productivity, waste recycling, weathering, and mineralization. Microbial capture of the sun's energy by photosynthesis is conducted by algae, a group of microbial organisms within the domain *Eucarya,* and cyanobacteria, a group of organisms within the domain *Bacteria.* Components of the third domain of life, *Archaea,* are not known to engage in traditional photosynthesis at this time. Once photosynthesis converts light energy into biomass, many other organisms, including animals, then thrive by consuming this energy. Photosynthesis thus provides the energy foundation for our macrobial-visible, eukaryotic world.

Photosynthesis by bacteria is visible all over Yellowstone, in the form of the many colors in and around hot springs (Figure 1). A walk around the park reveals a multitude of colorful microbial mats (whole assemblages of microorganisms) living at various temperatures and pHs under different site-dependent chemical regimes. These colors are often the product of photosynthetic pigments within the microbial cells. The green, black, orange, brown, and yellow mats around Grand Prismatic Spring, or around the boardwalks of the Lower Geyser Basin, are examples of photosynthetic mats. These microbial mats form their own complex ecosystems, composed of mixed communities of microorganisms living together with a few

photosynthetic members supplying energy to others. However, while photosynthesis is visible at the plant and microbial mat level, it is not the only kind of energetic fixation mechanism.

Yellowstone, with its more than 10,000 thermal features, is full of life at temperatures that exceed the limit of photosynthesis (~72°C; 158°F) (Figure 1, bottom). Some form of microbial life, generally bacteria or archaea, probably occurs in all Yellowstone hot springs, many of which are at boiling temperature. A different kind of energy, a chemical energy mechanism, must be available for life to thrive at these high temperatures. To survive anywhere, life needs four things: water, a carbon source, an electron donor—something to provide electrons (energy)—and an electron acceptor. Humans consume water, carbon, and electron donors; we inhale oxygen to accept the electron transfer from the donors, and exhale waste in the form of CO_2. Microbes in a Yellowstone hot spring do the same, but instead use CO_2 or small organic molecules for carbon, hydrogen sulfide (H_2S) or molecular hydrogen (H_2) as an electron donor, and molecules such as oxygen (O_2), sulfate (SO_4^{2-}), phosphate (PO_4^{2-}), nitrate (NO_3^{2-}), or various metals as electron acceptors. The result is that microorganisms can thrive both on the walls and in the water of hot springs (Figure 2).

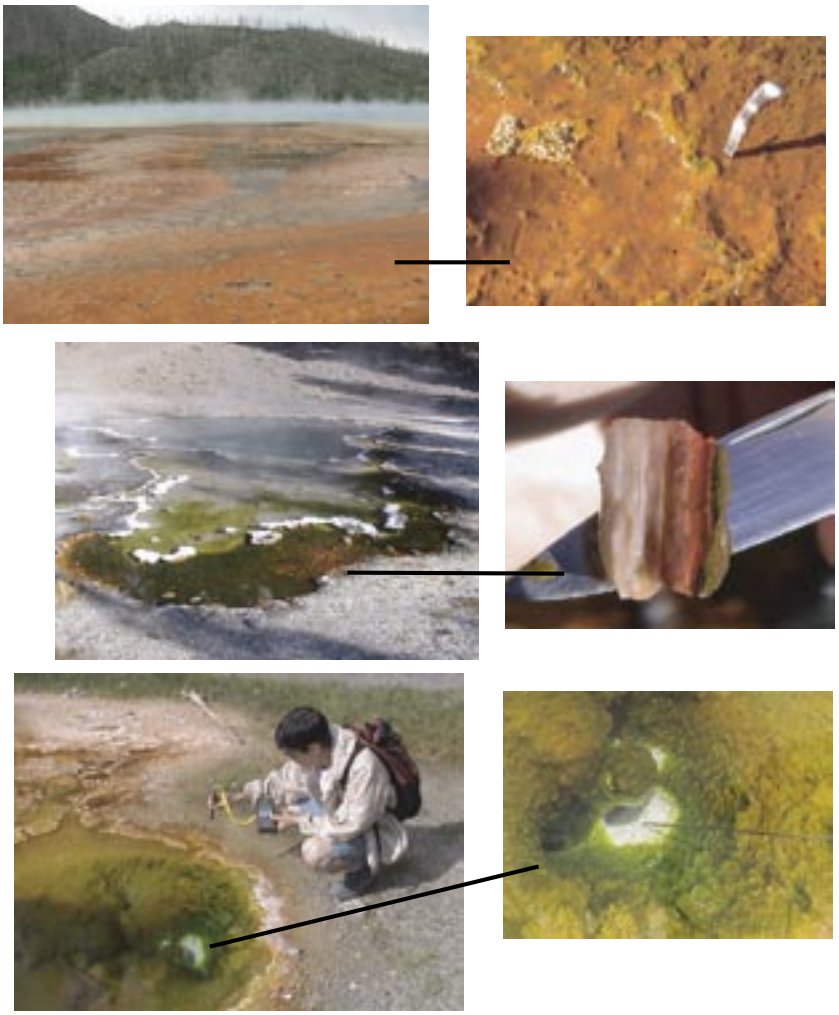

Figure 1. *Top:* Grand Prismatic Spring as viewed from the boardwalk. The presence of multiple colors around the edge of the world's largest hot spring are due to microbial mats. *Top right:* When viewed up close, oxygen bubbles from photosynthesis can be seen trapped within the multiple layers of the mat. *Middle:* Octopus Spring in the White Creek region, with colors imparted by microbial mats along its southern edge. *Middle right:* A close-up view of these beautifully laminated mats as seen on the blade of a pocket knife. *Bottom:* The green color of this hot spring in the White Creek region is due to the presence of photosynthetic microorganisms lining the walls of the spring. However, the photosynthetic temperature limit of ~72°C (158°F) is exceeded at the source vent, and a clear color delineation is observed along the walls from green (photosynthesizing) to white (if cells are there, living on chemical energy).

Until recently, little was known about microorganisms in any environment. Traditional microbiology has relied upon cultivation techniques. These have taught us a number of lessons about what organisms look like, how they metabolize different substrates, and how they live with each other. As more and more whole genomes from these cultured organisms emerge, information on their genetics and potential capabilities is also becoming available. We now know that <1% of Earth's microbes are able to thrive in a culture situation, simply because it is nearly impossible to duplicate an organism's natural environment in the lab (Amann, Ludwig, et al. 1995). With the advent of new molecular identification methods for microbes, we have gained a much broader knowledge of the microbial world than was possible with traditional cultivation methods. However, it is important to remember that modern microbiology relies upon information obtained from culture studies to infer information from molecular identification.

Molecular identification examines a gene, an amount of DNA sequence on a microorganism's chromosome. Differences in the DNA sequence on the 16S ribosomal RNA (rRNA) gene can be used to map relatedness between organisms and within groups (kingdoms within the *Bacteria, Archaea,* and *Eucarya* domains). The power of this process is that it can provide a definitive determination of who is who, provides data-baseable

Figure 2. *Top:* A hot spring in the West Thumb area. *Top right* provides a close-up view of the black color lining the walls of this 88°C hot spring. The color is imparted by non-photosynthetic pigments within microbial cells living on the sub-aqueous surfaces. *Bottom:* Octopus Spring in the White Creek region as seen from above. In addition to microbial mats, as seen in Figure 1, there are also organisms living within the water column itself, such as the many inch-long, white-to-pink filaments found in the immediate outfall channel of the hot spring (*bottom right*).

information (DNA sequences), and can be subject to thorough analysis (advanced statistical approaches).

The last decade has seen a number of these kinds of phylogenetic studies applied to several Yellowstone hot springs (Barns, Fundyga, et al. 1994; Reysenbach, Wickham, et al. 1994; Huber, Eder, et al. 1998; Hugenholtz, Pitulle, et al. 1998; Reysenbach, Ehringer, et al. 1998; Ward 1998; Ward, Ferris, et al. 1998; Reysenbach, Ehringer, et al. 2000; Blank, Cady, et al. 2002; Norris, Wraith, et al. 2002; Spear 2002). Upon review of the results, we observed a common theme. Several studies showed an abundance of members of the *Aquificales* bacterial division. Also present were representatives from the *Thermotogales*, *Thermus,* and *Proteobacteria* divisions. We know from traditional cultivation studies that many cultivars from each of these groups prefer or can only use H_2 as an electron donor. This is a surprising result, because when you walk around Yellowstone, you smell sulfide and see sulfur, both of which can act as strong electron donors for microbial life. It seemed as though, and was long thought to be, that the underlying energetic basis for life above the photosynthetic limit of 72°C was the microbial oxidation of these reduced sulfur compounds (Madigan, Martinko, et al. 2003). The phylogenetic results from these studies seemed to suggest otherwise. Could it be that instead, molecular hydrogen provides the electron

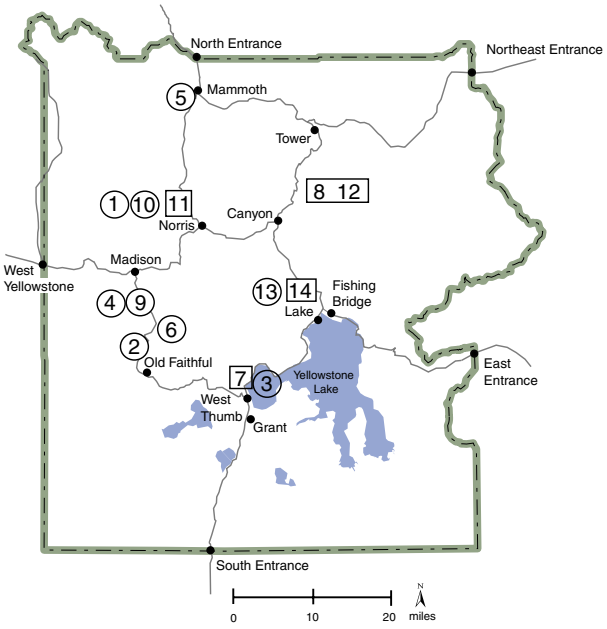

Figure 3. Map of Yellowstone National Park showing the authors' research locations by site number. Hydrogen measurements were conducted in the field by the method described at all sites. Site numbers in boxes represent sites with associated phylogenetic analyses conducted in the laboratory at the University of Colorado.

donor, the fuel, for these microbes in their environments? Thus began our study (Spear, Walker, et al. 2005).

Methodology

Sample collection and DNA extraction. Care was taken in the field to adhere to the idea of minimum impact research (Spear 2004). Samples for DNA extraction were collected from individual source springs in different regions of the park (Figure 3) by one of several methods, depending on the nature of the spring. In some hot springs (Washburn Spring, Queens Laundry), gram amounts of sediment could be collected, frozen in cryovials of liquid nitrogen in the field, and stored at –80°C in the lab. In others, glass slides were suspended in spring pools for periods ranging from 48 hours (Obsidian Pool Prime, Mud Volcano region) to two months (a West Thumb hot spring). Biofilms were then scraped from the slides and frozen in liquid nitrogen. Samples for analyses were collected in different seasons from 1999 to 2003. Total community DNA was prepared from ~1g of frozen samples (Dojka, Hugenholtz, et al. 1998). DNA was purified further to remove substances that could inhibit polymerase chain reactions (PCR) (Barns, Fundyga, et al. 1994).

PCR amplification of rDNA. Initial PCR amplifications of environmental DNAs were conducted to obtain the 16S rRNA genes from all members in the source community. This was conducted with the universal PCR primers 515F and 1391R (Lane 1991) that can amplify the small sub-unit rRNA gene from all three domains of life—*Bacteria, Archaea,* and *Eucarya.* Full-length bacterial rRNA gene sequences were obtained with the PCR primers 8F and 1492R (Lane 1991). Archaeal libraries were constructed with the PCR priming pairs 4Fa or 333Fa, with 1391R (Lane 1991). These primers were each combined with the environmental DNA sample in a PCR mixture including the enzyme *Taq* polymerase, which, when subjected to a certain temperature regime, makes many copies of each of the 16S rRNA genes in the sample. The end result of these amplifications is a tube full of many copies of mixed 16S rRNA genes representative of the source community.

Cloning and sequence analysis. Before DNA sequences can be obtained from PCR products, separation of the PCR product has to occur, because it is a mix of the many genes present in the source community. To do this, PCR products are cloned into *E. coli* cells with a virus-like vector that only puts one PCR product into each cell. The result is many *E. coli* colonies, each containing a unique PCR product

Glossary

Air-bubble stripping: A method of measuring the amount of dissolved gases in water. Similar to the way that CO_2 carbonation dissolves in soda or beer, most gases dissolve in water. Gases such as hydrogen and methane dissolve in Yellowstone's hot spring waters. To measure those, hot spring water is run through a plain atmospheric air bubble. In the process, gases leave the water and enter the air bubble. The air bubble is then extracted and analyzed for the types of gas it contains.

Anion: A negatively charged ion. Chloride (Cl-) and nitrate (NO_3-) are examples of anions.

Cation: A positively charged ion. Metals like copper (Cu_2+), Manganese (Mn_2+) and Sodium (Na+) are cations.

Dissociation constant: A value (K) that expresses the extent to which a substance (e.g., sodium chloride, NaCl) dissociates in solution. The smaller the value of K, the less dissociation. The value varies with temperature, ionic strength, and the nature of the solvent (water, in Yellowstone's case).

Environmental DNA: DNA extracted from the environment.

nM: Nanomolar. A molar solution is one mole of a substance in one liter of water. For example, there are 58 grams in one mole of sodium chloride, NaCl. If those 58 g are dissolved in 1 liter of water, the result is a 1M solution. One-billionth of that concentration is an nM solution.

Nucleotide: Basic structural units of nucleic acids (as RNA and DNA).

Obsidian Pool Prime: Obsidian Pool is a hot spring in the Mud Volcano region that has yielded enormous bacterial diversity, as shown by previous molecular analyses. Obsidian Pool Prime is a hot spring right next to it; the two have a common outfall channel.

Peristaltic pump: A mechanical pump used to pump fluid. The pump has no direct contact with the source fluid to be pumped. Instead, a mechanical wheel rubs a section of plastic tube in a pulse fashion to move the fluid through the tube. The pump head can have a variable-speed motor attached, in order to pump quickly or slowly.

Reduction gas chromatography: A method of separating the gases that comprise a source gas into their separate components. When a source gas is heated and passed through an analytical column, the different gases in the source gas separate into their constituent parts at different rates. From this, the types and amounts of gases that comprise the source gas can be determined. A reduction gas analyzer is used for gases like hydrogen and methane that contain several hydrogen atoms.

SSU: Small sub-unit, as in SSU rRNA, which stands for "small sub-unit ribosomal RNA." Ribosomes are the protein manufacturing facilities of all cells. They are composed of several sub-units, or components. Bacteria for example, have two RNA sub-units, and 52 protein sub-units that make up the ribosome. The authors of this article are tracking the genetic information (the DNA sequence that codes for the RNA) of one of those RNA sub-units.

Thermister: A temperature-sensing device, typically a thermally sensitive resistor that exhibits measurable change in electrical resistance. This can be read as temperature on a meter, much like a thermometer.

sequence. PCR products were cloned for sequencing.

Sequencing reactions were prepared and sequenced in 96-well format. Unique sequences were assembled and put through the Basic Local Alignment Search Tool (BLAST) algorithm (Altschul, Madden, et al. 1997) to determine approximate phylogenetic position.

Phylogenetic analysis. Chimeric sequences—two gene sequences from two different sources that appear as one—were identified using secondary-structure analyses in addition to software (Maidak, Cole, et al. 2001). Sequences that showed ≥99% identity to common contaminants of rRNA-based molecular surveys were excluded from further analyses (Tanner, Goebel, et al. 1998). The remaining environmental rRNA gene sequences were then aligned to other known SSU rRNA sequences.

Figure 4. Field-portable, bubble-stripping apparatus for measurement of H_2 in geothermal waters. An intake tube is wrapped in insulation to keep the water hot. A peristaltic pump pumps water through a glass jar (*right*) with a 20-ml atmospheric air bubble inside. After an amount of pump time at a given rate of flow, the bubble is withdrawn for analysis.

Hydrogen and water chemistry. To survey the distribution of hydrogen concentrations in high-temperature Yellowstone waters (pools, streams, geothermal vents, and a well; Spear 2002), we pumped source waters and performed air-bubble-stripping with H_2, CH_4, and CO_2 analysis by reduction gas chromatography (Chapelle 1997). A peristaltic pump was used to pump source waters through H_2-impermeable tubings into a 250-ml, glass-bottle, bubble-stripping device for triplicate analyses. A 20-ml atmospheric air bubble was introduced into the bottle after it was completely filled with the source water to be measured. Temperature of the bubble was measured by a thermister attached to a digital thermometer. Tubes were insulated from the hot spring water surface to the pump to maintain source water temperature in the bubble-strip apparatus (Figure 4). After bubble-stripping, bubbles were collected with an air-tight syringe and transferred to nitrogen-charged, H_2-impermeably sealed glass septum vials and sent to a geochemical research company in Pittsburgh, Pennsylvania, for immediate analysis on a reduction gas analyzer.

Sulfide measurements were conducted. Samples for water chemistry were collected by pumping water out of each spring, syringe-filtering it through a 0.2-µm filter, and acidifying it with ultra-pure nitric acid to preserve the sample. Samples were placed at 4°C for transport back to the lab. Anions, cations, and elemental analyses were conducted in the Laboratory for Environmental and Geological Studies of the Geology Department at the Uni-

Polymerase Chain Reaction (PCR)

IN MOLECULAR BIOLOGY, it is often necessary to know what one particular segment of DNA, a gene, does for a cell. However, the cell may contain only one copy of that gene (consisting of, e.g., 1,500 base pairs of nucleotides—A+T and C+G repeated in a certain order). One copy is a miniscule amount—too little to work with. In these cases, a process known as **Polymerase Chain Reaction,** or **PCR,** is used to repeatedly amplify a segment of DNA. PCR enzymatically replicates the one copy of the gene into millions or billions more copies.

To facilitate this process, PCR **primers** are applied to the source DNA sequence on either side of the gene. These primers consist of the building blocks of DNA (the A, T, C, and G nucleotides), appropriate to the gene being analyzed. From obtained DNA sequences, these oligonucleotide sequence primers (chains of up to 20 nucleotides) have come to be teased out of genomic DNA. For some genes, it has been observed that these sequences are often quite common between organisms. In the case of *Bacteria* and *Archaea,* for example, it is evident that on either side of the 16S rRNA gene, there is a like code of 15–20 base pairs that is always the same, no matter what kind of *Bacteria* or *Archaea* is examined. In fact, the 16S gene is very similar in actual base pair sequence among most organisms, with the occasional base difference.

It is the sum of these slight differences that allows geneticists to distinguish between organisms and infer relatedness to one another. PCR primers are used to match that 15–20 base pair difference on either side of the gene, thereby providing a starting point in PCR to amplify that one gene. To make the multitude of copies necessary for understanding the genetic function of the gene, the PCR reaction replicates the source gene many times in a series of heating and cooling reactions in the presence of an enzyme called DNA polymerase, commonly Taq polymerase—originally isolated from a Yellowstone hot spring.

versity of Colorado at Boulder.

Thermodynamic modeling. The amounts of chemical energy available from chemical primary producers were quantified with thermodynamic computer models. Species distributions for dissolved inorganic carbon and sulfide were calculated from the measured total amounts for these compounds, together with appropriate dissociation constants and the measured pH, assuming the species were in equilibrium.

Results and Discussion

Chemistry of Yellowstone hot springs. The first step in this work was to provide a chemical context to aid in the interpretation of the phylogenetic results of others (Reysenbach, Wickham, et al. 1994; Hugenholtz, Pitulle, et al. 1998; Reysenbach, Ehringer, et al. 2000; Blank, Cady, et al. 2002). We conducted chemical analyses of hot springs of different kinds of chemistries in different geological areas of the park. Hot springs in the Upper Geyser Basin, for example, contained little or no sulfide and had higher, alkaline pH (pH 8–9). Hot springs in Norris Geyser Basin and the Mud Volcano region sometimes contained higher concentrations of sulfide, and had low-to-neutral pH.

When we measured molecular hydrogen concentrations in hot springs in these different areas around the park, we found concentrations that ranged from 3 nM to over 325 nM of aqueous, dissolved, hydrogen (Spear, Walker, et al. 2005). From cultivation studies of microbes that oxidize hydrogen as an energy source, we know that these concentrations are in the range of, and often far exceed, the 5–10 nM concentrations sufficient to maintain growth in culture. We also measured carbon dioxide, methane (CH_4), and the light hydrocarbons ethane, butane, and butene that we will not cover here (Spear, Walker, et al. 2005).

Each hot spring has its own geochemistry. The H_2 concentrations are spring-dependent, and seasonally consistent when measured over time. Other potential energy sources for microbes in Yellowstone hot springs, such as iron (Fe(II)), manganese (Mn(II)), and aqueous ammonia (NH_4), also occur variably in hot springs. However, because of the chemistry of the springs, the potential for energy yield from such compounds is low relative to other sources like sulfide and hydrogen.

The results from these chemical analyses indicate that aqueous, dissolved hydrogen gas is ubiquitous in Yellowstone hot springs. The concentration varies from spring to spring for a number of possible reasons. But in most of the hot springs we tested, there was enough H_2 present to fuel the microbial cells that live there. The source of the H_2 in the water is probably chemical, not biologic—a geologic process in which heated waters in Yellowstone's subsurface react with iron-bearing rocks to produce H_2 (Gold 1992; Stevens 1995; Sleep, Meibom, et al. 2004). The actual measured presence of H_2 in Yellowstone waters provides our first line of inference that H_2 may be a common fuel for life at >72°C.

Phylogenetic analyses. For a second line of inference, we phylogenetically examined the microbes present in hot springs of >72°C, with both low and high hydrogen and low and high sulfide, to test the impact of reduced sulfur compounds on community composition. If sulfide or hydrogen is a dominant electron donor for the microbial community, this dominance should be reflected in the community composition. Microbes that use the dominant electron donor should be most abundant. To determine the community composition associated with these different chemical regimes, we PCR-amplified, cloned, and sequenced rRNA genes from crust and sediment communities. We also looked at pioneer communities that colonized on glass slides placed in hot springs for from two days to three months. We screened >2,500 randomly chosen clones from five different hot springs, and determined >400 new rRNA gene sequences for submittal to GenBank, a public repository of DNA sequence information.

To determine the phylogenetic types of organisms present in a given hot spring, we compared the obtained sequences to sequences of known organisms in public databases. Although the detail of hot spring composition varied, all of the five communities examined contained sequences representative of the same kinds of phylogenetic groups. As in previous studies (Hugenholtz,

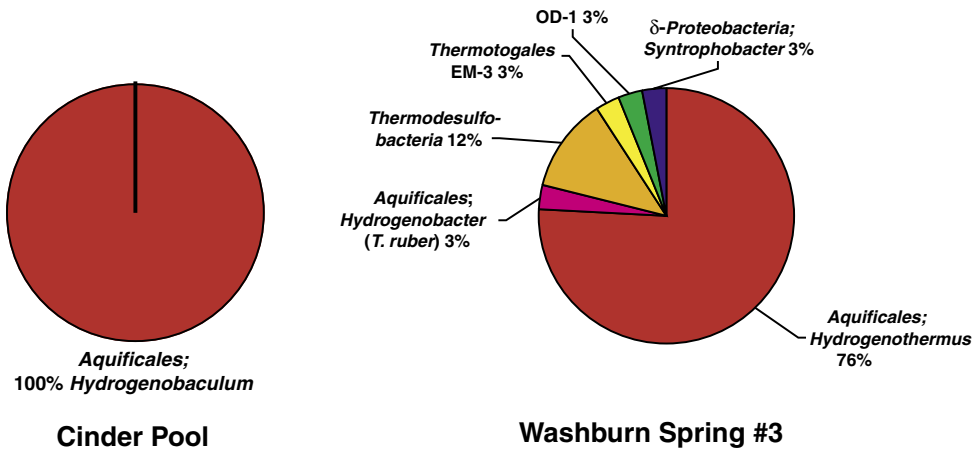

Figure 5. Results of phylogenetic analyses from Cinder Pool, Norris region, and a lower Washburn Spring, Canyon region, Yellowstone National Park. Cinder Pool is 88°C, pH 4.2, and contains 77 nM H_2, 1.2 µM CH_4, 47 µM sulfide, and 1 µM sulfate. One of the Lower Washburn Springs, #3, is 86°C, pH 6.2, and contains 19 nM H_2, 5.8 µM CH_4, 167 µM sulfide, and 44 µM sulfate.

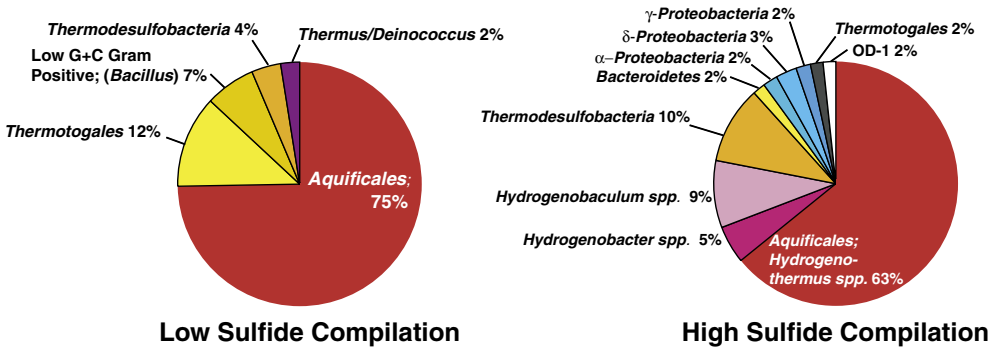

Figure 6. Results of community compilations from low- and high-sulfide Yellowstone hot springs. Left chart describes the phylogenetic distribution of rRNA gene sequences obtained from two low-sulfide springs in this study (a West Thumb hot spring and Obsidian Pool Prime), combined with the five low-sulfide springs of Blank, et al. (Octopus Spring, Queens Laundry, Eclipse Geyser, Spindle Spring, and Boulder Spring) (Blank, Cady, et al. 2002). Right chart describes the phylogenetic distribution of rRNA gene sequences obtained from three high-sulfide springs in this study (Cinder Pool, Washburn #1, and Washburn #3).

Pitulle, et al. 1998; Blank, Cady, et al. 2002), communities were dominated by bacterial rRNA genes. *Archaea* are considered common in these and other "extreme" environments, but as in the previous studies, our surveys indicated that these organisms are about one-tenth as abundant as bacteria. Most of the *Archaea* encountered in this study were relatives of an environmental Crenarchaeote observed in an earlier study of Obsidian Pool (Barns, Fundyga, et al. 1994; Barns, Delwiche, et al. 1996).

The several hundred unique rRNA gene sequences determined in this study fell into only a few phylogenetic groups. Sequences representative of the three main sub-groups of the bacterial division *Aquificales* (hydrogenobacter, hydrogenobaculum, and hydrogenothermus) were the most abundant in the five hot springs examined. The results of these analyses for two individual springs are presented in Figure 5. Sequences representative of the bacterial divisions *Thermotogales, Thermus/Deinococcus,* and *Thermodesulfobacteria* were also common. Cultivated representatives of organisms from all of these groups either exclusively or preferentially utilize molecular hydrogen as an electron donor. Collectively, >90% of the sequences obtained from these two hot springs came from these groups. If a characteristic is present in all of the cultivated members of a group, then other representatives of that group are assumed to also have those properties. Therefore, we can assume that the sequences obtained from the hot springs indicate that the organisms in the springs primarily use molecular hydrogen as their electron donor.

Communities of hot springs with both low and high sulfide concentrations did include some organisms of the kinds that can oxidize sulfide as an electron donor. However, these organisms were not as numerically abundant in the clone libraries as the H_2-oxidizers mentioned. Analytical results for hot springs of both high and low sulfide grouped together by spring type are shown in Figure 6. We did find a number of rRNA gene sequences representative of the δ-Proteobacteria, a group that includes many organisms that reduce sulfate to sulfide, often with molecular hydrogen as an electron donor. This would suggest that when sulfate is present in a Yellowstone hot spring, sulfate-reducing bacteria could then contribute to the energy budget of the community. As in the first two hot springs examined, the collected data from all of the springs indicate that, in hot springs of >72°C, >90% of community composition favors hydrogen oxidation independent of sulfide concentration.

Thermodynamic modeling. For a third line of inference that H_2 provides the fuel for microorganisms in Yellowstone hot springs >72°C, we thermodynamically modeled the potential energy available to the microbial communities present in a given hot spring. This model was based on the chemical compositions that we measured for the hot springs. Because photosynthesis does not occur at >72°C, most microbes in Yellowstone hot springs have to get their energy from reduced compounds, e.g., molecular hydrogen, sulfide, or methane (CH_4). The potential energy for the oxidation of these reduced compounds depends heavily on the availability of an electron acceptor in these hot springs. Molecular oxygen, O_2, diffusing into hot spring waters from the atmosphere, is the likely electron acceptor for most of the kinds of organisms we found in our phylogenetic survey.

Oxygen, however, is difficult to measure accurately in hot water, because of the low solubility of this gas at high temperature. Generally, the concentrations of this important electron acceptor are low, in the nM range, and we used a range of reasonable oxygen concentrations for our model. When we modeled the potential energy available in hot springs across a range of oxygen concentrations, the results indicated that the oxidation of H_2 was always favored under oxygen-limited conditions. This third line of experimental inference is consistent with the apparent dominance of H_2 oxidizers in our clone libraries of five hot springs examined.

Conclusions

With the combined use of chemical analyses, phylogenetic analyses, and thermodynamic modeling, we have shown that microbial life in Yellowstone hot springs at >72°C is most likely

fueled by aqueous molecular hydrogen, not sulfide. While actual hydrogen concentrations vary, along with microbial community structure and geochemistry in each spring within the park, we observed a trend that nevertheless indicates a favorable role for hydrogen to fuel microbial life. The importance of hydrogen-metabolizing microorganisms in environmental microbiology has long been recognized (Madigan, Martinko, et al. 2003). We have now determined that biologically significant levels of hydrogen occur in the waters of Yellowstone hot springs, and that there are a large number of organisms present of the kinds that oxidize hydrogen. This theme of hydrogen as a main fuel for Yellowstone hot springs likely resonates to other geothermal ecosystems around our globe, and maybe elsewhere in the universe.

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John Spear is an assistant professor in the Division of Environmental Science and Engineering at the Colorado School of Mines. John performed the work described here as a postdoctoral fellow in the laboratory of Dr. Norman Pace at the University of Colorado–Boulder. His laboratory at the Colorado School of Mines continues to focus on microbial diversity and energetic mechanisms relevant to environments such as those that occur in Yellowstone hot springs, as well as those of hypersaline microbial mats found near Guerrero Negro, Baja California Sur, Mexico.

Jeff Walker is a postdoctoral fellow in the laboratory of Dr. Larry Gold in the Department of Molecular, Cellular and Developmental Biology at the University of Colorado–Boulder. Jeff worked on the microbial communities associated with Yellowstone endolithic communities (life in the pore space of rocks) as a graduate student with Norman Pace.

Norman Pace is a Professor of Molecular, Cellular and Developmental Biology at the University of Colorado–Boulder. His laboratory has long been involved with microbial community characterization by applied molecular methodologies and has, for a number of years, focused on extreme environments like those found in Yellowstone hot springs. Dr. Pace is a member of the National Academy of Sciences and is a Fellow of the MacArthur Foundation.

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