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The evolution of geobiology in the context of living stromatolites

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ABSTRACT

Science has achieved tremendous success over the centuries, partly because the complexities of the Earth, the physical processes that sustain the planet, and the enormity of life were separated into disparate fields of study—mathematics, physics, chemistry, biology, and geology, to name only a few. Scientific compartmentalization was initially necessary to impart enough focus to make progress on complicated issues. However, as the knowledge base grew, it became more and more difficult to separate life and the history of the Earth, and vice versa. We now understand that to investigate the Earth's surface as an abiologic system is folly: Life and Earth processes are intimately linked. Hence, a new field was born at the interface between biology and geology: geobiology.

As a field, geobiology seeks to understand the intersection of life and the rock record across Earth's history: how organisms influence the physical Earth and vice versa, and how the marriage of physical and biological processes have transformed our planet over its long history.

The assessment of life's macromolecules of DNA, RNA, polysaccharides, proteins, and lipids, and their potential recalcitrance in an ecosystem, has opened up the field of geobiology to lead us toward a solid explanation of where life came from, how life has altered the planet, what may be possible for life elsewhere, and what represents one of the reasons for the explosion of geobiologic studies today. Here we outline how molecular biology has transformed our understanding of geobiology, describing a few of the essentials needed to understand geobiology and exploring an example of a modern geobiologically relevant system: a living stromatolite from the shore of a geothermal hot spring in Yellowstone National Park, Wyoming, USA.

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INTRODUCTION

Geobiology. From *geo*—and *logia*—earth discourse; and *biology*—the science of life.

Over the past fifty years, and in the past decade in particular, these two disciplines of science, geology and biology, have come together to form *geobiology,* an interdisciplinary study of the biosphere-geosphere-atmosphere interface; that is, the fundamental realization that Earth and life have co-evolved over the last approximately four billion years, and that one system cannot be understood without the other. The field has exploded in the last decade as molecular tools have arisen to understand the genetic basis for microbe-mineral interaction, and to construct meaningful phylogenies of morphologically conservative microorganisms. The best observations in science often happen at the interface of at least two things, and such is true with geobiology (Dilek et al., 2008). While geobiology relies on many of the tools of modern molecular biology, the field also relies upon the tools of geophysics, geochemistry, mineralogy, sedimentology, paleontology, genetics, physiology, biochemistry, and atmospheric science to tie site-associated "meta-data" with the biological data to generate a complete geobiological story. The generated information can then better inform, not only on the microorganisms present, i.e., who is there, but also on the kinds of chemistry that allow such a system to thrive. All of these different kinds of data can be statistically integrated and correlated to better understand what drives life in unique niches; what kinds of mineralizations are predictable and possible; what is the "genetic potential" of a particular environment-site-ecosystem; what is the "evolutionary temperature" (Woese, 1998, 2000, 2002) of a particular environment recorded in time and space within the rock record; and what does all of the above contribute to what is known about evolution, the rock record, humanity, and the Earth.

A subset of the field of geobiology is the specific discipline of geomicrobiology, a discipline that was born out of the recognition that microbes are literally everywhere. To have any environment, on Earth, totally free of any microbial organisms is in fact quite difficult to accomplish, short of that environment which exists within a flame. Microbiologists who studied microbes on surfaces quickly realized that, given the extent of the lithosphere of the planet, that the rock-microbe interface is prime habitat for the exploration of life's processes. A second subset of the field of geobiology is astrobiology, where practitioners take what is learned from geobiological studies and combine that with planetary science to think about life on other worlds. Astrobiology has greatly matured in the past dozen years, and both the Jet Propulsion Laboratory (JPL) and the greater National Aeronautics and Space Administration (NASA) must think about this fact in sending people, machines, and robots to other worlds in our solar system so as not to forward-contaminate those worlds with the kinds of microbiota that live on Earth while they seek an answer to the important question, are we alone? If not, that $n = 2$ for life in the universe, there are many implications from the origin of life, to evolution, to the kind of life possible biochemically, to the nature of human belief.

Early geomicrobiology grew from the discipline of microbiology by people who worked in the oil and mining industries. To be able to grow and determine the kinds of organisms that could "sour" an oil with the production of sulfide was a boon to the fields of petroleum engineering and of subsurface microbiology (Updegraff and Wren, 1954). The cultivation of sulfate-reducing bacteria (SRB) (Postgate, 1984; Odum and Singleton, 1993), most of which are obligate anaerobes that reduce sulfate to sulfide in an eight-electron transfer of energy, opened up much of what is known, not only of anaerobes, but of the kinds of life that can inhabit "unique" locations—a deep, anoxic, geochemically rich subsurface, for example. Because of early geomicrobiologists, we now appreciate that microbes are geologically significant from the upper stratosphere to the depths of tens of kilometers in the Earth's crust and are essential for global elemental cycles. Both weathering (mineral dissolution and precipitation) and aqueous redox processes name only a few of the metabolic processes that microbiota are capable of in allowing our Earth to be viewed in its entirety as "organism" with complex cycles of life, elements, rock, water, and atmosphere.

BIOLOGY + GEOCHEMISTRY + GEOLOGY = GEOBIOLOGY

As stated above, the field of geobiology lies at the intersection of many disciplines of science, so by nature there may be many "definitions" of geobiology. All heartily contribute to the evolution of the field, but the root of geobiology lies primarily within the disciplines of its namesakes, *geology* and *biology*. Geochemistry may be the glue that holds biology and geology together. Geochemistry can explain, predict, and model what is geobiologically possible in a given environment. A solid geobiological interpretation of a given place thus lies in the specific understanding of the kinds of biota that are/were present; the kinds of geologic forms and minerals that are present; and a characterization of the chemistry, the geochemistry, that likely drives or explains what is thermodynamically, biologically, and geologically possible in that environment. Geobiology requires a different kind of question to be asked; it is not about the abiologic system (which could be considered solely the realm of chemistry), nor the biologic system (the purview of biology), but how both interact to construct something greater than the sum of the parts, something that would not be understood with one or the other approach. This requires characterizing some aspects of biology, geochemistry, and geology in the field, with more in-depth analyses to follow in the laboratory by what is possible with the lab-based state-of-the-art instrumentation currently available, and what will become available in the near future. What is the question? is key, and to be able to ask that requires knowledge of some critical aspects in each of the fields of biology, geochemistry, geology, and other supporting scientific fields (Spear et al., 1999, 2000, 2007; Shock et al., 2005; Sahl et al., 2008; Berelson et al., 2011).

All organisms can leave an imprint on the environment, and vice versa. However, more often than not, geobiologic study tends toward investigating the role of microorganisms in the Earth system. This is not to say that "macrobes" (e.g., animals, plants, etc.) are excluded from geobiologic investigation, but in general, microbes are omnipresent on Earth and are responsible for cycling most if not all of the bioessential elements in the Earth system, as well as other Earth important processes (e.g., weathering–mineral dissolution–precipitation, etc.). Furthermore, as we will discuss, molecular biologic techniques have opened up the study of microbes in the environment in a way not possible in the past.

Geology and a Map of Time

The field of geology has studied and divided Earth's history into a series of geologic eons, eras, periods, and epochs that are well defined based on observed processes after examination of the rock record of the Earth. A basic chronology with "life processes" superimposed on geologic processes is afforded by DesMarais, Konhauser, and others (DesMarais, 2000; Konhauser, 2007) (Fig. 1). As depicted in Figure 1, the origin of the Earth occurred ~4.6 billion years (Ga) ago. What followed was a late heavy bombardment to 4 Ga, and speculation abounds that life likely arose shortly thereafter, perhaps at 3.8 Ga. Geologists long ago realized that the history and story of Earth is best told through the rock record. Geologic time is on a grand scale, almost imperceptible, with time measured in billions of years. Geologists have been able to employ various dating schemes to pinpoint Earth's processes across that time.

With decades of both great field- and laboratory-based geology, the science of geology has greatly advanced what is known of the Earth, and what is known of the life on Earth. Many time points are hugely significant across the time of Earth's existence (Fig. 1); the advent of photosynthesis, ca. 3.8 Ga; an oxygenated atmosphere at ca. 2.4 Ga, microbiologically created, that arose after a long period of a reducing atmosphere; a "snowball Earth" at ca. 2.3 Ga and ca. 730–635 Ma; the Cambrian explosion of ca. 540 Ma with the first abundant fossils of macrobial life; the first vertebrate land animals at ca. 360 Ma; the Permian–Triassic extinction at ca. 250 Ma that is thought to have killed >90% of all marine invertebrates; the Cretaceous–Paleogene extinction at ca. 65 Ma that is thought to have killed off the terrestrial dinosaurs; the first appearance of hominids at ca. 7 Ma; the first appearance of *Australopithecus* at ca. 3.9 Ma, an ancestor of humans, *Homo sapiens;* to 200,000 years ago, the first modern *Homo sapiens,* humans' direct ancestor, who appeared in East Africa.

Life arose early, on the bacterial line of descent, and likely shortly after the late heavy bombardment (Pace, 1997). From 3.8 to 2.5 Ga, microbial life arose with remarkable sophistication,

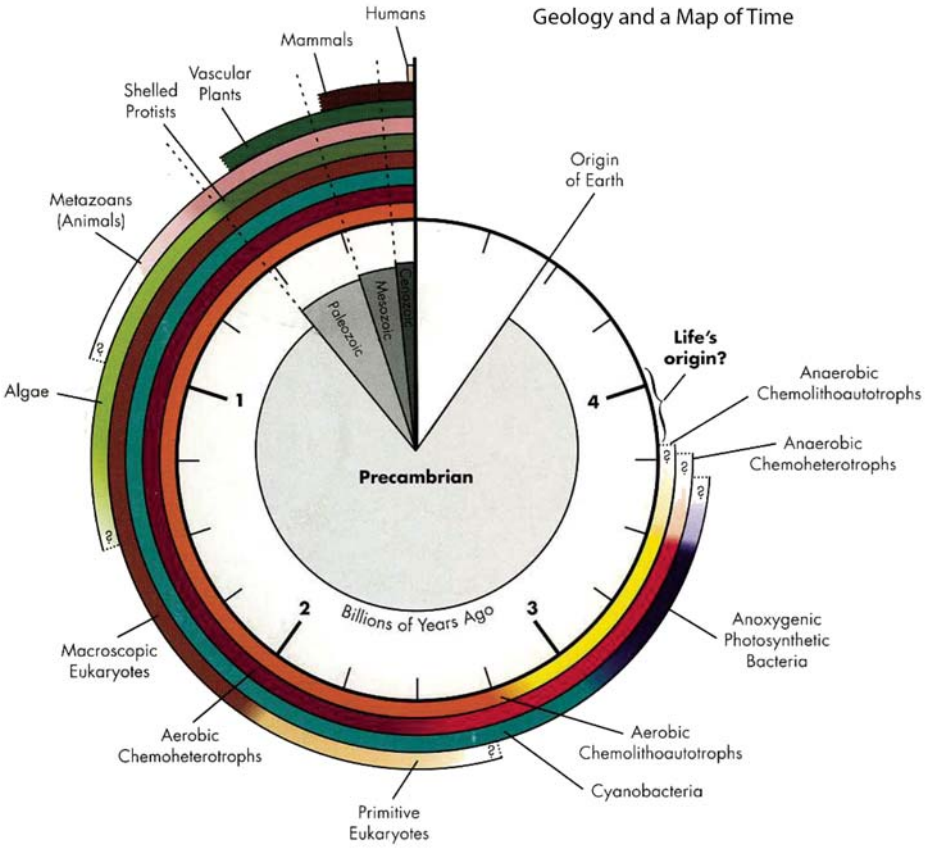

Figure 1. A biogeologic clock, as adapted from DesMarais (2000) and Konhauser (2007).

and that sophistication included photosynthesis, which produced an oxygenated atmosphere that macrobes, plants, and animals, still enjoy today. The billions of years of antiquity of Earth's biosphere likely illustrates how easily life can arise on a habitable planet, but it also presents a huge challenge and impedance that confound our efforts to become familiar with our earliest ancestors. The earliest sedimentary rocks have typically undergone extensive alteration by metamorphism, which takes a serious toll on microfossils, remnants of microorganisms, who only leave a trace of any kind of fossil behind via microbial mineralizations (DesMarais, 2000). Fortunately, our distant ancestors in all three domains of life are recorded not only in ancient rocks but also in biological macromolecules (Zuckerkandl and Pauling, 1965). DesMarais phrased it well in that "the two records are highly complementary: the geologic record offers the absolute timing of evolutionary innovations and their environmental context, while the living biochemical record can reveal the sequence of development of key pathways and biomolecules" (DesMarais, 2000, p. 1703).

Biology and a Map of Life

Humans like to classify things, but morphology can be problematic. Attempts to characterize and describe macrobial life, that which we see and can readily observe, occupies only a fraction of the total life on the planet. Traditional taxonomists relied upon visual-physical differences of the things they could see to both characterize and categorize life. While macrobial life can be (relatively) easily classified, microbial life appears morphologically simple. Taxonomy, by its nature, focused on classification, or "pigeonholing," and thus tends to break down at the microbial scale. Rather, phylogeny, the evolutionary interrelatedness of organisms, is the ultimate goal: but looks can be deceiving. The major leap forward occurred in the 1960s when Zuckerkandl and Pauling put forth the idea that evolution can be recorded in molecules, particularly the molecules that provide the code for the complexity of life, DNA and RNA, helping taxonomy and classification based on looks alone to move forward into meaningful molecular phylogenies (Zuckerkandl and Pauling, 1965).

Biological diversity can now be defined in terms of the relationships of DNA sequences (representing organisms) being compared between any organism and another. Comparisons of gene nucleotide sequences, the DNA code, can be used to construct "maps" of biological diversity. The process is relatively simple. Gene sequences from different organisms change with time owing to the pressures of natural selection, mutation, and the swapping of genetic information between organisms, termed lateral or horizontal gene transfer (HGT). Once DNA sequence is obtained from an environment, specific genes within that sequence are compared on a base-by-base basis, and the number of differences in the DNA sequence are considered to be some measure of "evolutionary distance" between the compared organisms. Just as maps can be made from distances between points, maps of evolution—"phylogenetic trees"—can be made with the use of evolutionary distances between organisms (Fig. 2).

Evolutionary maps can be made by comparing any gene sequence, or even complete genomes, but the small subunit ribosomal RNA gene sequences (SSU-rRNA), because of their ubiquity and high degree of conservation, have become the most widely used (Woese, 1987). DNA is the central processing molecule of life, as it prescribes the functions to be carried out in a cell by directing the manufacture of functional protein molecules. The proteins themselves are made by ribosomes, complex structures composed of both nucleic acids (RNA) and protein. Ribosomes are the protein manufacturing centers of all cellular life. Because of the ubiquity and necessity of ribosomes to make protein for all known life, the gene sequence for ribosomal RNA that lies within the DNA of a cell's chromosome is highly conserved, not subjected to too much base change with time nor HGT, making for a perfect molecule to compare and map life. Before the application of modern molecular methods (e.g., the polymerase chain reaction, PCR, with subsequent DNA sequencing), only cultured organisms could be used as references to deduce the paths of evolution. Because of the need for culture to examine the intricacies of microbial physiology in the laboratory, knowledge about the microbial world has been limited, and what we do know stems mostly from medically related organisms (pathogens) because of their importance to human and animal health. For example, 65% of published microbiology reports form 1991 to 1997 covered only eight genera of bacteria (Galvez et al., 1998). Sixteen years later, by 2013, we still have little information about how similar or dissimilar, and what metabolisms are exhibited by, uncultivated organisms in the environment, and these organisms likely make up the majority of the kinds of life in most environments (Pace, 1997, 2009).

Modern perspective on the nature of microbial diversity changed in the late 1970s with the development of molecular phylogeny. From this phylogenetic perspective has come the recognition that there are three distinct relatedness groups: the domains of life, as shown in Figure 2 (after Pace, 2009). This modern sequence-based definition of biological diversity replaces the traditionally taught view of biology as composed of "five kingdoms." Figure 2 provides a rendition for a map of evolutionary relationships between representative ribosomal RNA gene sequences. The diagram shows that all life on Earth is related, and that there are three main kinds of life, the Bacteria, the Archaea, and the Eucarya. There are not two kinds of life as has been historically implied by the terms *prokaryotes* and *eukaryotes*. This map is not a measure of time, but rather a measure of evolutionary distance. Lines in the map are from single sequences but represent members of relatedness groups, e.g., Animals, Plants, Stramenopiles, Cyanobacteria, etc. Line lengths are proportional to the evolutionary distance, the change in the ribosomal RNA gene sequence that separates the organisms represented. A similar relatedness map is obtained by comparative phylogenetic analysis of other genes involved in any of life's metabolic processes. All cells contain a core suite of genes necessary

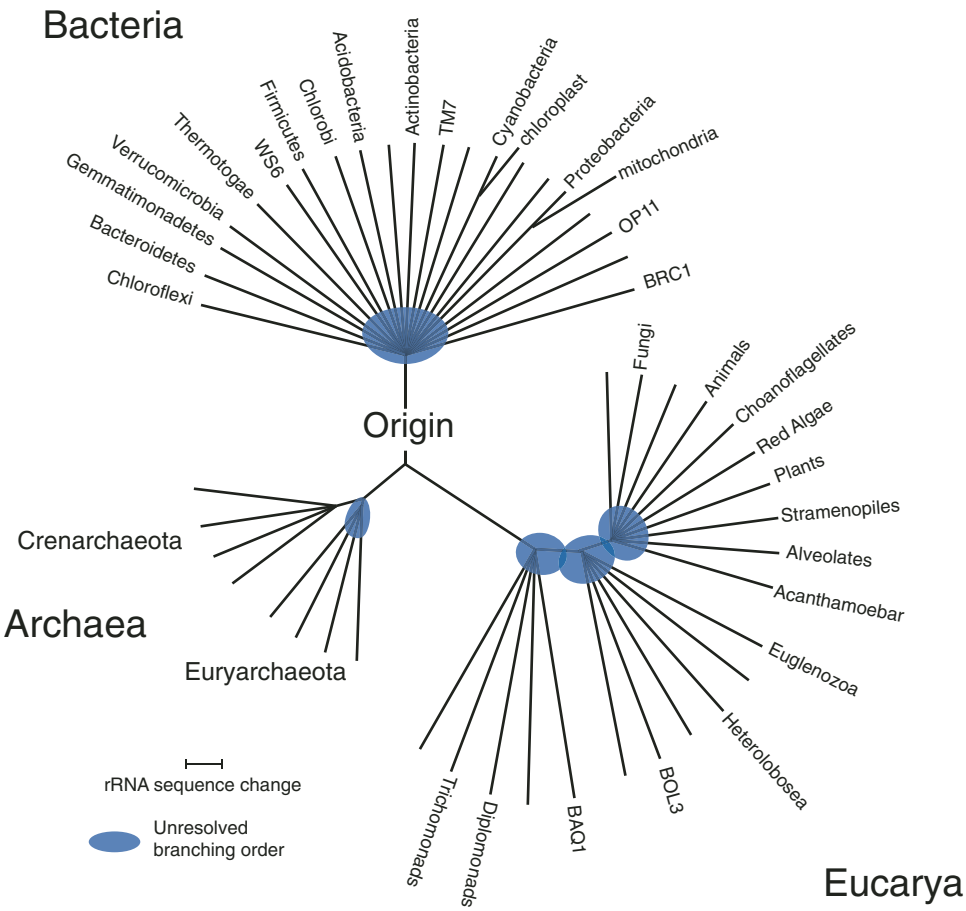

Figure 2. A map of life, adapted from Pace (2009). This map is generated by the comparison of DNA sequences from ribosomal rRNA genes (16S rRNA genes for Bacteria and Archaea; 18S rRNA genes for Eucarya). Each line segment in each of the three domains represents a line of descent to that particular kind of life, and each line in and of itself represents a complex radiation for that kind of life not depicted here. The opaque blue clouds at the roots of particular major radiations represent a blurred statistical branching order that may become more refined with bigger and more complete sequencing efforts. For full consideration of the figure, please see Pace (2009).

for life—replication and metabolism—which are required for vertical inheritance in a line of descent. That is to say that some cellular functions are so important, to an extent already highly evolved, and so necessary to carry out the function of "being alive," that very little change happens with time to these genes and that they are "highly conserved" and passed on to descendants in a close to "as is" state. Failure to pass along these genes would typically result in a non-viable organism. Comparisons of some metabolic genes can yield results that are inconsistent with Figure 2; however, there is no consistent alternative. These incongruities are due to, and part of, the extensive evidence for inter- and intra-domain lateral gene transfers (Woese, 2000).

The Geobiological Importance of Molecules

Nucleic Acids

The molecular perspective on organismal relationships is intellectually satisfying, because this method provides a far finer resolution of relatedness than that which is based on a purely morphological distinction, and it removes much of the inherent "expert opinion," or bias, present in traditional morphologically based phylogeny. A non-morphological phylogeny is particularly important for microbes; to be blunt, many microbes simply look the same (coccoids, rods, filaments, etc.), and they lack morphological diversity. It is the lack of morphological diversity in the microbes (e.g., the antiquated terms of "monera" and "prostista") that likely raised the animals, fungi, and plants to such a high position in the former "five kingdom" approach of classification, despite the now-understood huge diversity, and complexity, in microbial taxa. Importantly, it also provides a basis for the study of natural microbial communities without culture. Microbiologists have long realized that it is almost impossible to create an environment in the lab to grow an organism out of that environment. Typically, <0.1% of microbes are considered easily culturable. The microbes that do grow are often the most metabolically versatile organisms, "the weeds" of the microbial world. And while cultivation can only get so far with knowledge about an organism, it remains true that "to know it, you have to grow it." Cultivation remains a critical tool for the success of geobiological investigation.

From this nucleotide sequence-based perspective, organisms can be described as gene sequences of DNA instead of from their physiological properties. A problem of this perspective as it applies to geobiology is that it can only be used on living or recently alive organisms in a particular sample. The biological macromolecules of life, DNA, RNA, and proteins, are not recalcitrant in the environment, so the interpretation of life and geobiology from a nucleotide sequence approach is best made with living

systems. Currently, the "oldest" DNA is considered ca. 900 ka, and it originates from an ice core (e.g., a very unusual environment), and most DNA in sedimentary environments decays extremely rapidly. Figure 3 describes an interplay of the basic molecular methods that have been refined over the past decade to study microbes in the environment without culture. First, genomic DNA, representative of the resident organisms from a particular environment, is obtained by physically grinding or lysing cells and chemical extraction. The polymerase chain reaction (PCR) then is used to amplify small subunit ribosomal RNA genes (SSU rRNA) present in the extracted environmental DNA. The PCR amplified ribosomal RNA genes are, however, a mixture of the rRNA genes for the entire community. Initially, cloning of the PCR product from environmental DNA was carried out to separate the multitude of different rRNA genes. These clones were then typically subjected to Sanger sequencing techniques where "long-reads" of DNA sequence were possible to understand a gene's actual DNA sequence. Now, newer methods of DNA sequencing are employed (below), shorter reads are obtained, but identification is just as valid (Caporaso et al., 2012). Once sequenced, the problem shifts from the lab bench to the computer, where the (sometimes extremely long) nucleotide sequences are assembled and compared for interrelatedness. The ribosomal RNA gene sequence is different in different organisms and an *in silico,* i.e., microprocessor-driven computational comparison, termed a *bioinformatic comparison,* can then relate and map organisms on a "tree of life" (Fig. 2). The results provide a rough assessment of the extent of diversity in an environment. To date, however, we still have no idea of what the total microbial diversity on Earth is in terms of both the kinds of and numbers of phyla present in each domain of life.

The heart of the molecular methodology lies in the determination of the nucleotide sequences of DNA and/or RNA that are used to compare organisms. At this stage of the technology, sequences are determined by the use of automated DNA sequencers, that themselves have now gone through several stages of technological evolution. In 2013, we are now in a stage of "next-generation" DNA sequencing where Roche 454 Life Sciences pyrosequencing machines are capable of sequencing greater than one million, 400 base pair–long sequences in about eight hours; or where the Illumina HiSeq–MiSeq systems can sequence hundreds of millions of base pairs of shorter length (e.g., 125 base pairs) reads in a matter of days (Caporaso, et al., 2012); or where even the tested tools of electron microscopy can be applied for nucleotide base identification (Bell et al., 2012). The obtained DNA sequences can be compared to other DNA sequences in publicly available databases (e.g., GenBank, http://www.ncbi.nlm.nih.gov/genbank/), followed by thorough phylogenetic analyses with statistical applications in various software programs such as ARB (http://www.mikro.biologie.tu-muenchen.de) and the quantitative insights into microbial ecology portal (QIIME, http://qiime.org). With increased sequencing capacity, high-throughput, bioinformatic interpretation of generated DNA sequence via analysis methods that rely heavily on *in silico* biology, either on small servers or in large serving farms such as the cloud compute spaces afforded by the Amazon, Inc., Google, Inc., and Apple, Inc., have become commonplace. At some future date, the names and techniques listed above may have gone by the wayside because of the continued evolution of methodology, but the basic process, the generation of DNA sequences by whatever new technology, for comparison *in silico,* will remain. The main advantage offered by more DNA sequencing of a given sample is that we end up with a greatly more in-depth analysis of both "who is there" and "what are they doing," for example. This then allows for a far better interpretation of the true ecology of a microbial place, and with that understanding comes the knowledge to infer, interact, and interpret what has happened, is happening, or could happen in that place.

The most striking transformational phenomenon to affect the field of geobiology in its short history is the ability to sequence massive amounts of DNA cheaply with the next-generation sequencing methodologies. We used to answer the question of

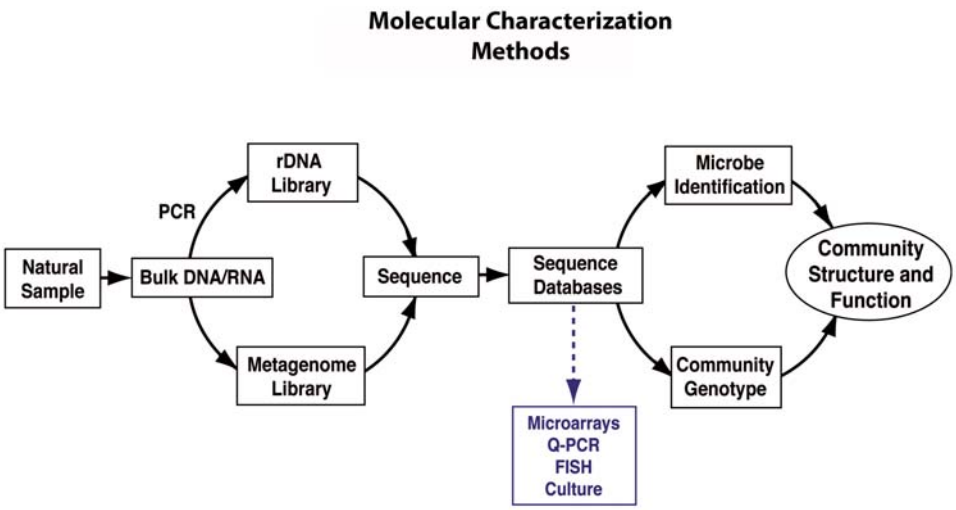

Figure 3. Methods used in geobiological investigation. PCR refers to the polymerase chain reaction, and Q-PCR refers to quantitative polymerase chain reaction. FISH—fluorescence in situ hybridization.

"who is there?" by sequencing SSU rRNA genes from all three domains of life, then assembling that sequence in long reads to cover the approximate 1500 base pair full-length gene, to then phylogenetically analyze the sequence to place the organisms of a particular sample in the context of all known life as well as to identify them. We can now ask this same question, and many others, to better understand the complexities of a geobiologically functioning community at a whole new ecosystem-wide and deeper level. With next-generation sequencing has come the advent of the "-omics" era.

We can now take an environmental sample, extract cellular DNA, RNA, protein, and lipids, and conduct next-generation analyses of biology's most important macromolecules. Random "shotgun" sequencing of DNA from a community has brought the field of metagenomics to fruition. Here, much can be learned about the total kinds of genes and organisms present in the source sample. Conclusions can be drawn from the data, but owing to the random nature of the sequence, the incompleteness of the gene sequences determined (as typically only snippets of their full length are obtained), the process really only works best on environmental communities that are not that complex, e.g., a community that contains ~10 organisms or less (Tyson, et al., 2004), though the technique is building into greater possibilities (Narasingarao, et al., 2012). This is now expanding into single-cell genomics, whereby cells from a community are individually sorted, and hundreds to thousands of individual genomes are generated for a full community genomic profile. The "genomics" era will redefine what we know about the rock-microbe interface, and likely will redefine what we know of life itself. Genomics can inform on the metabolic potential of a cell's or community's gene pool, but better is that RNA can also be extracted from cells within a community, a complementary DNA library obtained via reverse transcriptase PCR, and a "metatranscriptome" generated. A metatranscriptome, being generated from the RNA present in a community, not only reflects who is there but also who is alive and actively metabolizing. This can get at the heart of, for example, a geobiological mineralization phenomenon occurring at a particular site. In addition, amino acid sequencing of proteins via matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) has opened the door to understand the "proteomics" of a community. Together, all of these tools enable a much greater understanding of functioning geobiological communities.

Another important component of the molecular technology is to characterize cellular morphology and distribution in the natural environment for cells that are likely difficult to culture. For this, unique regions in ribosomal RNA gene sequences can be targeted with fluorescently tagged oligonucleotide (nucleic acid) probes, which can bind, in a hybridization step, to samples from the original environment. This process is known as "fluorescence in situ hybridization" (FISH) (DeLong et al., 1989). For example, a probe can be designed for a particular subregion of DNA, and when delivered to a sample it will bind to the organisms in that sample with that DNA sequence, regardless of the cultivability of the organisms. FISH can identify specific organisms in a mixture of different organisms from a particular environment (e.g., see St. Amand et al., 2005). Many variants of the FISH technique are relevant to geobiology, and catalyzed reporter deposition to greatly increase signal amplification of particular molecules in low concentrations is but one technique, so termed CARD-FISH (Pernthaler, et al., 2002). Fluorescent illumination of particular species among all species present can indicate cell quantity, morphology, and growth preferences of the organisms identified by the FISH probes.

Lipids

While nucleotide–gene sequence can inform on relatedness, a second important class of biological macromolecules are the lipids. Lipid molecules are hydrophobic, are essential for the "package" of the cell, that is, a lipid bag that holds all of the materials that make a cell a cell. This makes a cell capable of separation from its external environment, allows for a cell to be contained within a structure that can be differentially partitioned for different elements and compounds between an internal and external space across a membrane, and is capable of diffusive, passive, and active transport of compounds across a membrane (Madigan, et al., 2012). This is all made possible by the development of a lipid bi-layer membrane that surrounds most kinds of cells in all three domains of life.

In addition to these roles and functions listed above, lipids have another important quality. Unlike nucleic acids that have low recalcitrance in an environment and degrade quickly, lipids are degraded much more slowly and thus can serve as biosignatures of previous biological activity in a given sample. Biomarker molecules that are synthesized by cells (e.g., lipids and sterols) can have distinctive chemical, structural, and isotopic properties and forms, and as such can serve to identify the kinds of cells that may be (or were) present in an environment (that is, the cells may themselves be degraded and gone, but the lipids remain to indicate the cell's former presence). The attributes of the particular compounds are complex, and thus organism specific, and this specificity can be exploited to inform on the conditions that may or did allow for life to be present in a given environment. To be able to make inferences about the present or the past with natural compounds, molecules are needed that are recalcitrant, unique, and measurable in environmental samples (often in low concentrations), and which can survive in some form; the geologic pressures of diagenesis are ideal for such work. These compounds then can serve as tracers for ongoing biogeochemical processes in a living environment today, or as a "chemical fossil" that informs on the paleobiological record of past activity in a long-gone microbial community of the past (Summons et al., 1999; Gaines et al., 2009; Summons and Lincoln, 2012). Classes of lipid molecules can meet all of these requirements.

Lipid compounds that serve well as biomarkers can originate as cellular–biochemical pigments, cell wall or interior lipids that make up a cell's integrity or physiology, and sterols that can serve any number of cellular functions. Because lipids and sterols are hydrophobic hydrocarbon compounds, they are difficult to degrade

both chemically and biologically, particularly in anoxic sediments. Typical cellular lipid bilayer membrane lipids that are long-chain fatty acids linked to a glycerol molecule can survive chemical and biologic breakdown. Even more recalcitrant are polycyclic and branched hydrocarbons found in polyisoprenoid lipids and cyclic triterpenoids such as steroids and hopanoids, and they are well established biosignature compounds for Eucarya and Bacteria, respectively (Brassell et al., 1983). Many of these compounds with their hydrocarbon cores can remain intact and preserved in sediments and petroleum reserves for hundreds of millions to billions of years (Waldbauer et al., 2009; Peters et al., 2005).

As biological macromolecules, lipids serve vital functions for a cell. Typically, when a cell dies, the disequilibirum of energy that is life returns to equilibiria via the laws of thermodynamics. This means that a nucleic acid-rich, lipid-rich, protein-rich bag of tissue that is a cell will, upon death, lyse its nucleic acid-, lipid-, proteins- and polysaccharides into the environment and be itself a nutritionally rich food source for other members of the community. Upon cell lysis, biological macromolecules are then also subject to the geochemical pressures of the environment. That pressure initiates the process of diagenesis whereby lipid (and other) molecules undergo physical-structural transformations that change the original structure-function of the molecule. Lipids can have various rings (e.g., aromatic and cyclic), hydrocarbon alkyl chains, and subgroups that will be lost through environmental pressure, and only the most recalcitrant compounds will survive through time with some level of similarity to the original molecule. The preservation of such molecules is likely environmentally dependent, where the lack of an electron acceptor (e.g., oxygen or sulfate) in a sediment, for example, excludes or slows down the potential for decay of the molecule; or the presence of an electron donor (e.g., sulfide or hydrogen) as a strong reducing agent can remove unstable features like double bonds within the original molecule (Berner and Canfield, 1989; Adam et al., 2000). In addition, lipid molecules that are, or have become, associated with mineral particles in particular environments may afford some stability of the lipid molecule and perhaps could better allow for the molecule to become a "biosignature" in the long-term rock record (Hedges and Keil, 1995).

The beauty of lipid molecules that undergo the diagenetic process is that complex terpenoid lipids, such as carotenoids, sterols, and bacteriohopanepolyols (BHPs), can be reduced to a signature hydrocarbon skeleton that preserves a basic signature form of the original molecule. This signature form often has characteristic structural features that are diagnostic for the parent molecule, and inferences can be made as to the type or kind of organism(s) that synthesized the parent molecule in the biochemical life phase of the source organism within its ecological niche (Summons and Lincoln, 2012; Osburn et al., 2011). In addition, the process of diagenesis itself can impart predictable stereochemical and structural modifications to lipid macromolecules that are progressive with time. This then allows for the diagnostic interpretation, based on the kinds and states of the lipids present, for the kinds of organisms which produced the source compounds over all phases of the diagenetic process. This is particularly valuable, then, to better assess the age, maturity, or potential contamination of a sample to date, observe, or understand, respectively, the diagenetic processes that impart change to the source sample. However, because of the ubiquity of biochemical "core" metabolic processes—those that are shared between many kinds of organisms in all three domains of life—the number of truly unique lipids to a particular organism is rare.

Lipids as biomarkers are only as robust as our current knowledge of clade specificity. DNA is unique to each organism (but will not survive in the rock record); lipids may or may not be unique to certain clades, but they have a much better chance of survival over geologic time. For example, methylated hopanoids have been proposed as a biomarker for cyanobacteria (Summons et al., 1999) and have been found in rocks as old as 2.7 Ga (e.g., Brocks et al., 1999). However, recent work has demonstrated that other bacteria may also synthesize methyated hopanoids (e.g., Rashby et al., 2007), demonstrating that the clade specificity is key with respect to lipids as robust biomarkers.

Isotopes

The Periodic Table of the Elements is an absolutely wonderful tool to understand nature and the chemical basis of life, the Earth, and the universe. One aspect of the elements that is lacking in most forms of the Periodic Table are the variants of the elements that are primarily a natural reflection of the formation of the element. This variance is due to the fact that while the proton number in the nucleus defines all isotopes of the element, the neutron number can vary, affecting the mass of the element—e.g., ^{12}C, ^{13}C, and ^{14}C are all carbon with an atomic number of 6 but have masses of ~12, 13, and 14 g/mole owing to the presence of 6, 7, and 8 neutrons, respectively. These "stable isotopes" with the word *isotope* tend to reflect the chemical properties of an atom versus the nuclear properties of an atom, and thus are considered distinct from "nuclides," which generally refer to the radioactive elements and their isotopes. Stable isotopes then become an interesting tool for geobiological study, because life, with its billions of years of evolution, has had time to optimize the catalytic function of enzymes to a level that is isotope specific: for example, the oxidation and/or reductions of specific elements. The active sites of most enzymes have evolved to catalyze a specific elemental moiety to the angstrom level. For example, an organism may actively oxidize ^{12}C 99.9999% of the time, and only occasionally will a ^{13}C get metabolized. This metabolic difference is important, because it changes the stable isotope fractionation ratio of ^{12}C:^{13}C, and that can reflect the effects of biologic versus abiological activity across time, as measured across a rock record, for example.

The biogeochemically important elements of H, C, N, O, P, and S have the isotopes of ^{1}H, ^{2}H, ^{12}C, ^{13}C, ^{14}C, ^{14}N, ^{15}N, ^{16}O, ^{17}O, ^{18}O, P (mono-isotopic), ^{32}S, ^{33}S, ^{34}S, and ^{36}S. Redox-sensitive elements that are important for cellular processes-metabolisms that are also relevant in the rock record are iron (^{54}Fe, ^{56}Fe, ^{57}Fe, and ^{58}Fe), copper (^{63}Cu and ^{65}Cu), zinc (^{64}Zn, ^{66}Zn, ^{67}Zn, ^{68}Zn,

and ^{70}Zn) and molybdenum (^{92}Mo, ^{94}Mo, ^{95}Mo, ^{96}Mo, ^{97}Mo, ^{98}Mo, and ^{100}Mo) (Johnston and Fischer, 2012). Stable isotopes have been considered and studied as relevant and an important tool for geobiology for more than 60 years (Urey, 1947). With methods adapted from radionuclide chemistry and modern instrumentation, such as multi-collector inductively coupled plasma–mass spectrometry (ICP-MS), stable isotope geobiology, like lipid geobiology, has grown into its own subdiscipline.

Because of their variance in mass and disequilibirum of incorporation into biological molecules, isotope ratios become important tracers of mass flux across a microbiological (and macrobiological) ecosystem. Ecosystems are inherently complex, with huge time, space, and geologic scales, and the ability to understand an ecosystem is difficult at best, for both a modern, living system, and for the interpretation of an ancient ecosystem. Yet, the sensitive measurements afforded by the variance in mass of stable isotopes afford understanding across all of these scales. Mass spectrometer technology has allowed measurements between the most-more abundant isotopes and the native element (e.g., ^{13}C and ^{34}S or ^{13}C and ^{18}O, Eiler and Schauble, 2004; Fike et al., 2006; McFadden et al., 2008); the covariance of two stable isotopes in a mineral can inform on the geochemistry of mineral formation (Archer and Vance, 2006); and the variance of stable isotopes across a whole geologic stratigraphic section all make for a promising future for stable isotope geobiology. Major advances are on the horizon as mass spectrometers become more and more sensitive. For example, the rare isotopes of sulfur ($^{33, 36}$S) and oxygen (^{17}O) can now be measured and are being exploited for geobiologic investigation (e.g., Johnston et al., 2008; Johnston, 2011; Canfield, 1989).

Analysis and Interpretation of Geobiological Systems: A Case Study from Yellowstone National Park

By application of the tools and methods described above, the field of geobiology is breaking open what is known of the rock-microbe interface. The result is a far greater understanding of geology, geological systems, microbial mineralizations, and inheritable genetic capability to biologically synthesize both minerals and rock, as well as to biologically break down minerals and rock via microbiologically enhanced weathering. Geobiology as a science can thus have both basic and applied aspects to the field. The geobiological inquiry of a particular stratigraphic formation can inform on the entirety of geobiologic processes that made that formation over time, across a rock record, to answer a basic geobiological question. Or, for example, an in-depth analysis of the rock-microbe association between coal and methanogens can inform on how to mine methane from an in situ coal formation without having to remove the coal from the ground can answer an applied geobiological question. To that end, here we apply some of the tools available to the field of geobiology to interpret a living stromatolite system that both informs on a living microbial-mineral ecosystem as well as to stromatolites found throughout the rock record.

Stromatolites are perhaps the "poster child" for geobiologic investigation, as they are lithified structures thought by many to have been built by microbes. However, living examples of stromatolites are few and far between. Here, we will investigate a stromatolite actively growing in a hot spring in Yellowstone National Park as an example of a geobiologic investigation involving geologists, geochemists, geochronologists, microbiologists, and molecular biologists.

Stromatolites

Stromatolites, found throughout many time points in the rock record, are most commonly defined as laminated organosedimentary structures built by the trapping and binding and/or precipitation of minerals by microbial mats and/or cells (e.g., Walter, 1976; Riding, 1990; Riding and Awramik, 2000). Microbial mats themselves are best defined as complex microbial communities that are composed of many kinds of cells (from all three domains of life), sometimes in a layered association with cells surrounded and trapped with biologically produced extracellular polymeric substances (EPS). Though composed primarily of microbes, these microbial mats can be macro in scale, clearly visible to the naked eye, and can, for example, surround a hot spring in Yellowstone that adds color and tons of biomass to the thermal system (Ross et al., 2012). A morphologically similar microbial mat is a microbial biofilm. A biofilm is also composed of microbial cells and EPS, but biofilms tend to be less visible (those that coat the teeth of the human mouth, for example), no less complex than a macro-microbial mat, and are found on virtually every surface in any environment.

Likely being constructed by microbial cells, the presence of stromatolites is commonly taken as a biosignature of life at specific time points in the rock record, and they are presumed to constitute some of the oldest evidence for life on Earth, ca. 3.4 Ga (Hofmann et al., 1999; Allwood et al., 2006) (Fig. 4). However, as noted with taxonomy, morphology can be deceiving. Abiotic structures that mimic biogenic stromatolites are well known (e.g., McLoughlin et al., 2008), and numeric modeling demonstrates that stromatolite morphogenesis can be modeled with simple rules: Life may or may not be required for their formation (e.g., Grotzinger and Rothman, 1996; Grotzinger and Knoll, 1999). Thus the interpretation of ancient stromatolites—are they biotic or abiotic, for example—is not as straightforward as once thought.

There is a paucity of known, actively growing modern examples of stromatolites, whether biogenic or abiogenic, that make meaningful analyses with respect to growth rate, morphology, biota (if present), geochemical effect, environmental conditions, etc., difficult. Famous and well-studied modern marine examples thought to be representative of stromatolites in the rock record are known and include Shark Bay, Australia (e.g., Logan, 1961; Hoffman and Walter, 1976), and the Bahamas (e.g., Dravis, 1983; Dill et al., 1986; Reid et al., 2000). Unfortunately, most modern marine forms are crudely laminated at best, if laminated at all, and while they are instructive for our understanding of microbialite growth in general, they do not constitute a good textural

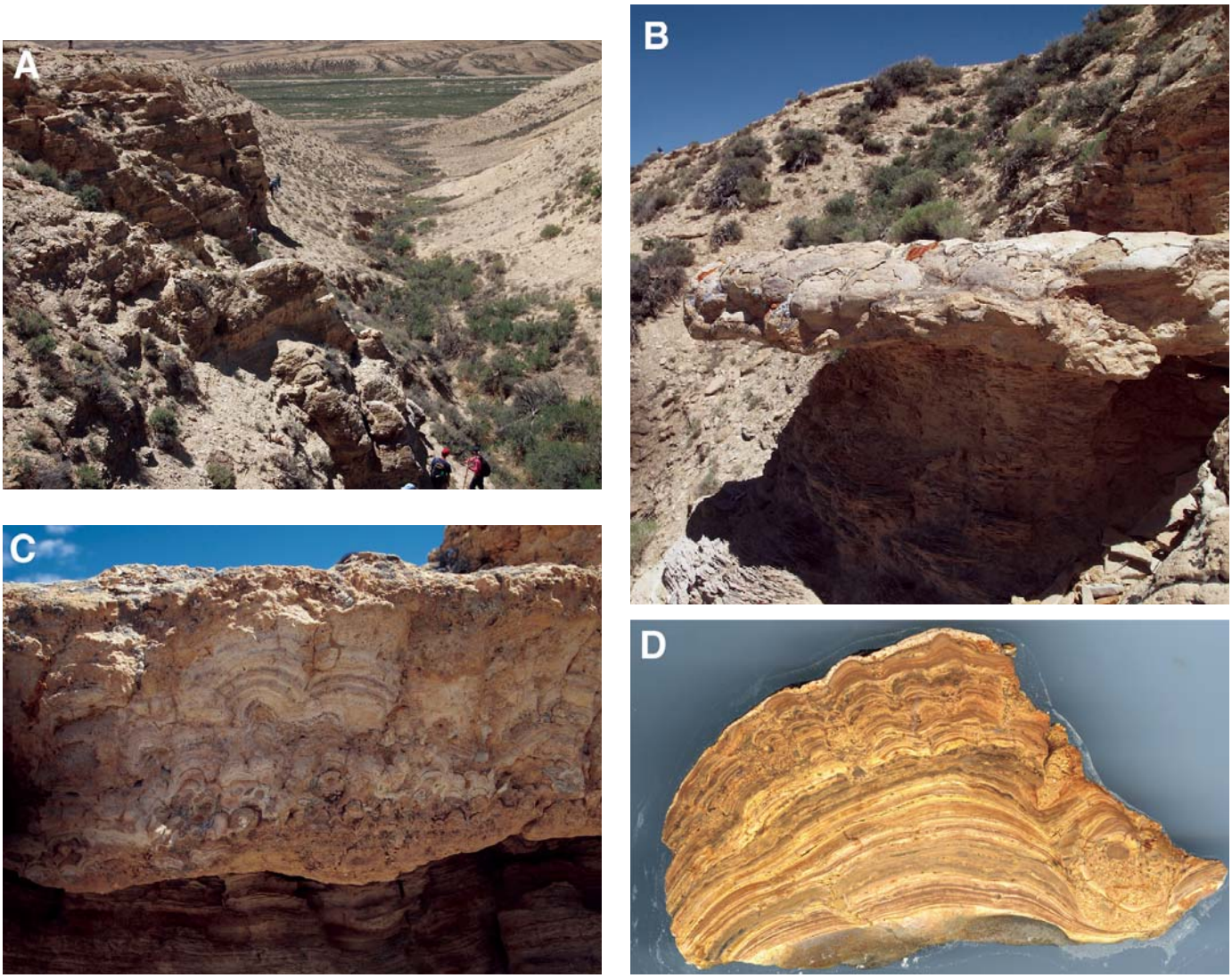

Figure 4. (A) The 50-million-year-old stromatolite outcrop of the La Clede Member, Green River Formation, southeast of Rock Springs, Wyoming. (B) A single stromatolite outcrop of the La Clede Member. (C) A close-up of one part of a single outcrop in B. (D) Slab cut and polished piece of La Clede stromatolite; piece is 15 cm across.

analogue for the more finely laminated Precambrian stromatolites found in the rock record (e.g., Awramik and Riding, 1988; Grotzinger and Knoll, 1999).

Living Stromatolites in Yellowstone

With over 10,000 geothermal features to consider for geobiological science, Yellowstone National Park (YNP) continues to be a locus of activity across both silica-rich and carbonate-rich systems (e.g., Spear et al., 2005; Walker et al., 2005; Berelson et al., 2011; Pepe-Ranney et al., 2012; Mata et al., 2012; Fouke, 2011). The spectrum of environments and microbiota reflected by hot springs alone is no less than amazing, even across a short spatial scale, with temperatures ranging from ambient to 94 °C (surface boiling at 2280 m; temperatures higher at depth in springs), and a pH between 2 and 10; each unique set of conditions provides a niche for unique communities of microorganisms. The geochemistry and microbiology can be completely different for two hot springs that are ~1 m apart.

Silica-rich hot springs provide an excellent environment for stromatolite morphogenesis, for several reasons (as first noted in Walter et al., 1972). First, it is thought that "harsh" acidic and high temperature conditions exclude most metazoan life that might graze on the microbial communities and disrupt stromatolite formation. Second, and most importantly, dissolved silica will adsorb and precipitate abiogenically on the cells within microbial mats as a spring water cools—regardless of the microbial metabolism. This can provide a condition where very early lithification of microbial mats can occur. There have been numerous studies

of YNP microbial mats (Ferris et al., 2003; Allewalt et al., 2006; Steunou et al., 2006; Bhaya et al., 2007; Bryant et al., 2007; Klatt et al., 2007; van der Meer et al., 2007; Steunou et al., 2008, Ross et al., 2012). However, a typical microbial mat is not likely to end up in the rock record as a stromatolite—the mat must be lithified or mineralized in some way to enter the fossil record as a stromatolite—and the silica-rich hot-spring environment is an excellent environment for early lithification to occur.

Stromatolites have been lightly studied and recognized in YNP for decades (e.g., Barghoorn and Tyler, 1965; Walter et al., 1972; Doemel and Brock, 1974; Awramik and Vanyo, 1986; Guidry and Chafetz, 2003). Additionally, several recent reviews discuss microbial hot spring lithification and its effect on sinter laminations and microstructure (Konhauser et al., 2003; Konhauser et al., 2004; Benning et al., 2005). Walter et al. (1972) give credit to Weed (1889) for first describing stromatolites in YNP (although the biogenic nature was not known at that time), but the fact that Yellowstone stromatolites were in the eye of geologists in the 1880s, shortly after Yellowstone became the world's first National Park in 1872, is remarkable. Barghoorn and Tyler (1965) recognized that the microstructure of YNP stromatolites was similar to some Precambrian forms. Laminated microbialite morphogenesis in YNP occurs in many ways, from subaqueous lithification of microbial mats (e.g., Walter et al., 1972; Walter, 1976) to subaerial, intermittently splashed microbial mats (e.g., Cady and Farmer, 1996; Blank et al., 2002).

Here, we describe some stromatolites that are microstructurally similar to the structures described by Walter et al., 1972; Walter, 1976) in that the laminations are composed of silica-coated filamentous bacterial sheaths, where the filaments are upright in one layer (usually a lighter colored layer) and lying down in the next (darker colored) (Fig. 5A) (Berelson et al., 2011; Pepe-Ranney et al., 2012; Mata et al., 2012). The YNP stromatolites differ in that they are domal or columnar on the mesoscopic scale (sensu Shapiro, 2000) rather than conical. Few modern living stromatolites have been age dated in order to understand their growth rate; for the few age-dated specimens, accretion rates do not match daily, seasonal, or yearly lamination accretion, most commonly cited for ancient stromatolite growth (e.g., Chivas et al., 1990). Based on a series of reports we have produced (Berelson et al.,

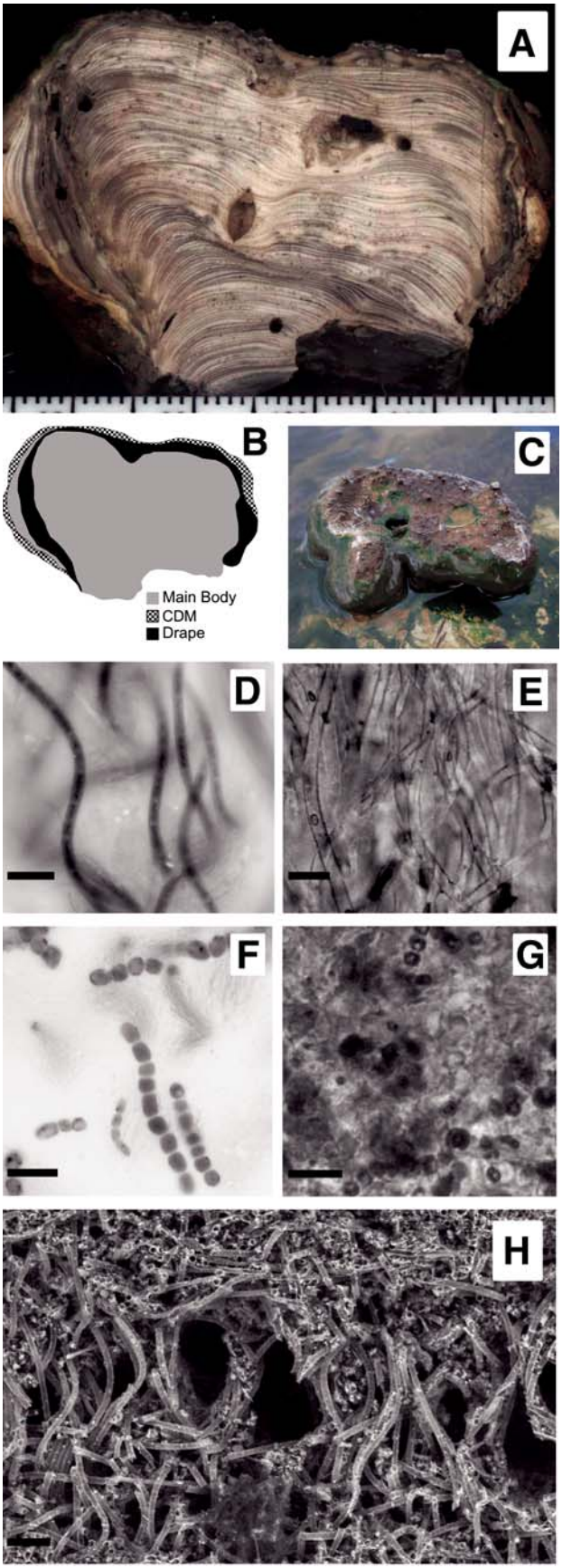

Figure 5. Selected images of different stromatolite facies from a Yellowstone National Park hot spring. All scale bars are 10 μm unless otherwise indicated. (A) Cross section of stromatolite from which Cocci/Diatom mat (CDM; the "drape facies") samples were imaged from a sample collected in August 2006. Gradations in scale bar across bottom of image are millimeters. (B) Drawing depicting the location of facies in cross section A. (C) Stromatolite from image A in situ. (D) Autofluorescence image of predominant cellular morphotype in the submerged, filamentous mat (SFM; the "main body facies"). (E) Thin-section image of "main body" lithofacies. (F) Autofluorescence image of predominant phylotype in CDM samples from August 2006. (G) Thin-section image of "drape" lithofacies. (H) Electron micrograph of silica tubes that constitute "main body" lithofacies. For full consideration of the figure, please see Pepe-Ranney et al. (2012).

2011; Pepe-Ranney et al., 2012; Mata et al., 2012) the described YNP stromatolites are biogenic, finely laminated, silica-formed structures that actively grow in an acidic hot spring in YNP. We describe their growth rate and morphology and the microbial community composition, and propose correlations between fine-scale morphologic changes in the stromatolites and the environment–tectonic evolution of the Yellowstone area.

Stromatolites occur as a continuous rim along parts of Obsidian Pool Prime, or as isolated mushroom-shaped structures on the shallow shelf adjacent to the rim of the hot spring (Fig. 5C). The isolated specimens range from a few centimeters to 30 cm across their top and can be 10–20 cm tall (Fig. 5C). A transverse section through an isolated, small stromatolite reveals the distinct laminated fabric and complex internal pattern of convex upward accretion (Fig. 5A). A cross section through the rim stromatolites reveals upward growth first (similar to the isolated stromatolites), followed by lateral accretion toward the center of the pool. There are two distinct styles of lamination observed in the stromatolites (Figs. 5A, 5B): The most common style makes up the majority of the stromatolite (the "main body" facies, Fig. 5B), while the other style is less common and less distinctly laminated (the "drape" facies, Fig. 5B).

The majority of the main body of the stromatolite is composed of alternating light and dark laminations that are easily visible and are themselves composed of amorphous silica-coated filamentous tubes (Figs. 5D–5H). Made of amorphous silica (SiO_2, opal), the structure remains wholly permeable; there is little silica cement between the filaments in either lamination. The dark layers are composed of tubes oriented parallel to the lamination, and the light layers are composed of what appear to be the same tubes oriented normal to the lamination. The darker layers are packed more densely with tubes. The lighter layers have greater porosity, and many have large cavities (Fig. 5H) and were likely formed as a function of photosynthetic oxygen bubbles within the microbial mat (Mata et al., 2012). Light and dark laminae both range in thickness between 100 and 200 µm, but in general the light layers average 150 µm and the dark layers ~100 µm. The silicified tubes range from 1 to 3 µm in diameter (Figs. 5D–5H) and appear consistent in shape in both the dark and light layers. The tubes are the sheaths of a filamentous, non-heterocystous cyanobacterium on the basis of their morphology and preliminary molecular analyses, but the silicification and subsequent organic-matter degradation are harder to determine.

It is apparent that the main body of the stromatolite grew upward, and when growth of this form paused, the whole structure was then "draped" by a somewhat dark, less distinct form of lamination (Figs. 5A, 5B). Ultimately, construction returned to the main body style of lamination, and the drape morphology thus represents a depositional layer within the stromatolite (note the two generations of "main body" lamination in Fig. 5B). The drape contains silicified tubes, some clearly branching, as well as coccoidal forms and pennate diatoms that are not found in the main body (Fig. 5F). Unlike the light-dark couplets in the main body, there is less internal structure to the drape, and the tubes are more tightly packed. In addition, the drape layers tend to be silicified more rigidly than the main body layers, where the pore space is entirely filled with silica (Fig. 5G). The drape and the main body fabrics alternate over time; in fact, the outer part of the stromatolite in Figures 5A and 5B represents a second drape, indicating at least two episodes of main body formation punctuated by two episodes of drape formation.

The employment of three radiometric dating techniques, $^{228}Th/^{228}Ra$, $^{228}Ra/^{226}Ra$, and ^{137}Cs are internally consistent and predict that stromatolite accretion occurs on the order of centimeters over the course of years rather than hundreds or thousands of years; the stromatolites grow remarkably fast (Berelson et al., 2011). To support the stromatolite growth, microbial cells must have an abundance of carbon. Shock et al. (2005) documented the gas bubbles that emerge from this spring as >95% CO_2. The original source of the dissolved inorganic carbon (DIC) contained in vent waters is likely the ancient limestones and other rocks through which the vent water flows before reaching the surface of the Earth in a pooled hot spring. Given the amount of cyanobacteria found, this implies that autotrophic fixation of CO_2 is the dominant carbon fixation mechanism, and that both the vent water and the atmospheric CO_2 can be a carbon source. Berelson et al. (2011) utilized ^{14}C content as a tracer for the CO_2 source utilized by the autotrophic, stromatolite-building communities. Two end-member sources, vent water CO_2 and atmospheric CO_2, were used to develop a mixing line on which stromatolitic samples reside. Parts of the well-laminated main body facies have ^{14}C signatures, indicating that a high proportion of their CO_2 originated from the hot spring vent (65%–80%). The drape fabric facies of a stromatolite has a ^{14}C signature that indicates that a larger fraction of atmospheric CO_2 (~46%) was fixed by autotrophs living in this layer. Thus the two prominent facies of these stromatolites, the main body and the drape, likely reflect different kinds of microbiota that utilize different CO_2 sources at different times, dependent on the environmental condition of the water level in the hot spring. Because of this, these stomatolites can likely record geological, geochemical, and physical factors that were present at the time of their construction. This could then be extrapolated to how the stromatolites grew, which are now part of the rock record.

Biologically, these living stromatolites are equally amazing. Molecular methods revealed that submerged mat cyanobacteria were predominantly one novel phylotype, while the exposed mats were predominantly heterocystous phylotypes (Chlorogloeopsis HTF [high-temperature form] and Fischerella) (Pepe-Ranney et al., 2012). The cyanobacterium dominating the submerged mat type does not belong in any of the subphylum groups of cyanobacteria recognized by the Ribosomal Database Project and has also been found in association with travertine stromatolites in a Southwest Japan hot spring. Cyanobacterial membership profiles indicate that the heterocystous phylotypes are "rare biosphere" members of the submerged mats (Figs. 6 and 7). The hypothesis behind the rare biosphere is that in a typical microbial community, some members can be dominant while others are less

abundant. When a physical or other condition changes, a member of the rare community can grow into a dominant role (Sogin et al. 2006). The heterocystous phylotypes likely emerge when the water level of the hot spring drops, and this allows for the community dominance to shift from one kind of cyanobacterium to another, also shifting the carbon source of CO_2 from vent CO_2 to atmospheric CO_2, recorded as described above (Berelson et al., 2011). Environmental pressures tied to water level, such as sulfide exposure and possibly oxygen tension, may inhibit the heterocystous types in submerged mats, or that in a zone of full emersion, the heterocystous kinds just cannot thrive owing to a lack of ability to fix nitrogen.

CONCLUSION

Geobiology has grown into its own field in the past decade. The intersection of geology with biology, linked by geochemistry, makes for a very exciting time in science! Some of the most important observations and discoveries of how the Earth works have been made at interfaces, and such is true with the interfaces of several different fields of science, coming together to form a new field and a new and better awareness of our planet. Technology has allowed for the development of some remarkable and sophisticated tools that allow for next-generation sequencing of DNA to unprecedented levels, a better understanding and more sensitive measurements of the elements for better geochemical interpretation, and sophisticated tracing of biosignature compounds and elements, taking geology to new levels of knowledge and the potential to better understand the encyclopedia of life recorded in the rock record. Inside every rock there is a potential story. The interpretation of that story, such as can be revealed by the analysis of a living stromatolite system, better informs on life, the Earth, evolution, and what may be possible. Geobiology allows for the asking and answering of both basic and applied research questions, and these will be needed to better use the resources of the Earth to support a growing humanity.

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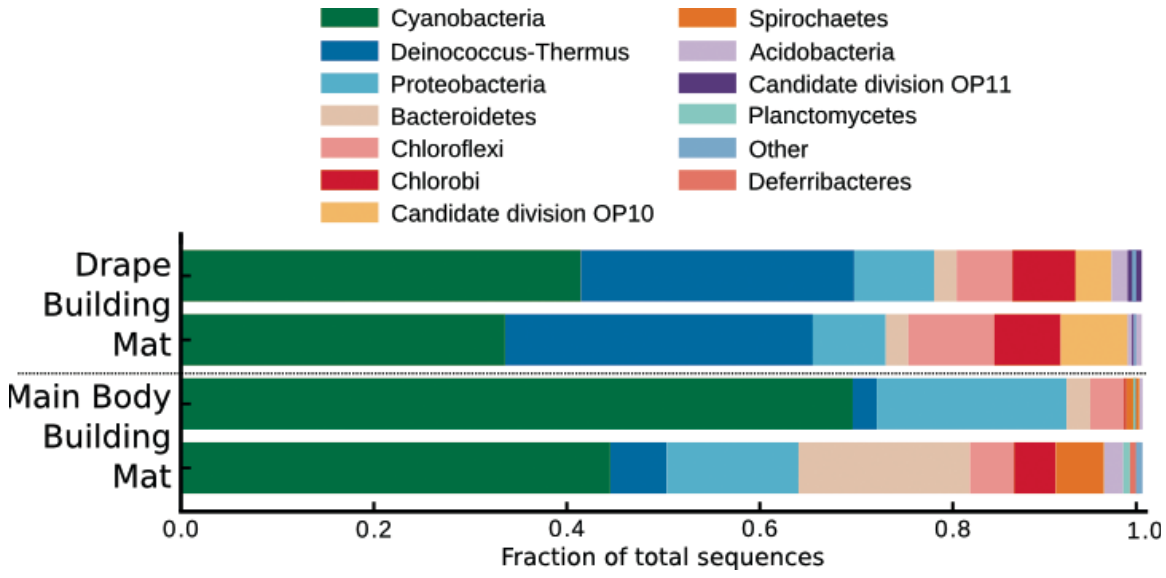

Figure 6. The microbiology of a living stromatolite. The bar chart depicts the distribution of pyrosequencing reads into phyla for each pyrosequence library. The top half shows the cocci-diatom morphotype-dominated drape building microbial mat; the bottom half describes the submerged, filamentous cyanobacterium main-body microbial mat. For full consideration of the figure, please see Pepe-Ranney et al. (2012).

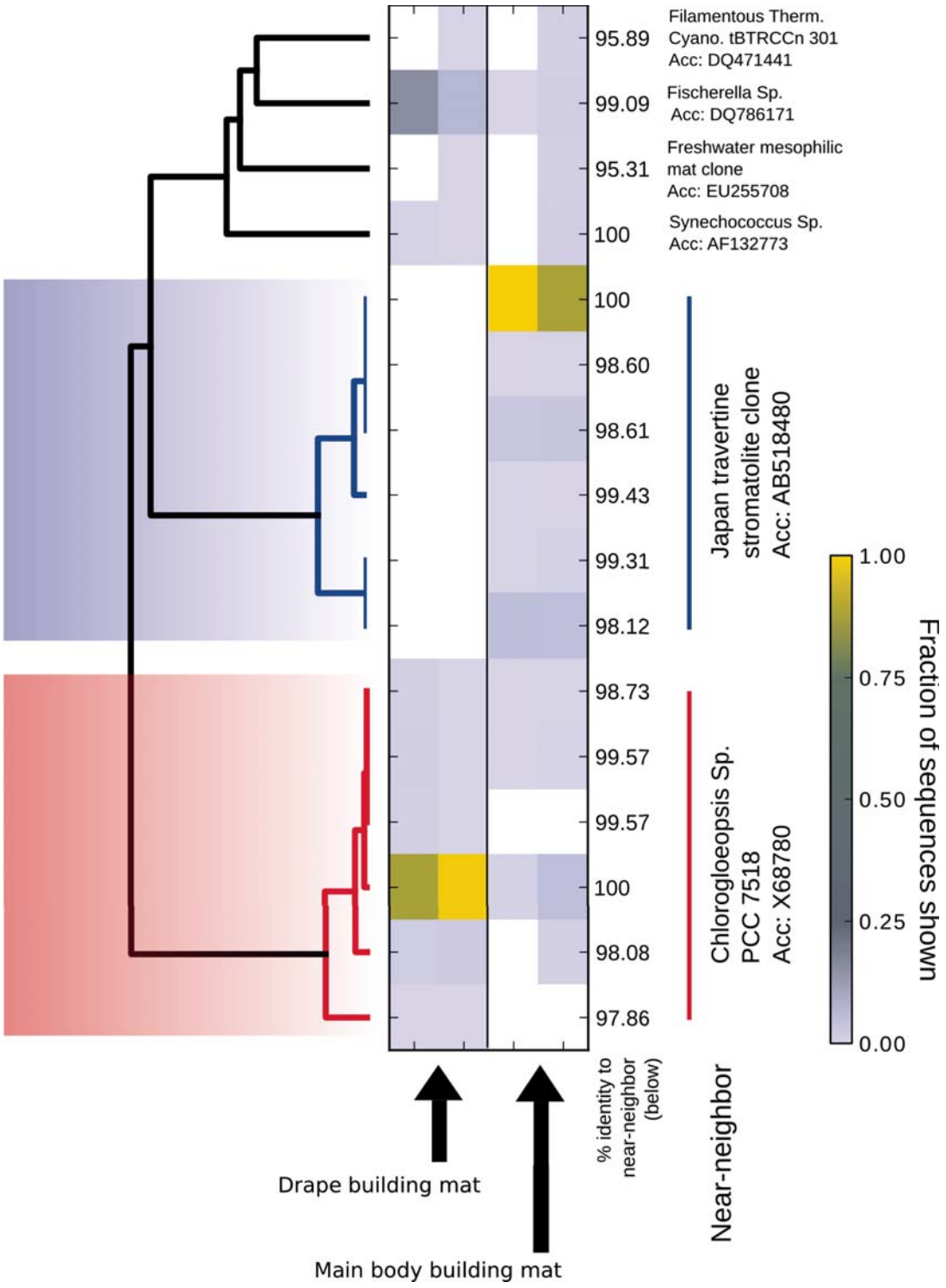

Figure 7. The microbiology of a living stromatolite. The Yellowstone National Park stromatolite shows two distinct lithofacies, the "drape" and the "main body." Each facies is built by a surface community that dominates the stromatolites under different geochemical conditions. The two surface communities occupy the same space; however, they are remarkably different. The heatmap (center) above shows the cyanobacterial 16S rRNA gene membership of two samples taken from each surface community. There is a profound shift in structure and membership from one community to the other, most notably in the highly abundant members. The dendrogram to the left of the heatmap is a clustering of each sequence by sequence identity. Notable near neighbors to each sequence shown are indicated to the right of the heatmap.

REFERENCES CITED

Adam, P., Schneckenburger, P., Schaeffer, P., and Albrecht, P., 2000, Clues to early diagenetic sulfurization processes from mild chemical cleavage of labile sulfur-rich geomacromolecules: Geochimica et Cosmochimica Acta, v. 64, p. 3485–3503, doi:10.1016/S0016-7037(00)00443-9.

Allewalt, J.P., Bateson, M.M., Revsbech, N.P., Slack, K., and Ward, D.M., 2006, Effect of temperature and light on growth of and photosynthesis by Synechococcus isolates typical of those predominating in the Octopus Spring microbial mat community of Yellowstone National Park: Applied and Environmental Microbiology, v. 72, p. 544–550, doi:10.1128/AEM.72.1.544-550.2006.

Allwood, A.C., Walter, M.R., Kamber, B.S., Marshall, C.P., and Burch, I.W., 2006, Stromatolite reef from the Early Archean era of Australia: Nature, v. 441, p. 714–718, doi:10.1038/nature04764.

Archer, C., and Vance, D., 2006, Coupled Fe and S isotope evidence for Archean microbial Fe(III) and sulfate reduction: Geology, v. 34, p. 153–156, doi:10.1130/G22067.1.

Awramik, S.M., and Riding, R.E., 1988, Role of algal eukaryote in subtidal columnar stromatolite formation: Proceedings of the National Academy of Sciences, USA, v. 85, p. 1327–1329, doi:10.1073/pnas.85.5.1327.

Awramik, S.M., and Vanyo, J.P., 1986, Heliotropism in modern stromatolites: Science, v. 231, p. 1279–1281, doi:10.1126/science.231.4743.1279.

Barghoorn, E.S., and Tyler, S.A., 1965, Microorganisms from the Gunflint Chert: These structurally preserved Precambrian fossils from Ontario are the most ancient organisms known: Science, v. 147, p. 563–575, doi:10.1126/science.147.3658.563.

Bell, D.C., Thomas, W.K., Murtagh, K.M., Dionne, C.A., Graham, A.C., Anderson, J.E., and Glover, W.R., 2012, DNA base identification by electron microscopy: Microscopy and Microanalysis, v. 18, p. 1049–1053, doi:10.1017/S1431927612012615.

Benning, L.G., Phoenix, V.R., and Mountain, B.W., 2005, Biosilicification: The role of cyanobacteria in silica sinter deposition, *in* Gadd, M.G., Semple, T.K., and Lappin-Scott, M.H., eds., Micro-organisms and Earth Systems: Advances in Geomicrobiology: Cambridge, UK, Cambridge University Press, Society for General Microbiology, 65th Symposium, p. 131–150.

Berelson, W.M., Corsetti, F.A., Pepe-Ranney, C., Hammond, D.E., Beaumont, W., and Spear, J.R., 2011, Hot spring siliceous stromatolites from Yellowstone National Park: Assessing growth rate and laminae formation: Geobiology, v. 9, p. 411–424.

Berner, R.A., and Canfield, D.E., 1989, A new model for atmospheric oxygen over Phanerozoic time: American Journal of Science, v. 289, p. 333–361, doi:10.2475/ajs.289.4.333.

Bhaya, D., Grossman, A.R., Steunou, A.-S., Khuri, N., Cohan, F.M., Hamamura, N., Melendrez, M.C., Bateson, M.M., Ward, D.M., and Heidelberg, J.F., 2007, Population level functional diversity in a microbial community revealed by comparative genomic and metagenomic analyses: ISME Journal, v. 1, p. 703–713, doi:10.1038/ismej.2007.46.

Blank, C.E., Cady, S.L., and Pace, N.R., 2002, Microbial composition of near-boiling silica-depositing thermal springs throughout Yellowstone National Park: Applied and Environmental Microbiology, v. 68, p. 5123–5135, doi:10.1128/AEM.68.10.5123-5135.2002.

Brassell, S.C., Eglinton, G., and Maxwell, J.R., 1983, The geochemistry of terpenoids and steroids: Biochemical Society Transactions, v. 11, p. 575–586.

Brocks, J.J., Logan, G.A., Buick, R., and Summons, R.E., 1999, Archean molecular fossils and the early rise of Eukaryotes: Science, v. 285, p. 1033–1036, doi:10.1126/science.285.5430.1033.

Bryant, D.A., Costas, A.M.G., Maresca, J.A., Chew, A.G.M., Klatt, C.G., Bateson, M.M., Tallon, L.J., Hostetler, J., Nelson, W.C., Heidelberg, J.F., and Ward, D.M., 2007, Candidatus Chloracidobacterium thermophilum: An aerobic phototrophic Acidobacterium: Science, v. 317, p. 523–526, doi:10.1126/science.1143236.

Cady, S.L., and Farmer, J.D., 1996, Fossilization processes in siliceous thermal springs: Trends in preservation along thermal gradients, *in* Bock, G.R., and Goode, J.A., eds., Evolution of Hydrothermal Ecosystems on Earth (and Mars?): Ciba Foundation Symposium 202: Chichester, UK, Wiley, p. 150–173.

Canfield, D.E., 1989, Sulfate reduction and oxic respiration in marine sediments: Implications for organic carbon preservation in euxinic environments: Deep-Sea Research, pt. A, Oceanographic Research Papers, v. 36, p. 121–138, doi:10.1016/0198-0149(89)90022-8.

Caporaso, J., Lauber, C., Walters, W., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G., and Knight, R., 2012, Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms: ISME Journal, v. 6, p. 1621–1624, doi:10.1038/ismej.2012.8.

Chivas, A.R., Torgersen, T., and Polach, H.A., 1990, Growth rates and Holocene development of stromatolites from Shark Bay, Western Australia: Australian Journal of Earth Sciences, v. 37, p. 113–121, doi:10.1080/08120099008727913.

DeLong, E.F., Wickham, G.S., and Pace, N.R., 1989, Phylogenetic stains: Ribosomal RNA-based probes for the identification of single cells: Science, v. 243, p. 1360–1363, doi:10.1126/science.2466341.

DesMarais, D.J., 2000, When did photosynthesis emerge Earth?: Science, v. 289, p. 1703–1705.

Dilek, Y., Furnes, H., and Muehlenbachs, K., 2008, Links Between Geological Processes, Microbial Activities & Evolution of Life: New York, Springer-Verlag, 348 p.

Dill, R.F., Shinn, E.A., Jones, A.T., Kelly, K., and Steinen, R.P., 1986, Giant subtidal stromatolites forming in normal salinity waters: Nature, v. 324, p. 55–58, doi:10.1038/324055a0.

Doemel, W.N., and Brock, T.D., 1974, Bacterial stromatolites: Origin of laminations: Science, v. 184, p. 1083–1085, doi:10.1126/science.184.4141.1083.

Dravis, J.J., 1983, Hardened subtidal stromatolites, Bahamas: Science, v. 219, p. 385–386, doi:10.1126/science.219.4583.385.

Eiler, J.M., and Schauble, E., 2004, (OCO)-O-18-C-13-O-16 in Earth's atmosphere: Geochimica et Cosmochimica Acta, v. 68, p. 4767–4777, doi:10.1016/j.gca.2004.05.035.

Ferris, M.J., Kuhl, M., Wieland, A., and Ward, D.M., 2003, Cyanobacterial ecotypes in different optical microenvironments of a 68 degree C hot spring mat community revealed by 16S–23S rRNA internal transcribed spacer region variation: Applied and Environmental Microbiology, v. 69, p. 2893–2898, doi:10.1128/AEM.69.5.2893-2898.2003.

Fike, D.A., Grotzinger, J.P., Pratt, L.M., and Summons, R.E., 2006, Oxidation of the Ediacaran ocean: Nature, v. 444, p. 744–747, doi:10.1038/nature05345.

Fouke, B.W., 2011, Hot-spring systems geobiology: Abiotic and biotic influences on travertine formation at Mammoth Hot Springs, Yellowstone National Park, USA: Sedimentology, v. 58, p. 170–219, doi:10.1111/j.1365-3091.2010.01209.x.

Gaines, S.M., Eglinton, G., and Rullkotter, J., 2009, Echoes of Life: What Fossil Molecules Reveal about Earth History: Oxford, UK, Oxford University Press, 376 p.

Galvez, A., Marqueda, M., Martinez-Bueno, M., and Valdivia, M., 1998, Publication rates reveal trends in microbiological research: ASM News, v. 64, p. 269–275.

Grotzinger, J.P., and Knoll, A.H., 1999, Stromatolites in Precambrian carbonates; evolutionary mileposts or environmental dipsticks?: Annual Review of Earth and Planetary Sciences, v. 27, p. 313–358, doi:10.1146/annurev.earth.27.1.313.

Grotzinger, J.P., and Rothman, D.H., 1996, An abiotic model for stromatolite morphogenesis: Nature, v. 383, p. 423–425, doi:10.1038/383423a0.

Guidry, S.A., and Chafetz, H.S., 2003, Depositional facies and diagenetic alteration in a relict siliceous hot-spring accumulation: Examples from Yellowstone National Park, U.S.A.: Journal of Sedimentary Research, v. 73, p. 806–823, doi:10.1306/022803730806.

Hedges, J.I., and Keil, R.G., 1995, Sedimentary organic matter preservation: An assessment and speculative synthesis: Marine Chemistry, v. 49, p. 81–115, doi:10.1016/0304-4203(95)00008-F.

Hoffman, P., and Walter, M.R., 1976. Stromatolite morphogenesis in Shark Bay, Western Australia, *in* Walter, M.R., ed., Stromatolites: Amsterdam, Elsevier, p. 261–271.

Hofmann, H.J., Grey, K., Hickman, A.H., and Thorpe, R.I., 1999, Origin of 3.45 Ga coniform stromatolites in Warrawoona Group, Western Australia: Geological Society of America Bulletin, v. 111, p. 1256–1262, doi:10.1130/0016-7606(1999)111<1256:OOGCSI>2.3.CO;2.

Johnston, D.T., 2011, Multiple sulfur isotopes and the evolution of Earth's surface sulfur cycle: Earth-Science Reviews, v. 106, p. 161–183, doi:10.1016/j.earscirev.2011.02.003.

Johnston, D.T., and Fischer, W.W., 2012, Stable isotope geobiology, *in* Knoll, A.H., Canfield, D.E., and Konhauser, K.O., eds., Fundamentals of Geobiology: Oxford, UK, Wiley Blackwell, doi:10.1002/9781118280874.ch14.

Johnston, D.T., Farquhar, J., Habicht, K.S., and Canfield, D.E., 2008, Sulfur isotopes and the search for life: Strategies for identifying sulfur metabolisms in the rock record and beyond: Geobiology, v. 6, p. 425–435, doi:10.1111/j.1472-4669.2008.00171.x.

Klatt, C.G., Bryant, D.A., and Ward, D.M., 2007, Comparative genomics provides evidence for the 3-hydroxypropionate autotrophic pathway in filamentous anoxygenic phototrophic bacteria and in hot spring microbial mats: Environmental Microbiology, v. 9, p. 2067–2078, doi:10.1111/j.1462-2920.2007.01323.x.

Konhauser, K., 2007, Introduction to Geomicrobiology: Malden, Massachusetts, Blackwell, 440 p.

Konhauser, K.O., Jones, B., Reysenbach, A.L., and Renaut, R.W., 2003, Hot spring sinters: Keys to understanding Earth's earliest life forms: Canadian Journal of Earth Sciences, v. 40, p. 1713–1724, doi:10.1139/e03-059.

Konhauser, K., Jones, B., Phoenix, V., Ferris, G., and Renaut, R., 2004, The microbial role in hot spring silicification: Ambio, v. 33, p. 552–558.

Logan, B.W., 1961, Cryptozoon and associated stromatolites from the Recent, Shark bay, Western Australia: Journal of Geology, v. 69, p. 517–533, doi:10.1086/626769.

Madigan, M.T., Martinko, J.M., Stahl, D.A., and Clark, D.P., 2012, Brock: Biology of Microorganisms: Boston, Benjamin Cummings, 425 p.

Mata, S.A., Harwood, C.L., Corsetti, F.A., Stork, N.J., Eilers, K., Pepe-Ranney, C., Berelson, W.M., and Spear, J.R., 2012, Influences of gas production and filament orientation on stromatolite microfabric: Palaios, v. 27, p. 206–219, doi:10.2110/palo.2011.p11-088r.

McFadden, K.A., Huang, J., Chu, X., Jiang, G., Kaufman, A.J., Zhou, C., Yuan, X., and Xiao, S., 2008, Pulsed oxidation and biological evolution in the Ediacaran Doushantuo formation: Proceedings of the National Academy of Sciences, USA, v. 105, p. 3197–3202, doi:10.1073/pnas.0708336105.

McLoughlin, N.A., Wilson, L.A., and Brasier, M.D., 2008, Growth of synthetic stromatolites and wrinkle structures in the absence of microbes; implications for the early fossil record: Geobiology, v. 6, p. 95–105, doi:10.1111/j.1472-4669.2007.00141.x.

Narasingarao, P., Podell, S., Ugalde, J.A., Brochier-Armanet, C., Emerson, J.B., Brocks, J.J., Heidelberg, K.B., Banfield, J.F., and Allen, E.E., 2012, De novo metagenomic assembly reveals abundant novel major lineage of Archaea in hypersaline microbial communities: ISME Journal, v. 6, p. 81–93, doi:10.1038/ismej.2011.78.

Odum, J.M., and Singleton, R., 1993, The Sulfate Reducing Bacteria: Contemporary Perspectives: New York, Springer-Verlag, 310 p.

Osburn, M.R., Sessions, A.L., Pepe-Ranney, C., and Spear, J.R., 2011, Hydrogen-isotopic variability in fatty acids from Yellowstone National Park hot spring microbial communities: Geochimica et Cosmochimica Acta, v. 75, p. 4830–4845, doi:10.1016/j.gca.2011.05.038.

Pace, N.R., 1997, A molecular view of microbial diversity and the biosphere: Science, v. 276, p. 734–740, doi:10.1126/science.276.5313.734.

Pace, N.R., 2009, Mapping the tree of life: Progress and prospects: Microbiology and Molecular Biology Reviews, v. 73, p. 565–576, doi:10.1128/MMBR.00033-09.

Pepe-Ranney, C., Berelson, W.M., Corsetti, F.A., Treants, M., and Spear, J.R., 2012, Cyanobacterial construction of hot spring siliceous stromatolites in Yellowstone National Park: Environmental Microbiology, v. 14, p. 1182–1197, doi:10.1111/j.1462-2920.2012.02698.x.

Pernthaler, A., Pernthaler, J., and Amann, R., 2002, Fluorescence in situ hybridization and catalyzed reporter deposition for the identification of marine bacteria: Applied and Environmental Microbiology, v. 68, p. 3094–3101, doi:10.1128/AEM.68.6.3094-3101.2002.

Peters, K.E., Walters, C.C., and Moldowan, J.M., 2005, The Biomarker Guide: Cambridge, UK, Cambridge University Press, 1155 p.

Postgate, J.R., 1984, The Sulphate-Reducing Bacteria (2nd edition): Cambridge, UK, Cambridge University Press, 151 p.

Rashby, S.E., Sessions, A.L., Summons, R.E., and Newman, D.K., 2007, Biosynthesis of 2-methylbacteriohopanepolyols by an anoxygenic phototroph: Proceedings of the National Academy of Sciences, USA, v. 104, p. 15,099–15,104, doi:10.1073/pnas.0704912104.

Reid, R.P., Visscher, P.T., Decho, A.W., Stolz, J.F., Bebout, B.M., Dupraz, C., Macintyre, I.G., Paerl, H.W., Pinckney, J.L., Prufert-Bebout, L., Steppe, T.F., and Des Marais, D.J., 2000, The role of microbes in accretion, lamination and early lithification of modern marine stromatolites: Nature, v. 406, p. 989–992, doi:10.1038/35023158.

Riding, R.E., 1990, Calcareous Algae and Stromatolites: New York, Springer.

Riding, R.E., and Awramik, S.M., 2000, Microbial Sediments: New York, Springer-Verlag, 331 p.

Ross, K.A., Feazel, L.M., Robertson, C.E., Fathepoure, B.Z., Wright, K.E., Turk, R.M., Chan, M.M., Held, N.L., Spear, J.R., and Pace, N.R., 2012, Phototrophic phylotypes dominate microbial mats of hotspring mesothermic zones in Yellowstone National Park: Microbial Ecology, v. 64, no. 1, p. 162–170, doi:10.1007/s00248-012-0012-3.

Sahl, J.W., Schmidt, R., Swanner, E.D., Mandernack, K.W., Templeton, A.S., Kieft, T.L., Smith, R.L., Sanford, W.E., Callaghan, R.L., Mitton, J.B., and Spear, J.R., 2008, Subsurface microbial diversity in deep granitic fracture water in Colorado: Applied and Environmental Microbiology, v. 74, p. 143–152, doi:10.1128/AEM.01133-07.

Shapiro, R.S., 2000, A comment on the systematic confusion of thrombolites: Palaios, v. 15, p. 166–169, doi:10.1669/0883-1351(2000)015<0166:ACOTSC>2.0.CO;2.

Shock, E.L., Holland, M., Meyer-Dombard, D.R., and Amend, J.P., 2005, Geochemical sources of energy for microbial metabolism in hydrothermal ecosystems: Obsidian Pool, Yellowstone National Park, USA, *in* Inskeep, W., and McDermott, T., eds., Geothermal Biology and Geochemistry of Yellowstone National Park: Thermal Biology Institute, Montana State University, p. 95–112.

Sogin, M.L., Morrison, H.G., Huber, J.A., Welch, D.M., Huse, S.M., Neal, P.R., Arrieta, J.M., and Herndl, G.J., 2006, Microbial diversity in the deep sea and the underexplored "rare biosphere": Proceedings of the National Academy of Sciences of USA, v. 103, p. 12,115–12,120, doi:10.1073/pnas.0605127103.

Spear, J.R., Figueroa, L.A., and Honeyman, B.D., 1999, Modeling the removal of uranium (U(VI)) from aqueous solutions in the presence of sulfate reducing bacteria: Environmental Science & Technology, v. 33, p. 2667–2675, doi:10.1021/es981241y.

Spear, J.R., Figueroa, L.A., and Honeyman, B.D., 2000, Modeling reduction of U(VI) under variable sulfate concentrations by sulfate-reducing bacteria: Applied and Environmental Microbiology, v. 66, p. 3711–3721, doi:10.1128/AEM.66.9.3711-3721.2000.

Spear, J.R., Walker, J.J., McCollom, T., and Pace, N.R., 2005, From the cover: Hydrogen and bioenergetics in the Yellowstone geothermal ecosystem: Proceedings of the National Academy of Sciences of USA, v. 102, p. 2555–2560, doi:10.1073/pnas.0409574102.

Spear, J.R., Barton, H.A., Robertson, C.E., Francis, C.A., and Pace, N.R., 2007, Microbial community biofabrics in a geothermal mine adit: Applied and Environmental Microbiology, v. 73, p. 6172–6180, doi:10.1128/AEM.00393-07.

St. Amand, A.L., Frank, D.N., De Groote, M.A., and Pace, N.R., 2005, Use of specific rRNA oligonucleotide probes for microscopic detection of Mycobacterium avium complex organisms in tissue: Applied and Environmental Microbiology, v. 43, p. 1505–1514.

Steunou, A.-S., Bhaya, D., Bateson, M.M., Melendrez, M.C., Ward, D.M., Brecht, E., Peters, J.W., Kühl, M., and Grossman, A.R., 2006, In situ analysis of nitrogen fixation and metabolic switching in unicellular thermophilic cyanobacteria inhabiting hot spring microbial mats: Proceedings of the National Academy of Sciences of USA, v. 103, p. 2398–2403, doi:10.1073/pnas.0507513103.

Steunou, A.-S., Jensen, S.I., Brecht, E., Becraft, E.D., Bateson, M.M., Kilian, O., Bhaya, D., Ward, D.M., Peters, J.W., Grossman, A.R., and Kuhl, M., 2008, Regulation of *nif* gene expression and the energetics of N_2 fixation over the diel cycle in a hot spring microbial mat: ISME Journal, v. 2, p. 364–378, doi:10.1038/ismej.2007.117.

Summons, R.E., and Lincoln, S.A., 2012, Biomarkers: Informative molecules for studies in geobiology, *in* Knoll, A.H., Canfield, D.E., and Konhauser, K.O., eds., Fundamentals of Geobiology: Oxford, UK, Wiley Blackwell, doi:10.1002/9781118280874.ch14.

Summons, R.E., Jahnke, L.L., Hope, J.M., and Logan, G.A., 1999, 2-Methylhopanoids as biomarkers for cyanobacterial oxygen photosynthesis: Nature, v. 400, p. 554–557, doi:10.1038/23005.

Tyson, G.W., Chapman, J., Hugenholtz, P., Allen, E.E., Ram, R.J., Richardson, P.M., Solovyev, V.V., Rubin, E.M., Rokhsar, D.S., and Banfield, J.F., 2004, Community structure and metabolism through reconstruction of microbial genomes from the environment: Nature, v. 428, p. 37–43, doi:10.1038/nature02340.

Updegraff, D.M., and Wren, G.B., 1954, The release of oil from petroleum-bearing minerals by sulfate-reducing bacteria: Applied Microbiology, v. 2, p. 309–322.

Urey, H.C., 1947, The thermodynamic properties of isotopic substances: Journal of the Chemical Society, p. 562–581, doi:10.1039/jr9470000562.

van der Meer, M.T.J., Schouten, S., Sinninghe-Damsté, J.S., and Ward, D.M., 2007, Impact of carbon metabolism on ^{13}C signatures of cyanobacteria and green non-sulfur-like bacteria inhabiting a microbial mat from an alkaline siliceous hot spring in Yellowstone National Park (USA): Environmental Microbiology, v. 9, p. 482–491, doi:10.1111/j.1462-2920.2006.01165.x.

Waldbauer, J.R., Sherman, L.S., Sumner, D.Y., and Summons, R.E., 2009, Late Archean molecular fossils from the Transvaal Supergroup record the antiquity of microbial diversity and aerobiosis: Precambrian Research, v. 169, p. 28–47, doi:10.1016/j.precamres.2008.10.011.

Walker, J.J., Spear, J.R., and Pace, N.R., 2005, Geobiology of a microbial endolithic community in the Yellowstone geothermal environment: Nature, v. 434, p. 1011–1014, doi:10.1038/nature03447.

Walter, M.E., editor, 1976, Stromatolites, *in* Developments in Sedimentology 20: Amsterdam, Elsevier, 790 p.

Walter, M.R., Bauld, J., and Brock, T.D., 1972, Siliceous algal and bacterial stromatolites in hot spring and geyser effluents of Yellowstone National Park: Science, v. 178, p. 402–405, doi:10.1126/science.178.4059.402.

Weed, W.H., 1889, Formation of travertine and siliceous sinter by the vegetation of hot springs, *in* Powell, J.W., Director, Ninth Annual Report of the United States Geological Survey to the Secretary of the Interior, 1887–1888: U.S. Geological Survey, p. 613–676.

Woese, C.R., 1987, Bacterial evolution: Microbiological Reviews, v. 51, p. 221–271.

Woese, C., 1998, The universal ancestor: Proceedings of the National Academy of Sciences of USA, v. 95, p. 6854–6859, doi:10.1073/pnas.95.12.6854.

Woese, C.R., 2000, Interpreting the universal phylogenetic tree: Proceedings of the National Academy of Sciences of USA, v. 97, p. 8392–8396, doi:10.1073/pnas.97.15.8392.

Woese, C.R., 2002, On the evolution of cells: Proceedings of the National Academy of Sciences of USA, v. 99, p. 8742–8747, doi:10.1073/pnas.132266999.

Zuckerkandl, E., and Pauling, L., 1965, Molecules as documents of evolutionary history: Journal of Theoretical Biology, v. 8, p. 357–366, doi:10.1016/0022-5193(65)90083-4.

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