

Microbiology in Yellowstone National Park

Yellowstone, a hotspot for unusual research in microbiology, also provides a setting for outreach education

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Yellowstone National Park plays host to numerous microbiological research projects — officially 23, according to the 1996 Investigators' Annual Reports. However, once categories such as aquatic ecology, the study of brucellosis and other wildlife diseases, environmental education, geochemistry and geothermal systems, and vegetation are included, the total came to at least 35 in 1996. Research projects in other scientific disciplines, including geochemistry, geology and environmental monitoring, fire impacts, and even studies of ungulates such as bison and elk, or of other mammals such as bears and wolves, are far fewer in overall numbers.

Yellowstone is one place where the importance of microorganisms is immediately noticed. Despite the park's charismatic megafauna, plentiful examples of fire's impact on megaflore, and its renowned geothermal features, Yellowstone is one place where the importance of microorganisms is immediately noticeable — even if not fully appreciated. As pointed out by my former research associate, Niels Ramsing of the University of Århus in Denmark, few who come to Yellowstone ever see a bear or wolf, while nearly everyone sees — but rarely appreciates — microorganisms that thrive in near-boiling water. If appropriately in-formed, however, some of the 3 million annual tourists may begin to ponder why these microorganisms live in water too hot for humans, why they exhibit more genetic diversity than all plants and animals, and that they may well represent the kinds of organisms from which all organisms, including plants and animals, evolved.



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Meanwhile, an avid contingent of park administrators and visitors with scientific training already are considerably interested in Yellowstone's microorganisms. On one level, this interest reflects a growing fascination with the opportunities for both academic and industrial microbiology research at Yellowstone. At another, it reflects concerns about how this research activity influences management of the park's microbiological resources. Beyond that, park officials, researchers, and educators also recognize that the noteworthy microbiota present opportunities for changing the way the public thinks about microorganisms.

Extensive Research Raises Questions for Park Officials

Most of the more than 60 investigators conducting research in Yellowstone Park are from universities and similar research institutes. Many others come from government agencies with basic research

FIGURE 1

interests, such as the National Aeronautics and Space Administration (NASA); with industrial or applied research interests, such as the Idaho National Energy Laboratory, the National Renewable Energy Laboratory, and the Alberta Research Council; or with natural resource management interests, including the U.S. Geological Survey and the National Park Service.

Of greatest concern to park managers worried about preserving the integrity of the park environment are the 11 projects sponsored by biotechnology companies, whose interests revolve around Yellowstone's thermophilic microorganisms and their potential to yield thermostable enzymes or unusual metabolites. The steadily growing interest in further exploiting such park resources faces officials who manage them with a serious challenge.

To assess available information about Yellowstone thermophiles and to discuss some of the broader issues involved in managing the park's microbial resources, park officials asked Anna-Louise Reysenbach of Rutgers University to organize a meeting several years ago. That conference, "Biodiversity, Ecology and Evolution of Thermophiles in Yellowstone National Park: Overview and Issues," held at Old Faithful in September 1995, helped to frame many of these complex issues.

In 1996, the World Foundation for Environment and Development (WFED), a non-profit organization based in Washington, D.C., that specializes in resolving environmental disputes, convened a follow-up workshop for park managers and academic and industry scientists. By holding that workshop in Costa Rica, participants experienced first-hand how that country, through the INBIO (Instituto Nacional de Bioversidad) organization, promotes a new paradigm for conservation that is based on renewable resource use by private industry combined with information flow to and profit sharing with the public.

Based on such discussions and the development of strategies since the two conferences, officials from Yellowstone National Park entered into a prototype relationship with [Diversa Corp.](#) of San Diego, Calif. This agreement exchanges access to thermophilic microbial resources for information learned from molecular studies of microbial diversity in Yellowstone along with a share in profits through royalties, which are to be used to improve the management of park resources.

Main Microbiology Focus is Extremophiles



The effluent channel of Twin Butte Vista Spring, a typical alkaline siliceous spring (background) with cyanobacterial mat in foreground. Note "V-shaped" green coloration, which marks upper temperature limit in effluent channel (ca. 74°C).

FIGURE 2



Close-up view of cyanobacterial mat sample (Octopus Spring, ca. 60°C), showing laminations that resemble those found in stromatolites.

Microbiological research activities at Yellowstone intensified several decades ago. My Ph.D. mentor Thomas D. Brock at the University of Wisconsin, Madison, and other microbiologists, notably Richard W. Castenholz at the University of Oregon, Eugene, established a tradition at Yellowstone through their intensive efforts in the 1960s and 1970s to cultivate thermophiles and to conduct direct ecophysiological studies on indigenous thermophilic microbial communities. That research laid a solid foundation to our knowledge of the diversity, ecology, and physiology of thermophilic microorganisms.

Though the majority of the microbiological research projects under way in Yellowstone involves basic studies on extremophiles, a few do not. For instance, two projects address the possibility of brucellosis transmission from bison to cattle (see p. 132). Two other projects involve microbe-plant associations, with one focusing on microorganisms in the rhizosphere of plants adapted to acid and thermal soils and the other on microorganisms which grow as epiphytes on plant surfaces. Several of the applied microbiology projects involving extremophiles are concerned with catalytic processes, such as denitrification, fossil fuel modification, chromate reduction, metal decontamination, metal resistance, and desulfurization.

FIGURE 3



Pink streamers of filamentous bacteria at ca. 85°C at Octopus Spring.

Most of the basic studies on microorganisms from Yellowstone involve prokaryotes. However, a few projects focus on eukaryotic diatoms and fungi, and one project focuses on thermophilic viruses. Even though several of the unusual thermoacidophiles, such as *Sulfolobus acidocaldarius*, obtained from Yellowstone hot springs are members of the archaea, only two current projects specifically focus on members of this evolutionarily novel group of microorganisms. Most Yellowstone-based microbiological research projects focus on bacteria, including cyanobacteria, anoxygenic phototrophs, and aerobic and anaerobic chemoorganotrophs. Following the tradition established by Brock and Castenholz, these range from descriptions of new isolates to studies of their physiology and genetics to ecophysiological studies of their activities in situ. For example, one of the studies from my laboratory involves detailed analysis of photosynthesis within cyanobacterial mat populations. Robert Lindstrom, a staff member of Yellowstone's Center for Resources and the park's microbiology liaison officer, is compiling a database derived from such studies of the thermophilic microorganisms of Yellowstone.

Molecular Methods Being Used To Explore Diversity of Uncultivated Yellowstone Microbes

The remarkable discoveries about evolution made by Carl Woese of the University of Illinois and other microbiologists since the late 1970s have significantly affected research on extremophiles from habitats such as those in Yellowstone. For example, 16S rRNA-based molecular methods that played an integral role in those discoveries are being used to analyze microorganisms that grow in hot spring habitats but resist cultivation in laboratories. Such analytical methods help to reveal the remarkable diversity of these microorganisms that exists in nature.

The work of Woese and others led to the hypothesis that hyperthermophiles are among the earliest organisms to have evolved. This hypothesis has become a major driving force behind the efforts of

Norman Pace of the University of California, Berkeley, and his collaborators to describe more fully the microbial diversity in Yellowstone's most extreme thermal environments. They recently reported finding diverse new lineages of archaea in a single hot spring source pool. Karl Stetter and his colleagues have used novel cultivation approaches to bring some of these microorganisms from hot spring habitats into pure culture.

Similarly, Reysenbach and her colleagues at Rutgers University have discovered new descendants of some of the earliest known bacterial lineages, such as Thermotogales and Aquifexales, in hyperthermophilic "streamer" communities. They are conducting in situ ecophysiology studies that may soon lead to cultivation of representative microorganisms from such communities.

Diversity Explained by Universal Principles of Ecology and Evolution

My students and I are using similar molecular approaches to study the evolutionary and ecological basis for the diversity within some of these microbiological communities, such as the cyanobacterial mats found in the alkaline siliceous Octopus Spring in Yellowstone. The diversity of uncultivated prokaryotes within such microbial communities is truly remarkable. For example, of 31 unique 16S rRNA sequences detected in the Octopus Spring mat, none match the 16S rRNA sequence of any previously cultivated isolate.

We have recently revised our estimates of diversity among the cyanobacteria dramatically upward. A decade ago, when researchers observed a single morphotype microscopically and could cultivate only a single genotype by standard methods, they reported that a single *Synechococcus* species made up the mat. We now have detected at least 11 distinct cyanobacterial 16S rRNA sequences in the mat, and their genetic diversity rivals that found among all cyanobacteria.

Culture and molecular methods give such different views of community composition in large part because readily cultivated bacteria are "lab weeds," and thus we have had to learn how to exclude them to cultivate predominant species which are more difficult to grow.

As ecologists, we are trying to understand why all this diversity exists, how it is organized, and what it contributes to overall community structure and function. Patterns of distribution of specific 16S rRNA sequences along environmental gradients clarify the relationship between 16S rRNA sequence diversity and organismic diversity, and we can see that bacteria obey ecological and evolutionary rules that apply to all species. For example, both cyanobacteria and green non-sulfur bacteria inhabiting the mat appear to have undergone evolutionary radiations, much like Darwin's Galapagos finches, with species adapted differently to temperature and light. We are also beginning to see that diversity measurements based on 16S rRNA sequences underestimate microbial species diversity.

According to Michael Kuhl and colleagues at the Max Planck Institute for Marine Microbial Ecology, Bremen, Germany, species diversity may be important to stabilizing guild activities within the community. They have observed that the cyanobacteria that coexist in the mat are adapted to different temperatures. These cyanobacteria reduce the sensitivity of oxygenic photosynthesis, the guild activity to which they contribute, to temperature fluctuations.

Park Provides Insights into the History of Life on Earth (and Mars?)

Yellowstone provides settings that are of special value to microbiologists who are addressing questions of intense interest in exobiology. For example, David Des Marais, Jack Farmer, and their collaborators at NASA Ames Research Center in Moffett Field, Calif., speculate that life existed on early Mars in geothermal settings. To model martian environments and the evidence of microbial life that might be found there, Des Marais and Farmer are studying how thermophilic microbial communities occur in relation to the geothermal landscape and how they are preserved as fossils. Such studies will influence site selection and sample collection on future missions to Mars.

Other scientists are working in Yellowstone to probe other issues concerning ancient microbiology. For instance, I am collaborating with organic geochemists Geoffrey Eglinton at Bristol University, in Bristol, United Kingdom, and Jan de Leeuw at the Netherlands Institute for Sea Research, Texel, the Netherlands,

studying the relationship between mat-forming microorganisms and their recalcitrant organic remains. We are trying to understand better how these "chemical fossils" and their stable isotope signatures are preserved in mat communities, considered to be analogs of stromatolites, the predominant fossils of the Precambrian era. Some efforts involve investigating Chloroflexus mats found in sulfide-rich springs in Yellowstone Park. These anoxygenic phototrophs seem to have arisen before cyanobacteria. If true, the mats they formed would predate cyanobacterial mats.

Similarly, Beverly Pierson from the University of Puget Sound in Tacoma, Wash., is investigating the relationship between photosynthetic prokaryotes and minerals found in iron-rich springs. She hopes to determine whether banded iron formations constitute a "chemical finger-print" of oxygen derived from cyanobacterial photosynthesis.

Yellowstone Also Provides Valuable Setting for Educators

Educators find Yellowstone a valuable setting for science teaching projects. Included in this category are high school student projects, a workshop for high school educators, an undergraduate program sponsored by the National Science Foundation, and a microbial diversity field course for graduate students.

Yellowstone is also being used to educate the public through several outreach projects. For example, WFED is coordinating production of a video for educating park visitors about the microbial life associated with the many thermal features of the park. Meanwhile, Crest Films of Santa Monica, Calif., is producing a film on western national parks for the Discovery Channel that includes a segment on Yellowstone's charismatic microbiota. Kurtis Productions, Ltd. of Chicago, Ill., is producing an hour-long New Explorer's program, "From Yellowstone to Mars," for the Arts and Education Network that features thermophiles. Both programs are scheduled to air in spring 1998, the latter program being tentatively scheduled for 2 April.

WFED, in conjunction with ASM, is producing a guidebook for the Midway Geyser Basin that will introduce tourists to the microbiological features there. These kinds of projects have the potential to change the way the public thinks about microorganisms, providing a more comprehensive and naturalistic view of microorganisms instead of the conventional view of microbes as "germs."

This article is dedicated to Yellowstone's "chief geyser gazer," Rick Hutchinson, whose tragic death last year will be a setback to all microbiologists working in the park. A man of numbers, Rick wondered about the number of days till Christmas, about the number of snow fleas beneath his skis, and about the diverse unexplored thermal habitats of Yellowstone that will undoubtedly occupy the interests of microbiologists as we proceed without his guidance.

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