



Codiscoverer of the polymerase chain reaction that is essential to forensic analysis and detection of DNA based diseases, David Gelfand collects hot water organisms from a Lower Geyser Basin hot spring. Researchers discovered *Thermus aquaticus* here in 1966, giving science and industry the source for the revolutionary DNA amplification technique and riches, topics of debate at the Yellowstone conference.

BIODIVERSITY, ECOLOGY, AND EVOLUTION OF HOT WATER ORGANISMS IN YELLOWSTONE NATIONAL PARK:

Symposium and Issues Overview

By BOB LINDSTROM

THE GREATEST CONCENTRATION of experts in the field of Yellowstone microbiology held a highly successful 4-day symposium at Old Faithful, September 17-20, 1995. Organized by the Yellowstone Center for Resources and aquanaut-microbiologist Anna-Louise Reysenbach of Rutgers University, the symposium aimed at increasing communication and establishing contacts among the academic, biotechnology, and resource management communities. Three government agencies (NASA, National Science Foundation, and the Department of Energy) and 17 biotech companies, all interested in advancing knowledge and generating research into the fascinating world of life at high temperatures, funded the symposium. The synergy, communication, and contacts fostered amongst scientists, biotech companies, and resource managers in this es-

teric research field went a long way toward elevating the profile of this important Yellowstone natural resource.

The 4-day conference delved into the state of the art of research into Yellowstone microbial resources, the colorful prokaryotic inhabitants of the Yellowstone geothermal ecosystem. Attended by 110 scientists from around the world, the conference brought together micro- and molecular biologists whose primary focus is thermophilic (heat loving) microorganisms and their heat-stable enzymes, the protein macromolecules that make up the building blocks of life. A conference publication, being prepared by the American Society of Microbiology, will serve as a milestone, updating modern thermophilic research and synthesizing NPS management options towards commercial development of research specimens.

The keynote address by Dr. Thomas Brock, an introduction to modern Yellowstone microbiological research, included

his story behind the 1966 discovery of *Thermus aquaticus* (*Taq*). This was the first life-form detected growing above the known upper temperature limit for life, then believed to be 72° C (162° F). A heat-stable enzyme from this organism, *Taq*DNA polymerase, was essential in establishing the polymerase chain reaction (PCR) process. This DNA amplification technique revolutionized DNA science and earned its inventor, Kary B. Mullis, a Nobel prize in 1993. The gene expressing *Taq* polymerase was removed from specimen YT1-25104 (Yellowstone Type 1, deposited in the American Type Culture Collection as sample 25104) and inserted into *E. coli* ("microbial livestock") producing a genetically engineered organism called pLSG1. Polymerase chain reaction made possible a quantum leap in the DNA diagnostics industry including forensic analysis and detection of any type of DNA based disease; it grosses \$200 million per year for the patent holder, Hoffmann-LaRoche, a Swiss pharmaceutical company. According to David Gelfand (see photo this page), codiscoverer of PCR, in his presentation on DNA polymerase, "PCR generated revolutions within the revolution" in molecular biology by providing new tools in amplifying DNA. O.J. Simpson's PCR evidence, and Michael Crichton's Jurassic Park scenario are spin-offs of how PCR could be used to make millions of copies of DNA. Medical technology is perhaps the greatest beneficiary of PCR. For example, Amplification of the Human Immunodeficiency Virus (HIV) DNA provides the only reliable early detection of AIDS. Polymerase chain reaction allows scientists to create any quantity of any type of DNA at will, opening up to humankind what until now has been the elusive domain of fundamental natural processes.

Biological diversity represented in the Yellowstone thermophiles is of special interest to biotechnology companies. Since microbes can perform most biochemical reactions known, their enzymes are used in manufacturing chemicals, antibiotics, plastics, detergents, and fermentation products. The recent development of heat-stable enzymes is increasingly important to such companies as Lily,

Exxon, E.I. DuPont, Roche Molecular Systems, and the dozen others with representatives attending the conference. Thermostable enzymes lend themselves to vast industrial processes and are less susceptible to denaturation than their mesophilic (body temperature) counterparts.

A good example of what national parks contribute to society is habitat protection and resulting preservation of biological diversity. In the case of thermophiles, conservation has yielded great utilitarian value in the enzymatic diversity that has been preserved, inadvertently, along with the geological curiosities and wonders for which the park was established in 1872. The Yellowstone geothermal ecosystem consists of the world's greatest concentration of thermophilic biodiversity in its 10,000 thermal features, and the companies want to contribute to the preservation of this unique biosphere reserve. They discussed voluntary contributions, royalties, foundations, and user fees as means by which companies could financially support National Park Service resource management efforts. Such funding could sponsor public and legislator education (through interpretive presentations) as to the value of maintaining biological diver-

Old Faithful Protection Act, which would prohibit geothermal drilling activities within 15 miles of the park boundary.

A full day of presentations and roundtable discussions centered on the management of publicly owned resources and included active audience participation. The National Park Service does not encourage commercial development of natural resources within its jurisdiction. If, however, during the course of investigation, researchers make a commercially significant discovery, a means of sanctioning that discovery is now available through their research permit agreement with the superintendent and according to ongoing revisions in the Code of Federal Regulations. Intellectual property rights, patenting organisms, their products, and genes, trade secrets, and material transfer agreements are all issues related to research specimens that participants also addressed in their presentations and discussions. The symposium failed to reach consensus on royalties from profitable discoveries but did initiate a workshop entitled "Conservation and sustainable use of thermophilic microbial biodiversity at Yellowstone National Park: consensus building initiative" being conducted at the National Biodiversity Institute (Instituto Nacional Biodiversidad or INBio) of Costa Rica, January 20-27, 1996.

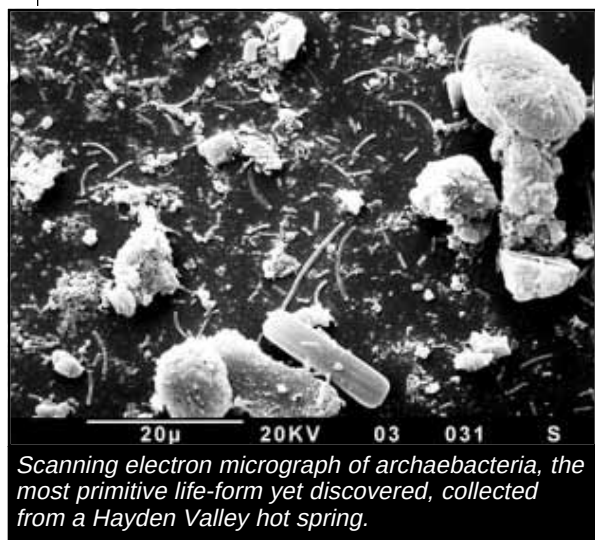
Thomas Lovejoy, science advisor to the Secretary of the Interior, spoke of Yellowstone thermophiles as "environmental extremists." Living in the extremes of temperature and pH, thermophiles clearly point out the importance of preserving biological diversity. He spoke of the biotechnology age (the interface of technology and biodiversity) where, through use of modern research, tremendous wealth is generated, exemplifying the utilitarian value of species preservation. He implied that in this era of hyperextinction, if we cannot preserve biodiversity

for the obvious ethical reasons, then we should preserve it for the potential unknown benefits to humankind, such as the polymerase chain reaction.

During his presentation, "The Biological Wealth of Nations," Dr. Lovejoy also introduced the concept of INBio, the Costa Rican quasi-government organization that funds biodiversity preservation through private sector cooperative agreements. In exchange for access to Costa Rican National Park genetic resources (and excellent public relations), companies such as Merck Pharmaceutical and Bristol-Meyers invest large sums on rain forest preservation. Although distinct, INBio has evolved a biodevelopment track record Yellowstone could draw upon in respect to thermophiles. Indeed, Ana Sittenfeld, Director of Biodiversity Prospecting at INBio, gave a presentation on this issue and offered to share their experience, a wealth of details, on how to manage microbial resources in Yellowstone. In a presentation on high-tech molecular approaches to assessing biodiversity, Eric Mathur, Director of Recombinant BioCatalysis, Inc., of La Jolla, California, linked resource preservation to private industry in these days of public fiscal austerity by saying that "if industry does not support preservation of biodiversity, it probably won't happen."

Natural history presentations of thermophiles included an outline by Dave Ward of Montana State University on microbial ecology and the impacts of increasing numbers of researchers on bacterial mats. Since the small samples (a few milliliters) needed to start tissue culture collections are usually gathered with tweezers, and since the high growth rates of thermophiles revegetate disturbances quickly, no long-term harm to the resource is apparent. Human impacts are monitored closely by resource managers who emphasized that the research community must police itself with respect to minimal sampling and minimal impact to the system. Research permits are granted on a yearly basis on the premise that "no harm" to the resource will result from the research.

Other presentations included newly discovered species by Jurgen Wiegel and Beverly Pierson. Their work is being included in the NPS database known as the



Scanning electron micrograph of archaeobacteria, the most primitive life-form yet discovered, collected from a Hayden Valley hot spring.

sity. Biotech companies could also support political activism in lobbying for passage of conservation efforts such as the

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thermophilic microorganism survey, a baseline inventory of these species which is being established on the World Wide Web. Other topics included physiology, distribution, evolution and techniques used to study these enigmatic creatures, most of which cannot be grown in tissue culture.

The symposium generated suggestions on how we might proceed with the Yellowstone microbiology program. They included the establishment of an independent blue-ribbon panel of senior scientists connected via an Internet list server. Representing the three constituencies of academia, industry and resource management, the expert panel could suggest solutions to commercial use issues, review technical material concerning research permits, develop or review protocols for field researchers working in the geothermal ecosystem, and provide insight into the long term preservation and management of this national treasure.

The symposium drew to a close with a field trip led by Dr. Brock to Black Sand Basin, within walking distance from Old Faithful. It was made clear that the private sector is enthusiastic about working with the NPS in formulating strategy on the preservation and maintenance of thermophilic biodiversity in Yellowstone National Park. With an estimated 99% of Yellowstone's thermophiles yet undiscovered, according to Norm Pace of Indiana University, who developed a new way of detecting enigmatic species called "phylogenetic analysis," our 40 ongoing microbiology research projects have much work to look forward to.

As with many other issues in conservation biology, Yellowstone has a long history of setting precedents. To me, the National Park Service conference organizer, the end of this symposium marks the beginning of the Yellowstone Microbiology Program, an initiative to preserve, protect, educate and attract funding for the benefit of all participants interested in this nontraditional, superheated field of resource management.

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Bob Lindstrom is Management Assistant with the Yellowstone Center for Resources. His phone number is (307) 344-2234.