Perspectives

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The Value of Basic Research: Discovery of *Thermus aquaticus* and Other Extreme Thermophiles

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POLYMERASE chain reaction (PCR), the revolutionary technique for DNA research, depends on Taq polymerase, an enzyme from *Thermus aquaticus*, an organism that I first isolated from a hot spring in Yellowstone National Park. The PCR technique is so important that Hoffmann-LaRoche, the giant Swiss pharmaceutical company, paid more than $300 million to acquire world rights for this procedure. Essentially, Hoffmann-LaRoche was acquiring the rights to two patents, those of GELFAND *et al.* (1989) on Taq polymerase and of MULLIS *et al.* (1990) on the PCR technique.

The importance of Taq polymerase was recognized by the White House in 1991. D. ALLAN BROMLEY, the Director of the Office of Science and Technology Policy and the President's chief science advisor, testified before the House of Representatives:

> Different kinds of research and development tend to have different kinds of returns. With basic research—the majority of which is done by individual scientists and small groups of scientists at universities—it is very difficult to predict when, where, and to whom the returns will eventually accrue. Yet even work that can seem highly abstract can have surprisingly immediate impacts. To take just one example, in 1968 Thomas Brock, a microbiologist at the University of Wisconsin, discovered a form of bacteria in the thermal vents of Yellowstone that can survive at very high temperature. From these bacteria an enzyme was extracted that is stable at near-boiling temperatures. Nearly two decades later this enzyme proved to be vital in the process known as the polymerase chain reaction, which is used to duplicate specific pieces of DNA. Today, PCR is the basis of a multimillion dollar business with applications ranging from the rapid diagnosis of disease to forensic medicine.[1]

In 1989 *Science* magazine (December 22, 1989 issue) established a new award called "The Molecule of the Year" award. Taq polymerase from *T. aquaticus* was designated the first awardee.

Discovery of *T. aquaticus*: I began my scientific career in 1951 as a microbial physiologist and did extensive work in molecular biology and microbial genetics through the mid-1960s. Although I found this work satisfying, I had always been interested in the outdoors. In 1963 my professional circumstances were such that I could branch out into new areas of research. It was at this time that I initiated what was to become a long-term research program in microbial ecology.[2] My ecological work focused on the study of microorganisms directly in their natural environments. I worked on a variety of habitats, including marine intertidal pools, freshwater lakes, and soils, but for 10 years I concentrated my research on hot springs and geysers, which I viewed as model systems for basic research in microbial ecology.

My discovery of *T. aquaticus* and other high-temperature bacteria could not have been made without studies directly in the natural environment in Yellowstone National Park. At the time I began this work, thermophilic bacteria were thought to have temperature optima of only 55°, and the upper temperature for life was stated as 73° (KEMPNER 1963). However, I was not initially interested in defining the upper temperature limits of life. My first Yellowstone work involved a study of the distribution of photosynthetic microorganisms (primarily cyanobacteria) along the thermal gradients created by the outflow channels of hot springs. The research was part of a broader study on the thermal control of photosynthesis and primary productivity in natural environments (BROCK and BROCK 1967, 1968). The hot springs were viewed as "steady state" ecosystems or what I came to call "experiments in nature" where critical ecological research could be carried out. High-temperature bacteria were an unexpected discovery.

I did my first research in Yellowstone National Park

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[1] Testimony of D. ALLAN BROMLEY, Director, Office of Science and Technology Policy, before the Committee on Science, Space, and Technology, House of Representatives, February 20, 1991. BROMLEY's statement should be corrected. The discovery was made in 1966 (published in 1969), and I was then at Indiana University in Bloomington.

[2] For background on my career, and how I came to work on thermophilic bacteria, see BROCK (1995a).

in June 1965 when I was Professor of Microbiology at Indiana University. I studied a large group of thermal pools, many of which had good effluents that provided thermal gradients from boiling down to ambient temperature. I was able to show that there was a definite upper temperature for photosynthetic life, at around 70–73°, but higher temperatures were not devoid of life. I observed that in the effluents of certain springs there were masses of filamentous bacteria living at temperatures much hotter than those at which photosynthetic organisms were present. One particular spring, Octopus Spring, had large amounts of pink filamentous bacteria at temperatures of 82–88°. Here were organisms living at temperatures above the reputed "upper temperature for life."

Attempts to cultivate these pink bacteria were initially unsuccessful, but during the next several years, as I continued broader work on the ecology of the Yellowstone springs, I continued to make occasional observations on these bacteria. At that time, I was doing a large amount of work on the photosynthetic microorganisms of Mushroom Spring, a large spring in the Lower Geyser Basin (BROCK 1967a). The source pool of this spring had a temperature of 73°, just at the known upper temperature limit for photosynthetic life. Because of the difficulty I had cultivating bacteria at temperatures above 80°, I decided to study the bacteria in Mushroom Spring that lived at 70–73°. Beginning in the fall of 1966, with undergraduate student HUDSON FREEZE, I began the work that was to lead to the discovery and culture of *T. aquaticus*. It was from a sample collected from Mushroom Spring on September 5, 1966 that culture YT-1 of *T. aquaticus* was isolated. This is the culture that is used today as the source of Taq polymerase for PCR and is the culture specified in the Taq polymerase patent (GELFAND *et al.* 1989).

HUDSON FREEZE and I isolated *T. aquaticus* culture YT-1 in October 1966. During various travels to study thermal areas in other parts of the world, I isolated a number of other cultures of *T. aquaticus*. An interesting culture was isolated from the hot water system of a building on the Indiana University campus. Subsequently, I was able to show that *T. aquaticus* was widespread in artificial hot-water environments, and other workers have isolated it from hot tap water in other parts of the world.

All of the cultures had an optimum temperature for growth at around 70° and were able to grow at temperatures up to 79°. FREEZE also showed that enzymes from *T. aquaticus* were able to tolerate temperatures higher than the maximum growth temperature, even surviving boiling water (FREEZE and BROCK 1970). Another student in my laboratory, J. GREGORY ZEIKUS, studied aspects of the protein-synthesizing system of *T. aquaticus* and showed that ribosomes and amino acid-activating enzymes were also active at high temperature (ZEIKUS *et al.* 1970; ZEIKUS and BROCK 1971).

By the fall of 1968, I had obtained a large number of cultures of *T. aquaticus* and had characterized them. Most of the thermophilic bacteria that had been described by earlier researchers were members of a group called the spore-forming bacteria (because they produced heat-resistant spores). The new bacterium was definitely not a spore-former, and it was clear that it was a member of a new genus of organisms.

In preparing a paper for publication, I needed to create a name for this new genus. I did an extensive survey of the literature of thermophilic bacteria, listing all the names that had been used over the years. The new name I first selected was *Caldobacter trichogenes,* reflecting the fact that the organism lived in hot water (*caldo* is Italian for hot), and under some conditions forms filaments (*tricho* derives from the Greek for filament or thread). However, after the first draft of the manuscript was written, I decided that this name was too fanciful. I chose instead the name *T. balnearius* (*Thermus* referring to heat and *balnearius* to a spa or thermal bath). Later, for reasons that I no longer remember, I replaced *balnearius* with *aquaticus* (*aquaticus* referring to water, where the bacterium grows). This last name, *T. aquaticus,* became official when the paper describing this organism was published (BROCK and FREEZE 1969).

The name for Taq polymerase is often abbreviated "Taq" in common usage. Is a name really a trivial matter? The word Taq rolls readily off the tongue and also fits well into molecular biology jargon and advertising copy. I suppose if I had kept my original name, *Caldobacter trichogenes,* the enzyme might be called "Cat!"

At the time that the paper on *T. aquaticus* was being written, I also deposited representative cultures of the organism in the American Type Culture Collection in Washington, D.C. Among these cultures was YT-1, the culture later to be used in PCR. Culture YT-1 became ATCC 25104. When DAVID GELFAND of Cetus conceived of the idea of using a thermophile as a source of DNA polymerase for PCR, he tested all of the available cultures of *T. aquaticus* from the American Type Culture Collection. Strain YT-1 turned out to have the enzyme with the best properties for PCR (D. GELFAND, personal communication).

Long before GELFAND's work on Taq polymerase, biochemists had started work on thermostable enzymes from *T. aquaticus*. Even before my first papers on *T. aquaticus,* I had been sending out cultures to biochemists who had seen a feature article I had written in Science (BROCK 1967b). A variety of thermostable enzymes were discovered. One enzyme that worked with DNA was Taq I restriction endonuclease (SATA *et al.* 1977). Also, JOHN TRELA at the University of Cincinnati described a thermostable DNA polymerase from *T. aquaticus* (EDGAR *et al.* 1975). Although there have been some fairly expensive legal arguments about whether TRELA's enzyme is the same Taq polymerase that GEL-

FAND *et al.* (1989) patented, the fact remains that there was a long history of research on thermostable enzymes from *T. aquaticus* before PCR was discovered.

It is fair to say, however, that it was PCR that put *T. aquaticus* on the map, and there has been a veritable "feeding frenzy" among the media on this subject. Television has particularly liked to discuss Taq polymerase in the context of forensic medicine, because footage of Old Faithful erupting can be shown. I have ceased trying to note all references in the popular literature to *T. aquaticus,* but a brief listing through mid-1995 can be found in BROCK (1995a).

In 1991, when I did a retrospective computer search, over 1000 papers on *T. aquaticus* had been published, and the number continues to increase. Truly, one does not know where a research study will lead!

Bacteria in boiling water: Although I had convinced myself in 1965 that bacteria were living at much higher temperatures than had been previously suspected, I had still not realized that they were living in "boiling" water. My ideas had been based only on observations in outflow channels where "visible" accumulations were present (such as the pink bacteria at temperatures above 80° in Octopus Spring). Culture studies on extreme thermophiles are technically difficult, and if I had relied on standard bacteriological isolation procedures I might never have been successful. Here is where the ecologist's approach, study of organisms directly in nature, comes to the fore.

Near the end of the summer of 1967 I started using a sensitive immersion slide technique to demonstrate microscopically the presence of living bacteria in boiling water. This immersion slide technique had been widely used by microbial ecologists studying "normal" environments. With this technique, I was able to show that although there were no visible accumulations of microorganisms in the boiling pools themselves, bacteria were present in microscopic amounts in virtually every boiling pool I looked in.

In 1967 I initiated a systematic search for the presence of bacteria in Yellowstone boiling springs. The experiment was simple: tie one or two microscope slides to a piece of string, drop in the pool, and tie the other end of the string to a log, rock, or nail. Return later, retrieve the slides, and examine under the microscope. The results were dramatic. Virtually every slide, from every boiling or superheated pool, had heavy bacterial growth, readily visible microscopically. In some cases, the density was so heavy that the slides had a film visible to the naked eye. The following summer (1968) THOMAS BOTT did an outstanding job of proving that these bacteria were really growing, by measuring their growth rates *in situ* (BOTT and BROCK 1969).

Because of the altitude, water boils at only 92.5° in Yellowstone. Once I knew that bacteria could live in boiling springs here, it was natural to plan field trips to thermal areas at lower altitudes, where higher temperatures could be found. I made visits to Iceland and New Zealand, where the boiling springs are at low altitudes and temperatures range up to 100° or a little higher in superheated waters. I was able to find bacteria in virtually every boiling spring of neutral or alkaline pH in these areas, thus extending to a somewhat higher value the upper temperature limit for life.

Deep sea vents: For many years my Yellowstone work had seemed somewhat "exotic" to many microbiologists, perhaps because of the presumed restricted distribution on earth of hot springs. This attitude changed after the discovery of the deep sea vents, with their very high temperatures and the associated diverse and flourishing life forms. Deep sea thermal vents are widespread in the oceans, usually associated with volcanic activity and tectonic movements of the earth. After the deep sea vents were discovered in the late 1970s, my Yellowstone work took on broader significance, because it not only made it reasonable to hypothesize that microbes might be present in some of these high temperature systems, it also provided the essential foundation for studies on the microbiology of thermal vents. The techniques and principles that I had developed for proving that bacteria live and reproduce in boiling water could then be applied to the deep sea habitats.

The upper temperature limit for life has still not been completely resolved, but the chance to study microorganisms living in deep sea vents, where temperatures range up to greater than 300°, has opened up a whole new approach. Although it is clear that no organisms exist at such high temperatures (even amino acids are not stable under these conditions), there are also thermal vents in the sea at temperatures between 100 and 150°. The German microbiologist KARL STETTER in particular has done an excellent job of isolating bacteria capable of growth at temperatures above 100° from some of these vents. One microorganism, *Pyrolobus fumarii,* has an optimum temperature for growth of 105° and can still grow at 113° (MADIGAN and MARRS 1997). (This organism finds 90° too cold!)

Microbial prospecting in Yellowstone: Although it was clear when I ended my project in 1975 that there were many interesting organisms with potential practical applications in hot springs, it was not until the advent of PCR that widespread attention really focused on thermophiles. Not only has the biotechnology industry discovered Yellowstone, but the National Park Service itself has finally realized that there is more of biological interest in Yellowstone than grizzly bears and lodgepole pines (MILSTEIN 1994). Dozens of research groups now have permits to collect microbial samples in Yellowstone. Although another discovery as valuable as Taq polymerase may not come out of this work, it is certainly possible that some of these groups may discover organisms of value. Never before has industry profited directly from living creatures taken from a national park, and the Yellowstone administrators are concerned

about whether the Park itself should participate in the largess. Yellowstone, of course, has no monopoly on thermophiles, but it provides the most accessible location where a wide variety of thermal habitats are available.

Also, thermophilic bacteria are now just one example of "extremophiles," microorganisms capable of living under extreme conditions. Other extreme habitats where interesting bacteria live are those with high or low pH, high salt, and low temperature. Extremophiles are suddenly "hot" research topics with the biotechnology industry (MADIGAN and MARRS 1997).

EPILOGUE

The work I have discussed here is just a small subset of the various research areas that I have been involved in throughout my career. A more detailed overview is given in my memoir for the *Annual Review of Microbiology* (BROCK 1995a) and in two supplements to this memoir (BROCK 1995b,c). However, of all the work I have been involved with, the Yellowstone project has been the most exciting and has continued to elicit the most interest from others. For 10 years I operated a field research laboratory in West Yellowstone, Montana, which provided facilities for research by a variety of students and postdoctorates, as well as visitors from across the country and around the world. Much of this work is discussed in a book (BROCK 1978) and in over 100 research and review papers (see BROCK 1995c for listing).

Thermostable enzymes were only a small part of this work, since my focus was not on biotechnology but on basic research. However, the new microorganisms that have been discovered in Yellowstone and elsewhere around the world have been made available to the scientific community. Only one of these organisms led to PCR, but others have provided interesting research problems for microbiologists, biochemists, geneticists, evolutionists, geologists, and ecologists.

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