Some Like It Hot: The Discovery of Thermus Aquaticus

By James Coppinger

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In July 1964, Thomas D. Brock, Ph.D., a professor and microbial ecologist at Indiana University, stopped by Yellowstone National Park on his way to the west coast to study streptococci in marine animals. At Yellowstone, he observed distinct color patterns in outflows from hot springs and noticed various color bands of biomass in 82˚C (179.6˚F) water.

Brock recalled that day during an interview with me in January 2018 at his home in Madison, Wisconsin. He said he recognized that the color changes of this bio-matter followed what he called "temperature gradients" as the water cooled in flowing away from its source. He realized these patterns indicated distinct colonies of organisms separated within well-defined optimal temperature ranges.

On his return trip home, he again stopped by Yellowstone to look for sulphur springs—possible habitats of a bacteria called *Thiorhrix*. He says, "I had not expected such an enormous development of microorganisms as were present in the runoff channels of the Yellowstone hot springs" (Brock, "Life"). He followed the outflows and took temperature readings, made additional observations, and collected specimens for his lab in Bloomington.

In July 1965, Brock returned to Yellowstone to study thermal algae found in outflow channels. "The trip was one of the most exciting two weeks of my career," he later wrote (Brock, "Life"). Brock's previous observations indicated hot spring channels contained extensive developments of photosynthetic organisms—thermal algae and cyanobacteria.

His field notes from that trip record a key discovery in the outflow channel from "Pool A" (Octopus Spring): "I saw pink gelatinous masses of material, obviously biological, at surprisingly high temperatures" (Brock, "Life"). Further analysis showed while this material had considerable quantities of protein, there was no chlorophyll. "I became convinced that the pink material was definitely bacterial and that bacteria, not phototrophs, were living at temperatures near boiling," he later wrote, and those observations led him to "commit early to the idea of what I later called extreme thermophiles (hyperthermophiles)" (Brock, "Life").

In the fall of 1965, Brock submitted a research grant proposal to the National Science Foundation to study thermal springs at Yellowstone. The proposal was funded and Brock's research at Yellowstone began in earnest in June of 1966 from a temporary lab in a rented cabin.

Limits on Life

Consider the environmental constraints for life—temperature, liquid water, carbon, and an energy source. What happens when environmental conditions are too hot?

Living cells are made of proteins, which are chains of amino acids. There are but twenty major amino acids in plants and animals—yet these twenty can construct an infinite number of proteins.

As proteins form, they are held together by two types of chemical bonds—covalent and non-covalent. Covalent bonds occur when pairs of atoms share electrons. Covalent bonds are strong. Non-covalent bonds do not share electrons—the atoms combine through electromagnetic attraction. Non-covalent bonds allow proteins to fold into unique shapes, and the protein's shape determines its function. Individually, non-covalent bonds are weak, but in aggregate, are relatively strong.

Heat affects the two kinds of bonds differently. Eggs, for example, are rich in protein. Crack open an egg, and it is loose and viscous. However, bring an egg to a boil, and it solidifies into a hard-boiled egg. When heat is applied to proteins, energy transfer agitates atoms and the non-covalent bonds break apart. The process is called "denaturing." This is permanent: once an egg is hardboiled, it is irreversible.

For a long time, it was thought the boiling point of water was the upper temperature limit for life.

Denaturing can actually be an advantage in fighting pathogens and disease.

Pasteurization and Pathogens

High temperature is used in autoclaves to sterilize medical equipment, but a more familiar example of the effect of high temperatures on living organisms is likely as close as your refrigerator. In 1864, microbiologist and chemist Louis Pasteur discovered and the following year patented a process that took his name—*pasteurization*. The process was utilized with milk and other food products to prevent tuberculosis.

Pasteurization is a function of both temperature and time. Guidelines by the Department of Health and Human Services specify 72˚C (162˚F) for 15 seconds to pasteurize milk (*Grade "A" Pasteurized Milk Ordinance*). 162˚F is 60˚ below the boiling point of water; 15 seconds indicates how quickly bacteria are destroyed. At 100˚C (212˚F) the time requirement is .01 seconds.

For a long time, it was thought the boiling point of water was the upper temperature limit for life. A milestone in changing that well-established threshold was discovered in a popular tourist location visited by thousands each year. It just took the right person to find it.

The Making of a Scientist

Born in 1926, Thomas Brock grew up in Cleveland, Ohio. His mother, Helen Sophia Ringwald, was a nurse. His father, Thomas Carter Brock, had only an eighth-grade education, but through self-study and correspondence courses became a power plant engineer in Cleveland. Brock's father always encouraged Thomas to get a good education and brought home discarded electrical equipment to teach Thomas to make coils, electromagnets and radios. Then came a crucial gift: "When I was 10 years old, I received a chemistry set for Christmas, and he helped me set up a simple laboratory in the basement" (Brock, "Road," 7).

Brock's father died when he was only fifteen, and the family moved to Chillicothe, Ohio, where his mother's family lived. There he made a friend, David Thornburgh, who was also intrigued by chemistry. The two built a research lab in the loft of a barn next to Brock's home. During my interview with Brock, he related that the Thornburgh family vacationed in Florida each winter. On one return trip, David collected "red-soil" samples from Georgia, and together they taught themselves to assay iron concentrations in those samples. When Brock's high school chemistry teacher was called away for military service, the school hired a chemist from a local paper company to fill in, but as Brock recalls, he and Thornburgh soon realized they were already advanced beyond this teacher's abilities.

Brock graduated from high school in 1943 and immediately enlisted in the Navy. After discharge from the military in 1946, he took advantage of the GI Bill and enrolled at Ohio State University where he earned his bachelor's in botany. Brock went on to receive a master's degree in 1950 and a PhD in 1952 in the field of mycology, studying mushrooms and yeast.

Brock came to Kalamazoo in 1952 as a bacteriologist in the Antibiotics Research Department of the Upjohn Company and lived in Kalamazoo until 1957, when he left Upjohn to take his first academic position at Case Western Reserve University in Cleveland, teaching bacteriology and microbiology. After two years of teaching, he took a postdoctoral position at WRU's medical school's Department of Microbiology where, with the aid of two National Institute of Health research grants, he worked on yeasts and antibiotics.

In 1958, he published his first book, *Milestones in Microbiology*, with Prentice-Hall—a publishing company with which he continued a long relationship—and went on to become Assistant Professor of Bacteriology at Indiana University in Bloomington.

His having many outdoor interests and experience led him to pursue aquatic and marine microbiology. He says, "One reason I became firmly field-oriented in my research may have been because I had become so enamored of the outdoors." (Brock, "Road," 8). In the spring of 1963, which Brock calls a major turning point in his career, he applied for a research project at the University of Washington's Friday Harbor Laboratories to study the presence of certain bacteria in marine animals. That research transitioned into research on Leucothrix mucor—a marine microorganism. The resulting paper, "Knots in *Leucothrix Mucor*," became a cover story of *Science* magazine and subsequently featured in the May 15, 1964 edition of the *New York Times*.

His work on *Leucothrix murcor* spurred Brock's curiosity about sulfur springs, the habitat for bacterial species of the related genus *Thiothrix*. His research expanded from cold to hot springs.

And that led to the moment at Yellowstone.

In 1966, Brock, along with Hudson Freeze, an undergraduate honors student, cultured specimens from sources above 69˚C (156˚F) and isolated a bacterium which Brock would name as both a new genus and species–*Thermus aquaticus*. The following year, Brock wrote "Life at High Temperatures" for the journal *Science*, in which he stated, "Bacteria are able to grow […] at any temperature at which there is liquid water, even in pools which are above the boiling point" (Brock, "Life").

Professor Patrick Forterre, head of the Department of Microbiology at the Pasteur Institute in Paris, writes in *Microbes from Hell* that "He [Brock] is considered today to be the father of microbes living at high temperatures, known as thermophiles" (20-21).

Extremophiles: Pushing the Boundaries of Life's Limitations

The upper temperature limit for organisms was long considered 50˚C (122˚F)—the point at which proteins denature. Today, the boundary is far exceeded by *simple thermophiles* (50˚C–64˚C) to *hyperthermophiles*, who can live at temperatures from 80˚C (176˚F) up to 122˚C (251.6˚F). Thermophiles are just one group of organisms under the heading *extremophiles*. There are organisms which live in extreme conditions of many kinds: acidity, alkalinity, salt concentrations, cold, deprivation of oxygen or sunlight, pressure, radiation, high levels of dissolved heavy metals, dryness, or far below the earth's surface in porous rock.

So, what exactly enables these specialized organisms to live in such extreme environments?

The "doubling time" (a way of measuring rate at which a population grows) of *Thermus aquaticus* is two to six hours in boiling water. These bacteria "were not struggling to survive but were thriving at these high temperatures," as Brock stated in an article published in the American Society of Microbiology's ASM News in 1998. How is *Thermus aquaticus* able to not only survive but actually thrive in extreme temperatures?

DNA

In the April 25, 1953 edition of *Nature*, James Watson and Francis Crick published a now famous article about the structure of DNA (*deoxyribonucleic acid*), research for which they would receive a Nobel Prize in 1962. DNA, the "construction manual" of life, is a polymer (a compound made up of several repeating units) strand of nucleotides in a double helix comprised of four base molecules: *Adenine* (A), *Guanine* (G), *Thymine* (T) and *Cytosine* (C). Adenine only links with Thymine, and Cytosine only links with Guanine.

To replicate DNA, an enzyme separates the strands at each nucleotide pair. Think of it as a zipper; once unzipped, the two sides are separated. Now imagine each zipper tooth is made of one of four materials that can pair up with one and only one of the other three materials. One strand thus serves as a template for the other strand.

Once the strands have separated, an enzyme, DNA polymerase, catalyzes a process to replace the missing nucleotides from each half. Since only one sequence combination is possible, two exact duplicates of the original DNA molecule are made from the separated strands.

Another way to separate DNA is high temperature. However, beyond certain temperatures, as we saw in the example of the egg, heat "denatures" enzyme proteins employed to replicate DNA. In a few species, though, extreme heat actually *enables* these enzymes.

Thermozymes

Within cells, enzymes and nucleic acids carry out the production of proteins. All living organisms have similar, but not identical, DNA polymerase enzymes. Mesophiles, (that is, plants and animals, including humans, who live in moderate environments between 20˚ and 45˚C) have DNA polymerase constructed with lots of ionic bonds. Thermophile DNA polymerase, however, has a higher proportion of heat resistant bonds. As mentioned above, proteins fold into unique shapes, but these thermozymes do not easily unfold and lose their structure. As a result, thermophile DNA polymerase performs identical functions replicating DNA, but at different temperatures. The operational range for mesophile DNA polymerase is 10˚C to 40˚C, but for thermophiles, between 60˚C and 90˚C. Outside of these 30˚ ranges, enzyme activity ceases. No enzyme exists that is functional across all temperatures.

The discovery of *Thermus aquaticus* is so remarkable because the specialized enzyme, unique to this organism, *Taq polymerase*, is not only heat stable but also functional at high temperatures.

Polymerase Chain Reaction

In 1993, biochemist Kary Mullis, Ph.D., received the Nobel Prize in Chemistry for his work on *polymerase chain reaction* (PCR) techniques. The breakthrough utilized *Taq polymerase*, "Taq" being an acronym derived from the name *Thermus aquaticus*, the species Brock discovered at Yellowstone.

The National Center for Biotechnology Information provides the following definition: "DNA polymerase [is] a type of enzyme that synthesizes new strands of DNA complementary to the target sequence. The first and most commonly used of these enzymes is Taq DNA polymerase (from Thermus aquaticus)." Such enzymes enable many kinds of research because they " 1) can generate new strands of DNA using a DNA template and primers, and 2) they are heat resistant" ("Polymerase Chain Reaction"). PCR is used to make numerous copies of a segment of DNA, producing the quantities of DNA investigators need for various procedures in molecular biology, forensic analysis, evolutionary biology, and medical diagnostics. PCR enables a fast, reliable, and cost-effective means to replicate DNA from even trace samples—e.g., DNA recovered from saliva found on a cigarette butt.

Taq polymerase is *the* essential

factor in the PCR process. High temperature is used to split DNA; Tag polymerase is the catalyst which facilitates replication, exponentially replicating DNA. Within 30 cycles, a single strand of DNA is duplicated into more than a billion copies.

PCR has made contributions to the fields of medicine, biotechnology, genetics, and genetic engineering, with tangential industrial, agricultural, and pharmaceutical applications. It is used to identify previously unknown pathogens and viruses, to detect HIV antigens (and other disease agents), and to screen blood donations. PCR is instrumental in tissue-typing for organ transplants, in cancer research, and in the detection of genetic disease mutations. It is also used to analyze tissue samples of mammoths, Neanderthal skeletons, and Egyptian mummies. PCR enjoys worldwide acceptance in forensics to help solve murder, rape, and other criminal cases as well as to exonerate innocent individuals accused of such crimes. PCR and Taq polymerase are indispensable in paternity tests and the ubiquitous ancestry DNA tests—all thanks to Brock's discovery of *Thermus aquaticus* at Yellowstone.

The discovery of *Thermus aquaticus* not only provided greater understanding about the inner-cellular functions of microbial life, DNA, and expanded known limitations for survival, but has also opened doors to look back billions of years to the origin of life on earth and, perhaps, a path to discover life beyond earth—in environs once considered impossible to sustain life. Although one would assume these bacteria must have evolved to withstand extreme environments, they actually may have appeared soon after the earth's molten crust formed and water vapor from volcanic eruptions condensed into primal seas. Extremophiles may represent the earliest forms of life on this planet.

They may even hold clues to life's final existence. Bacteria's advantage is its ability to rapidly reproduce. "At some point in time […]" says Sebastian G.B. Amyes, in *Bacteria: A Very Short Introduction*, "this planet is likely to become uninhabitable for human life and perhaps also for all vertebrates." He adds, "The organisms most likely to survive are bacteria" (120).

Conclusion

In 1970, Brock authored and published *Biology of Microorganisms*—now in its 15th edition, translated into several languages, and recognized as the standard microbiology textbook.

In 1971, Brock joined the faculty of the University of Wisconsin–Madison and in 1979 became chair of the Department of Bacteriology.

Now retired from teaching, he lives in Madison with his wife, Kathie, and involved in land conservation throughout southwestern Wisconsin.

While primary credit goes to Brock for his Yellowstone discovery and thermophile research, credit must also be given to his parents who inspired their son's educational desire and purchased a chemistry set for Christmas. That chemistry set produced more than acrid fumes from experiments; it ignited the passion for a lifelong pursuit of curiosity and knowledge–perhaps the greatest gift of all. We might all keep this in mind next time we have to find a birthday or holiday present for a young, growing mind.

And, finally, one does not have to travel to Yellowstone to discover *Thermus aquaticus*; it is probably living in your basement's water heater. Brock told me he had such an inspiration while washing lab equipment and cultured a sample from the water heater—and there it was—*Thermus aquaticus*.

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