Chapter 9

Yellowstone Extremeophiles: The Life of Heat-Loving Microbes

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The hot springs of Yellowstone are fatal to several unfortunate visitors every year. The hottest springs are close to boiling and some are as caustic as battery acid. Yet, even in these extreme conditions life thrives. The rainbows of colors seen in the park's thermal features are thermophilic (heat loving) microbes and represent a level of biodiversity unrealized until just a few years ago. The multicolored slimes flowing along the edge of thermal water are actually ecosystems of microbes.

Mark Young, along with others at the MSU Thermobiology Institute (TBI), investigates the myriad of life forms in the microbial mats and open water of thermal features; they have found life virtually everywhere they look. Mark's main interest is viruses – the most abundant biological units on the planet and also among the smallest. Working with samples from the park and a powerful electron microscope, Mark has identified elaborate structures in viruses with an evolutionally history extending back more than 3 billion years. One of his goals is to figure out how to use these intricate virus lattices to deliver drugs to humans. These "virus cages" could act like tiny Trojan horses, carrying therapeutic drugs directly to the cells that need them while minimizing unintended damage to healthy cells. The fact that they survive in extreme environments like thermal features means they are robust vehicles.

One of the unique features of the TBI group is their emphasis on teaching others about the thermobiology of the Park. They have produced educational videos, books, and courses for the general public so they can share what they are learning about the unseen life in Yellowstone. Discoveries in the bacterial mats promise to continue to be exciting and may one day unlock novel medical treatments or help solve our energy dependence. The thermophiles will continue to teach us the complexity of Yellowstone.

J. Johnson



Norris Geyser Basin (Trey Ratcliff)

Chapter 9

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The crime was hideous, a double murder late in the night on the boardwalks surrounding Old Faithful. The perpetrator seems to have disappeared without a trace. But not quite. The perpetrator's bloody handprint remains in the mud by the victim's side. Today, as any fan of crime mystery television can tell you, DNA sequencing can quickly and accurately identify the owner of any microscopic pieces of evidence left behind.

The handprint alone is enough to identify the perpetrator using methods that had their origins in discoveries made in Yellowstone years before. What most people do not know is that the ability to sequence tiny amounts of DNA is due to the discovery of a bacteria that grows in the hot springs in Yellowstone National Park and other thermal areas around the world.

In the 1960s, microbiologist Thomas Brock was studying microbes that grow in Yellowstone's hot springs. He isolated a novel bacterial species, *Thermus aquaticus*, from the Great Fountain area of the Lower Geyser Basin. This organism was thriving at 70°C (158°F). This meant that all of the enzymatic machinery inside this single cell organism was functioning at a temperature much higher than that of any other known organisms. *Thermus aquaticus* has to copy its own genetic information with an enzyme called a DNA polymerase in order to survive and replicate. If *Thermus aquaticus* lives at 70°C, its DNA polymerase must function at that temperature as well.

The discovery of this DNA polymerase enzyme and its application in a process called *Polymerase Chain Reaction* (PCR for short) resulted in a Noble Prize and a multi-billion dollar industry. PCR essentially operates as a molecular photocopy machine, allowing for the copying and amplification DNA from a very small starting amount. PCR has become a routine tool in laboratories all over the world. It is the basis for our molecular understanding of the relationships between all forms of life, it allowed scientists to sequence the DNA genome of any organism, including the complete human genome, it allows the medical world to better understand and design treatments for genetic diseases, and it lets the CSI technician ID the culprit in our crime scene.

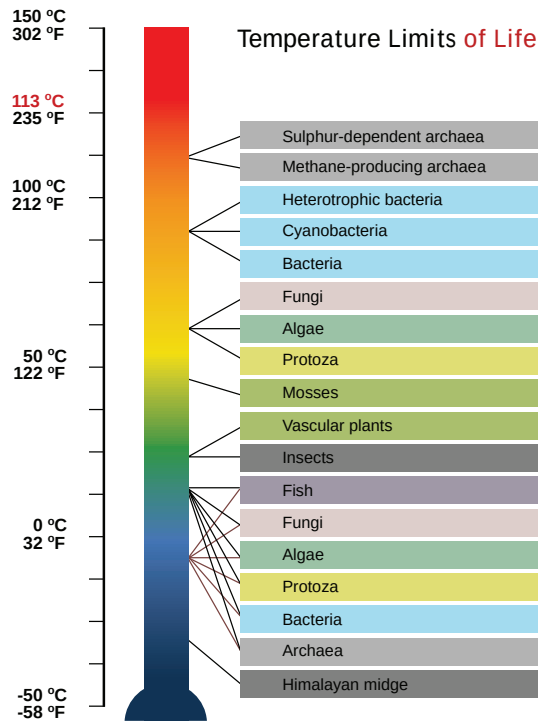
Upper Temperature Limits of Life

Microbiology has long focused on organisms with medical relevance. Microbes that can infect us and other vertebrates thrive at the temperatures in which the host organism lives; 37°C for humans. Few scientists thought that life could exist in the extremes of temperature and other parameters, that exist on the planet. Extreme heat in Yellowstone or cold in Antarctica, high salt concentrations as in the Dead Sea, heavy metals concentrations found in abandoned mines, among other stresses, were all thought to inhibit life. However, a closer look at these environments reveals that they not only support life, many organisms actually thrive there. We now know that life exists at temperatures up to and exceeding 121°C. This is remarkable, considering that 121°C is considered the "gold standard" of sterilization and is used to kill all previously known microbes. Most organisms are literally cooked at these temperatures, with their proteins denaturing, much like a cooked egg; lipids and other molecules becoming unstable and melt. Understanding how this organism thrives at such extremes can help us understand the fundamental nature of these biological molecules and may even help us understand how life first began on earth.

A Short Explanation of Microbial Life

The word microbe is general term and simply refers to the small size of an organism. If you need a microscope to see an organism it's likely a microbe. Some microbes are pathogenic; *Bacillus anthracis*, causes anthrax, *Yersinia pesitis*, the causative agent of bubonic plague (the Black Death), are but two famous examples. Other,

benign, microbes that surround us, cause no harm, and often aid us. There are more than 2000 commensal (beneficial) microbes in and on our bodies, while there are only approximately 100 known pathogens. Few of these pathogens are in residence in us at any given time. Those thatare present are being kept in check by the commensals. In our gut, they help metabolize food and glean all the available nutrients that we could not access without them, they synthesize vitamins that we cannot make or get from our normal food sources. For example, the human gut cannot efficiently extract nutrients from complex carbohydrates (starches). The end products of human digestion of these starches become the energy source for colonic microbiota like *Clostridium and Firmicutes* species which then provide as much as 15% of the human energy requirements. These beneficial bugs also keep pathogens at bay simply by outcompeting them for space and nutrients.



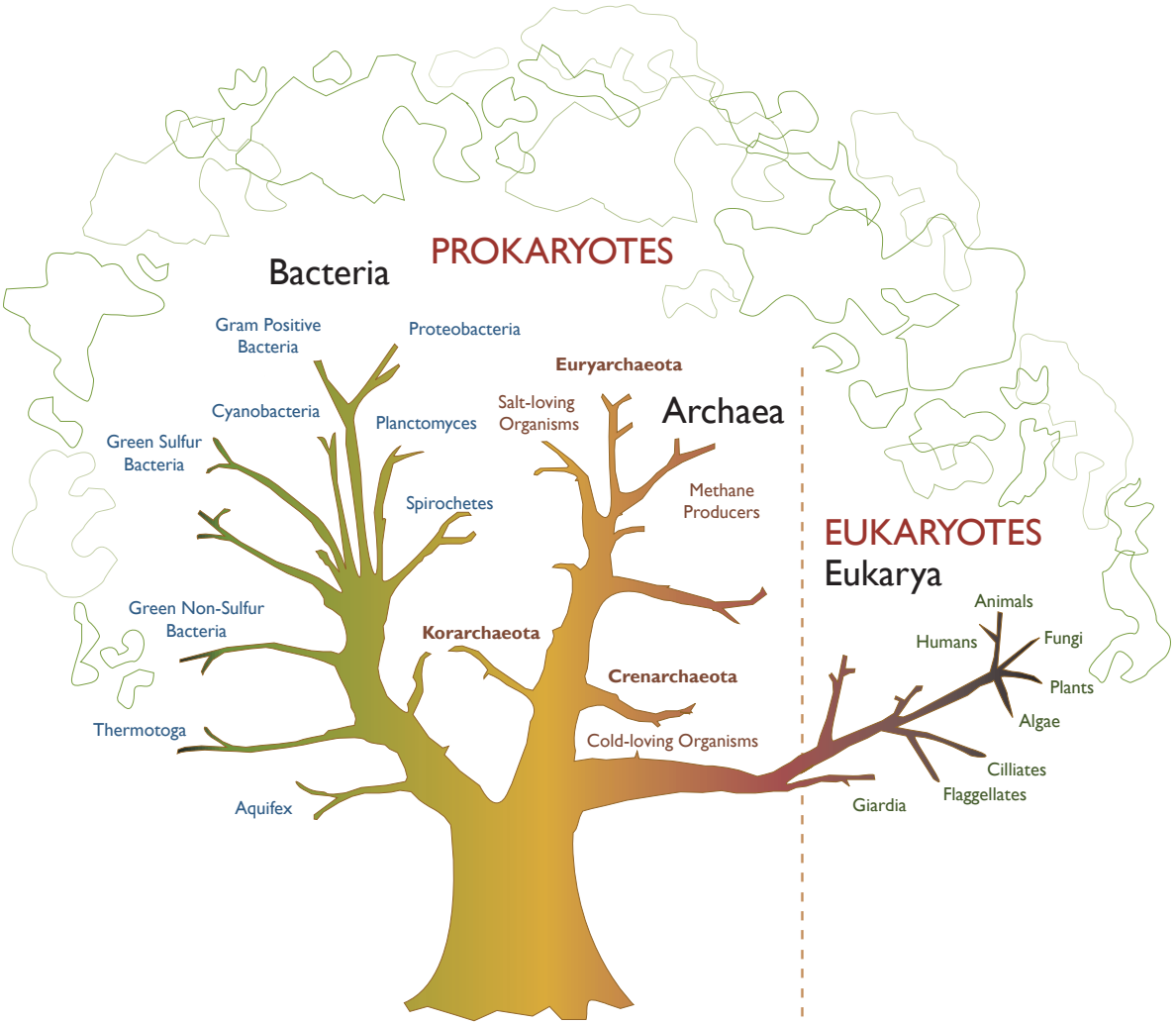
▲ FIGURE 9.1 Most multicellular life forms (Eukaryotes) live at temperatures below 113° F (45 C). Hyperthermophiles, single celled microbes, live at temperatures greater than 176 °F (>80° C). The highest temperature known than life can survive is 266° F (130° C). Thermophiles thrive between 113 -175° F (45-80 C). (Thermal Biology Institute, MSU)

DNA Analysis and PCR

The ability to read the genetic code of an organism has become indispensable for biological research. By reading the sequence of the four nucleotide - adenine (A) , guanine (G), cytosine (C), and thymine (T), that make up a molecule of DNA (Deoxyribonucleic acid), scientists can read the genetic instructions used in the development of all living organisms. Knowing how to decipher the DNA information is a central theme of the life sciences and impacts the understanding of evolutionary biology, the diagnosis of disease, and the law, to name a few.

The ability to sequence DNA is significantly enhanced by a process called PCR (polymerase chain reaction). PCR is used to amplify or replicate a single or few copies of a piece of DNA. In many ways PCR is like a molecular photocopy machine that allows for the specific amplification of small amounts of DNA. PRC allows researchers to generate thousands to millions of copies of a particular DNA sequence. This breakthrough has multiple uses. If doctors can identify the DNA segment that provides for immunity to a disease, they can replicate the strand and use it to treat patients, food crops can be genetically manipulated to select for desirable traits, small tissue samples can be traced to help solve crimes. Mark Young and his crew use PCR analysis to discover new life forms in Yellowstone hot springs.

PCR requires a small sample, often less than a drop, from which the DNA is amplified for sequencing. During the PCR amplification process, each type of nucleotide (A, C, G, or T) is chemically marked with its own unique fluorescent tag. The sequence of the amplified DNA is determined from the labeled DNA when it passes through a sizing matrix that can separate DNA molecules that differ by only one nucleotide. As the DNA molecules in the sizing matrix pass by a laser, a detector records the color of the fluorescent tag. A computer is then used to assemble the sequence of the DNA analogous to determining the order of four different colored beads on a long string of beads. The order of the long string of DNA is the genome of the organism and it can be millions to billion of nucleotides long. From there, specific gene sequences can be studied and tested for composition and function. In the case of thermophiles and hyperthermophiles there are genes allow the organism to make a living in high temperatures, allow them to exist in extremes of pH (potential of hydrogen) or low oxygen environments.



▲ FIGURE 9.2 Recent advances in molecular biology allows determining the DNA (gene) sequences of all organisms present in a Yellowstone hot spring. In fact, a key component that made DNA sequencing possible was originally and enzyme isolated from a bacteria living in a Yellowstone hot spring. Determining the gene sequences helps scientists understand how life functions at high temperatures. (Thermal Biology Institute, MSU)

Microbes also live in the soil, in frozen glaciers, in the depths of the oceans and in the boiling acid hot springs of Yellowstone National Park. Microbes are by far the most abundant forms of life on the planet. They are major contributors to mortality and carbon cycling in all environments. They contribute to global energy pools via photosynthesis, and thus sit at the base of the food chain. They break down everything from dead trees in the forest to other microscopic organisms. All higher life forms such as plants and animals are absolutely dependent on microbes for their survival.

Sheer numbers make microbes incredibly important to global ecology. There are currently approximately $7x10^9$ humans on the planet. Microbes are estimated to number 10^{29} cells on the planet. If you lined up the microbes present on the planet end-to-end, it would take more than 200 million light years to travel from one end to the other! If all 'higher' life forms completely disappeared from the planet, the microbes would hardly notice. However, if all the microbes suddenly were gone, the entire food web of the planet would collapse in short order.

Life, as we understand it today, is divided into three major domains: Eukarya, Bacteria and Archaea. The divisions being determined by fundamental ways in which they live. Many biology students are familiar with the distinction between Eukaryotes and Prokaryotes.

Eukaryotes are thought to have arisen late in the evolution of life. They have a complex cellular structure - a nucleus and membrane bound organelles, and thus division of labor within the cell. All macroscopic life, with the exception of communities of single celled organisms, are eukaryotes. All animals and plants are eukaryotes. But many microbes are also eukaryotes, including the protists, yeast, paramecium and algae. In general, Eukaryotes are larger, more complex and less adapted to extreme environments than prokaryotes.

The term prokaryote was used to define single celled organisms that lack a nucleus and defined membrane bound organelles. However, prokaryote is an overly broad term that encompasses organisms that are as different from each other as *E. coli* is from humans. Today, Prokarya is divided into two major domains, Bacteria and Archaea. At first glance, these two groups might seem to have much in common: they are all single celled organisms. They lack membrane bound organelles, and are generally similar in size and shapes. Distinctions, it seemed, were primarily based on habitat, with the Archaea generally occupying the more extreme environments and Bacteria the more moderate climes. However, with advances in technology scientists discovered other profound differences between these two groups. The ability to sequence DNA has enabled us to precisely define the degree to which organisms are related and it is now well accepted that the Bacteria and the Archaea are not at all alike. In many ways, the Archaea more closely resemble Eukaryotes than Bacteria. Both Bacteria and Archaea can be found in a wide range of habitats. Archaeans live in soils, in our guts, in the oceans, and in fact, in almost every environment in which we look. Some Bacteria thrive at temperatures up to and beyond 90°C. However, Archaea tend to be more dominant in extreme environments while Bacteria are the major players in the more moderate environments. Most pathogenic microbes are Bacterial species, while, to date, no Archaeans have been identified that cause disease in humans. In extremely hot (>80°C) very acidic (<pH4), or high salt environments we see predominantly members of the domain Archaea. Molecular techniques have shown us that the composition of the cell walls of Archaeans is very different from Bacteria. Key genes and proteins within these different groups are also profoundly different. The ability to reduce carbon dioxide into methane and water (methanogenesis) is also uniquely Archaean.

These insights into the uniqueness of extremophiles are what lead researchers to believe they hold the key to some sticky scientific issues. For example, cellulases (an enzyme that plays a key role in transforming plant cellulose into ethanol) from high temperature organisms could make ethanol fuel production more efficient and more viable as a renewable energy resource. Other microbes, like *Deinococcus radiodurans*, which thrives in extremely radioactive environments, or *Geobacter* species, which reduce heavy metals like Mercury and Chromium, might provide clues as to how to deal with anthropogenic environmental degradation like mine waste or industrial pollution.

Viruses are the fourth major piece of the puzzle that makes up life on earth. Viruses occupy an odd position outside of the tree of life, but interacting with all members of the tree and helping shape both the genomes and the population structures of the organisms they infect. Viruses are obligate intracellular pathogens meaning they spend most of their "life cycle" inside a host cell and their replication and assembly takes place entirely inside this host. They are not technically life forms, but are made up of biological molecules; DNA, RNA, proteins and lipids – the biochemicals of life. Viruses are the most abundant biological entities on the planet, estimated at 10^{31} particles on the planet, and are found in every environment we look. There are likely viruses for every cell type that exists on earth. They play a major role in cellular mortality, transfer genetic material between distantly related cells, and exert evolutionary selection upon the cellular life forms they infect. Hot acidic environments, like we find throughout Yellowstone, may be one of the few environments in which we see fewer extra-cellular viruses than cells. In the oceans, scientists routinely observe ten times the number of free viruses as cells in a milliliter of water. However, in the harsh environments we find in Yellowstone, viruses seem to have adapted lifestyles to minimize the time they spend outside of their cellular hosts.

Multi-cellular Consortia of Single-Cell Organisms

In recent years an additional layer of complexity has been added to our understanding of single-cell life forms. It turns out, that while we often study these organisms as isolated cultures in liquid or solid media in the laboratory, in the real world few organisms pursue such a solitary lifestyle. Instead, most species are living in communities that function together to do much more than any individual species could do alone; in this respect they are not all that different than the animal communities with which we are most familiar.

Biofilms are a relatively well-studied example of this. Once numbers are high enough in any given culture, there is a signaling event within the community that triggers a change in behavior. These behavior changes include moving from planktonic (free-swimming) lifestyle to attached and highly ordered structures growing on surfaces, exuding a protective slime layer (exo-polysaccharide) to exclude antibiotics or other organisms, as well as an increase in communication between individual cells through a process called quorum sensing. Biofilms are found throughout our bodies and in almost every other ecosystem.

Microbes of Yellowstone

Yellowstone is full of thermal features, including hot springs, mud pots, fumaroles, and geysers. Over 10,000 thermal features are found within Yellowstone, making it one of the largest and most diverse thermally active sites on the planet. A vast array of different temperatures, pH, and geochemistry can be found associated with Yellowstone's thermal features. We find sites in Yellowstone that are as acidic as your stomach (pH~1) and as alkaline as soap or the Great Salt Lake in Utah (pH~10), with temperatures ranging from cool to boiling. The chemicals and minerals in these pools vary widely as well. They contain sulfate, chloride, arsenic, mercury, boron, nickel, zinc, cadmium, and ammonia, among other "noxious" chemicals. Despite the seeming harshness of these environments, we find that the diversity of life here has exceeded all expectations. Each of these pools supports unique communities of Bacteria and Archaea, along with some thermophilic eukaryotes, including plants and even some animals.

How Do These Microbes Make Their Living? The Geochemistry of Yellowstone Hot Springs

Yellowstone sits on top of a volcanic hot spot, an area where the earth's crust is much thinner than normal. Underneath this thin crust is a huge magma chamber. While this is as much as 10,000 feet or more below the surface, this is much closer to the surface than in most areas of the world. Water seeps into the ground and moves deep underground through fissures, cracks, and faults, where it gets heated to extreme temperatures, and is forced back to the surface under great pressure. During this process it leaches minerals out of the rocks through which it moves.

BIOLOGY

The geochemistry of these sites impacts the biology of the organisms living in them, but this is not a one-way street. Biological processes also have a major impact on the geology and geochemistry of the hot springs as well. At Angel Terrace in the Mammoth Hot Springs area of Yellowstone, huge terraces have been created by



▲ PHOTO 9.1 The different brightly colored microbial communities are formed along gradients of temperature and water chemistry. Each colored microbial community requires its own unique set of water chemistry and temperatures to grow. (Thermal Biology Institute, MSU)

precipitation of carbonite. The precipitation is controlled by water chemistry, physical processes (temperature, evaporation, degassing, steam), hydrology (flow rates, surface area) and biology (photosynthesis and respiration of microbes). The water coming out of the ground at these springs flows over mats of filamentous bacteria of the *Aquificales* family. Aragonite, a carbonite mineral, precipitates on the surface of these filaments to form tubes millimeters in diameter and centimeters in length. Inside, the microbes die and decay, leaving behind the hollow tubes. Different sizes and shapes of precipitates form depending on the particular combination of chemical, biological and physical factors coming into play in any given micro-zone of the terraces. Surprisingly, these terraces form very rapidly, up to 30 cm/year.

ENERGY PRODUCTION

Many organisms survive by their ability to take advantage of the geochemical energy sources available in these environments. Some archaeans make methane from carbon dioxide and hydrogen. Other archaeans, and many bacteria, reduce sulfur to hydrogen sulfide to produce energy.

There is a wide range of metabolic strategies employed by various organisms living in geothermal hot springs. Some organisms are oxygen respirators, or aerobes, while others shun oxygen completely (anaerobes) or to a lesser extent (microaerophiles). Some organisms, heterotrophs, utilize organic carbon available in these pools that has been produced by other organisms (i.e., they graze on other cells as food sources). Others, chemolithoautotrophs, can convert carbon dioxide and chemical sources of energy into organic carbon for their food source.

Many organisms in hot springs are free-living cells, floating, and sometimes actively "swimming" in the aqueous environment. However, many of the cells have also adapted other strategies to find niches within these environments. Some grow attached to solid surfaces, like sediments in the pools. Others grow in communities with other organisms, either of the same or different type. Many organisms are motile and can respond to changes in their environments. For example, in a hot spring, the temperature might be 70°C at the outflow, but closer to 80°C near the source. An organism with an optimal temperature of 80°C might be able to sense temperature changes and move towards the optimal temperature, a process called thermotaxis. They can employ similar strategies to move towards food sources or required chemicals, or away from too extreme of conditions or toxins. Many organisms employ multiple strategies depending on the situation in their environment.

In recent years it has come to be recognized that many organisms which we have traditionally grown as single isolates suspended in liquid media or as colonies on plates, might actually prefer to live as members of complex communities as biofilms or, in one well studied example from Yellowstone, as microbial mats. In Yellowstone, these communities are some of the most visible and beautiful examples of microbial life. Vibrantly colored mats of bacteria grow around the fringes of many hot pools in the park. Streamers flowing in the outflow channels of hotsprings are also microbial in origin.

The mat communities in Yellowstone were quite famous long before anyone recognized that they were composed of bacteria. In these communities there is a stratification based, in part, on the ability to utilize light. In fact, the mat communities exist as relatively self-contained ecosystems interacting with each other and with their environment. The surface consists of a green layer of cyanobacteria (*Synechococcus*) which, as one might guess from the greenish color, is a photoautotroph, or organism capable of utilizing light and **inorganic** carbon for its food and energy source. The lower layer consists of green nonsulfur bacteria (*Chloroflexus*), that is a photoheterotroph, requiring light and **organic** carbon sources. *Synechococcus*, living at the top of the mat has the best light situation and can efficiently use light to fix inorganic carbon into organic carbon. *Chloroflexus*, living deeper within the mat, still requires light, but uses the byproducts of *Synechococcus* for its carbon source. Studying either of these organisms alone in culture would not reveal the true nature of how they make their living in their native environments.



▲ PHOTO 9.2 The bright colors seen in Yellowstone hot springs are often formed by microbial communities that form mat-like structures. The color of the mat can often be used to identify the organisms and the temperature at which they are growing. The green colored mat is made up of a photosynthetic organism that grows at a lower temperature than the bacteria that makes up the red colored mat. (Thermal Biology Institute, MSU)

How Do These Organisms Survive Temperatures That Would Kill Most Life?

This question has been one of the fundamental questions driving research in Yellowstone hot springs. Answering this question would not only provide insights into these unusual organisms, but also how life may have first evolved, as well as give us biotechnology tools for working with biological systems in these extremes.

Unlike warm blooded animals, microbes have no ability for internal thermo-regulation in the face of non-optimal external conditions. Instead, all of their internal cellular "machinery" must adapt to the temperature



▲ PHOTO 9.3 At Mammoth Hot Springs the terraces are built from the precipitation of carbonite. Different sizes and shapes of precipitates form depending on the particular combination of chemical, biological and physical factors coming into play in any given micro-zone of the terraces. This complex relationship creates one of the most iconic thermal features in the Park. (Thermal Biology Institute, MSU)

of their environment. Some of the adaptations we see in thermophiles include unusual lipids (fats) not found in mesophilic (moderate temperature loving) organisms. Most of these organisms have very efficient DNA repair systems and proteins that coat or coil the DNA to protect it from an environment that is inherently damaging to genetic material. The proteins produced by these organisms often fold into conformations that are inherently stable and thus resist denaturation at high temperatures. However, the bottom line is that we still know relatively little about many of these heat adaptations.

How We Do Our Work

For centuries microbiologists have studied organisms that grow in medias cooked up in the laboratory. This is true for both medically relevant and environmental organisms. This has served us well. It allows us to grow a single organism to high concentration and study it in detail. Experiments are reproducible, and we can often manipulate these organisms *in vitro* to produce proteins, antibiotics and even genetic material that have numerous beneficial applications in medicine and biotechnology. Petri dishes of bacteria are familiar to everyone who has taken high school biology.

However, there are certainly enormous numbers of organisms that simply do not grow well in these conditions. Altering media to expand what we can grow has worked well to date. But, we are still faced with a

Microscopy

While molecular tools have come to prominence in the last few decades, the microscope remains a fixture in most microbiology labs. The microscope has been a fundamental tool in understanding microbes since the birth of microbiology more than 300 years ago. Huge advances have been made since Antony van Leeuwenhoek's first design. Today we have powerful electron microscopes capable of viewing single viruses and strands of nucleic acid at 100,000x amplification. Improvements in the light microscope and the use of fluorescent dyes or antibody-linked dyes have resulted in incredibly powerful tools. Observation of unstained cells can tell us about numbers of cells in a culture or the morphology of the cells. Observation of differentially stained or immuno-flourescently labeled organisms can provide information about composition of the cell wall, whether the cell is alive or dead, the number of chromosomes present and even the localization of specific proteins within the cell.



▲ PHOTO 9.4 The first light microscopes were made in the late 1590s which allowed some of the first single celled microbes to be seen. Modern light microscopes are indispensible for observing the microbes that live in Yellowstone hot springs. (Allan Wissner)

vast level of diversity in the environment that doesn't match what we have in culture. Furthermore, in many instances, the organisms that we are able to consistently culture out of an environmental sample are not the major players in the environment. *Sulfolobus* species are one such example. Various species and strains of *Sulfolobus* have been grown in enrichment cultures out of hot acidic pools all over the world. In fact, if a pool is between pH 2-4 with a temperature between 70-80°C, some species of *Sulfolobus* is likely to grow if the right media is present. One result will be the rotten egg smell ubiquitous at thermal sites. If we study these environments,by other techniques we find that *Sulfolobus* species often make up only a small fraction of the inhabitants.

To understand better who all the players are in these environments scientists have been recently using culture-independent techniques. What this means is that we spend a great deal of time in the thermal regions of Yellowstone sampling hot springs, mud pots, and the other thermal features. When we return these samples to the lab, we can sequence specific genes, aided once again by the *Thermus aquaticus* DNA polymerase, and we find organisms that we would never detect by trying to grow them in the laboratory. What we discovered when we started to look at pools in Yellowstone using these methods, was that the diversity was astounding. The vast majority of the organisms were ones that had not been previously identified.

In just the past few years sequencing technology has once again made huge leaps forward. It is now possible to sequence entire communities of organisms at once. The speed and ease of sequencing has created a situation in which we can quickly obtain more information than we are currently able to process. We can see that these environments are full of novel life forms and viruses that are going about their business in ways that we don't yet fully understand.



▲ PHOTO 9.5 A modern electron microscope allows observing details of cellular structure at much higher resolution than is possible with a light microscope. Using an electron microscope to look at microbes from Yellowstone hot springs allows new insights into the cellular structures that allow life to exist at high temperatures. (Thermal Biology Institute, MSU)



▲ PHOTO 9.6 Scientists carefully sample Yellowstone hot springs. Ongoing research in Yellowstone's hot springs is making fundamental scientific discoveries about the diversity of life, our understating of life at high temperatures, and about the evolution of life on earth. (Thermal Biology Institute, MSU)

A Day in the Field

Scientists are increasingly interested in studying these microbes and viruses in their natural environment, essentially using the hot springs themselves as the incubators and culture flasks. This work obviously requires new skills not usually associated with bench science. Yellowstone National Park is a dynamic environment for both the researchers and the organisms we are studying. Some studies are looking at changes in microbes over time, requiring monthly or weekly sampling points. In the summer, researchers spend long days hiking in the backcountry of Yellowstone locating hot springs and sampling over several time periods. The backcountry of Yellowstone is not only inhabited by a number of intimidating macrofauna, like bison and bears, requiring scientists to travel in groups of at least two, but the terrain itself can be quite treacherous. More than one graduate student has been initiated into field-work by stepping through the thin crust around a boiling acid hot spring.

Winter sampling requires long days outside in frigid conditions, often below zero (Fahrenheit). Travel into the backcountry might require snowshoes or skis. Working around boiling hot springs provides an unexpected challenge in the winter as well. While the boiling springs provides a relatively mild environment for collecting the sample itself, upon departing for the long hike back to the road, one discovers that all their warm winter layers are soaked through from the steam. Hypothermia in such an environment is a serious risk.

Extracting water samples from boiling acid often requires special tools to avoid falling into the pool. Scientists suspend sampling bottles and measuring instruments like pH meters and temperature probes from telescoping poles in order sample from the relative safety of the more stable ground away from the pool's edge. Often samples must be collected in as sterile a manner as possible in order to avoid contamination from surrounding soils or the researcher herself. This is no small challenge in the backcountry. Pre-sterilized vials and on-site filter sterilization along with working as close to the hot springs as possible, helps avoid contaminants.

Once samples are collected they are generally returned to the laboratory as quickly as possible. Sometimes, samples need to be kept anaerobic (without oxygen) or at environmentally relevant temperatures. Other times, we stop all growth and metabolism by flash freezing them in liquid nitrogen at -80°C, and transporting them frozen back to the lab for further analysis.

Evolution of Life on Earth and the Search for Extraterrestrial Life

Yellowstone's geothermal environment is a keyhole



▲ PHOTO 9.7 Thermal features can take on an otherworldly appearance. It is possible that discoveries made in Yellowstone will lead to understanding lifeforms on other planets. (Cathy Whitlock, MSU)

through which we can potentially view the evolution of life from its very beginnings on Earth. The hot springs are possibly more similar to earth just after the inception of life than any other environment. While the organisms that live there have changed and evolved through the eons, they have a record in their genomes of the changes that have occurred. The ways in which they interact with each other and with their environment may give us insights into how early cells, in the absence of oxygen, with an atmosphere of noxious chemicals, may have made their living and began to shape the planet into what we know today.

Early microbes are generally thought to be responsible for generating the oxygen-rich atmosphere that has made earth hospitable to all other subsequent life forms. But research in Yellowstone is showing that they may also have played a significant role in forming the geology of the planet as we see it today as well. These sorts of studies will enable us to look at similar formations if we find them on meteors or on other planets and more easily recognize signs of life.

Understanding these processes may also give us clues as to what to look for as we venture farther out into our solar system and beyond in the search for evidence of life elsewhere. It seems highly unlikely that our first contact with extraterrestrial life will be the green men in space ships or even intelligently formed messages via radiowaves from deep space. More likely, we will find evidence of biological processes that may have ceased millennia ago. Evidence of biologically driven geological depositional processes in the water channels on Mars might tell us of the previous existence of life elsewhere in our solar system. Fossils of mineralized filamentous organisms might be recognized because of our knowledge of similar organisms in Yellowstone and the processes that lead to their mineralization and fossilization.

Back on Earth, it is not unreasonable to expect big things from thermophiles. As the planet warms, our ability to practice agriculture will inevitably change. Previously fertile regions will become too hot and dry to support crops; farming will move into new regions. Meanwhile scientists look for ways to grow crops in warmer, drier environments. Hot springs panic grass, common in geyser basins throughout Yellowstone, can withstand temperatures up to 140 degrees Fahrenheit. However this heat tolerance is the product of a three way mutual relationship between the plant, a stringy microscopic fungus on the plant's roots and a virus that infects the fungus. Thermotolerance imparted on the plant via the virally infected fungus could help create heat tolerant crops for a warming environment. It's not inconceivable that thermophilic microbes and the viruses that infect them may play a significant role in future food and energy supplies well into the next century.

Yellowstone's thermophile ecosystems, it turns out, have a great deal in common with the more familiar macro ecosystem. Each displays an amazing array of biodiversity and interconnectedness. Both have adapted to their environments in complex ways by evolving coping mechanisms to climate and changing conditions. Both hold promise for producing an even wider assortment of ecosystem services beneficial to humans. Already, thermophiles have helped solved murder cases; we anticipate they will eventually improve our lives in other ways as well.