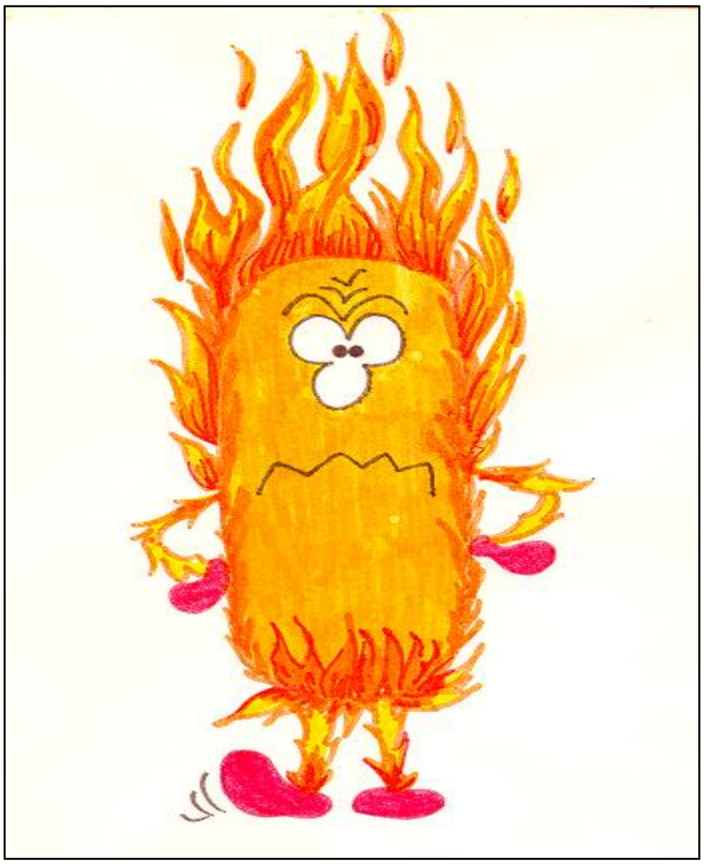
Hyperthermophiles

I thought that microorganisms can't live in boiling water. Well, guess what? Some can. They not only survive – they thrive!



Pyrococcus furiosus" The rushing fireball" Image credit: Ilse Blumentals

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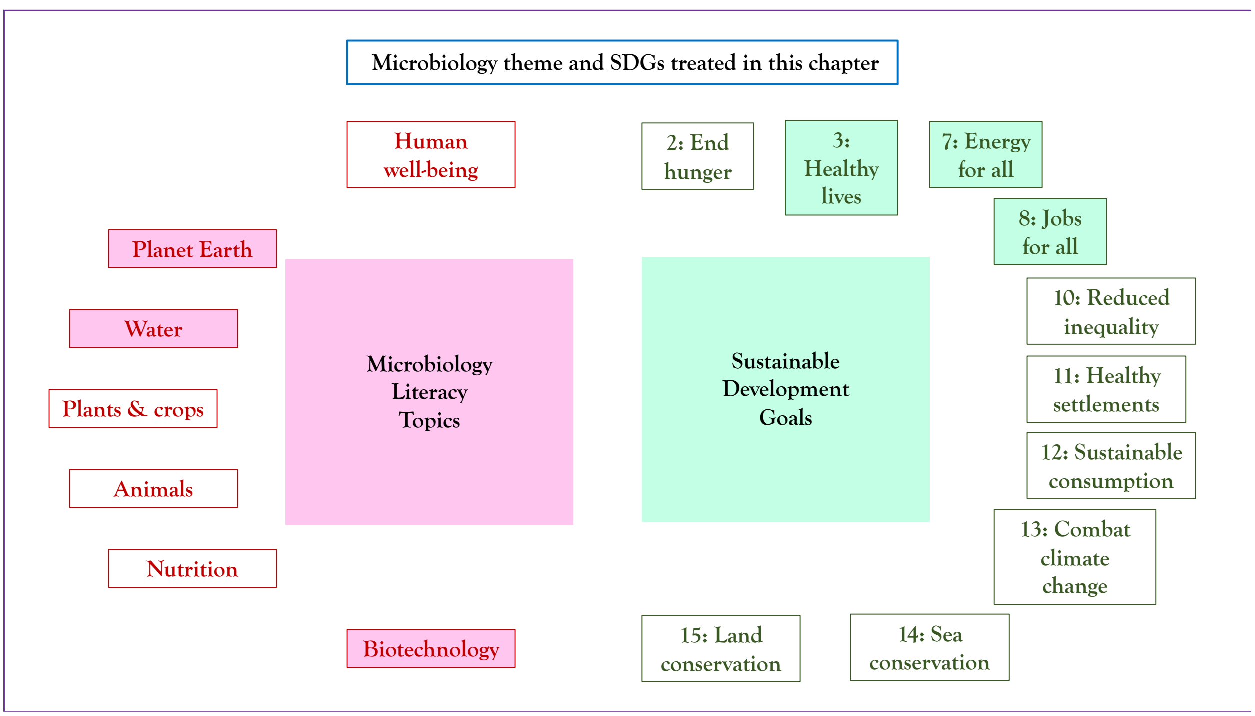
Hyperthermophiles

Storyline

Microbes that thrive at significantly higher temperatures than we do are known as heat-loving or ‘thermophilic’ organisms, while those that grow near and even above water’s boiling point are known as *hyperthermophiles*. So, how does life go on in boiling water? How are hyperthermophiles different from microbes commonly found in less thermal and “normal” environments (normal for you but freezing from them)? Are these microbes useful in industry and biotechnology – can they help us make useful products? And what-how life evolved on this planet over four billion years – what do present-day hyperthermophiles tell us about that?

The Microbial and Societal Context

The realization that life occurs at very high temperatures raised many important questions and suggested technological opportunities. Are these organisms dangerous (pathogenic)? So far, the answer is ‘No’. Are they very different from microbes we have long-studied? Well, ‘Yes’ and ‘No’. Did their discovery change the way we think about the evolution of life on earth and possibly elsewhere? ‘Certainly, Yes’. Are they important in biotechnology? ‘A resounding ‘Yes’.



Hyperthermophiles: The Microbiology

1. In the beginning. Microorganisms were the first form of life on our planet and evolved more than four billion years ago. At that time, conditions on earth were nothing like we see today. In fact, the earth was covered in oceans of boiling water. The first simple microorganisms are thought by many to have arisen from non-living chemicals near volcanic vents in a hot primordial soup containing the essential elements needed for life, that include carbon, hydrogen, oxygen, sulfur and phosphorus, as well as metals such as iron. The hot vent waters were likely enriched for such chemicals as they flowed upwards from the crust to emerge at the earth's surface, both in the shallow and deep sea and on land. These chemicals were the precursors to ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and to the amino acids that make up proteins, all essential components of all life forms, including us, that we know of today.

In the deep sea, the emerging hot volcanic fluids did not boil but remained in a liquid state because of the extreme pressures of hundreds of atmospheres in the depths, and temperatures could approach 600°F (300°C). However, this is far hotter than any life form that we know of today can tolerate, and living organisms probably first evolved at temperatures below the current upper limit for microbial life, which is near 250°F (120°C). So, the theory goes that life started in the volcanically-heated waters near so-called hot water or hydrothermal vents.

2. Discovery (not surprisingly, hyperthermophiles are found in hot places). The first explorations to the ocean floor that discovered the deep-sea vents at temperatures far above the normal boiling point (212°F, 100°C) was not until the 1970s. Remarkably, they found flourishing ecosystems in the surrounding cold seawater also consisting of higher life forms, like shrimps and crabs, not just microbes, that obtained energy to grow from the nutrient-rich vents. Hence, in the otherwise cold desert-like environment of the deep ocean floor, there were, as some would say, a proverbial 'Garden of Eden', near hydrothermal vents, elaborate vestiges of where microbial life may well have first arisen.

Over the past century, we have become increasingly aware that microbes on earth flourish in a variety of high temperature ecosystems. These include hot springs like you see in Yellowstone National Park, and in steamy fissures around volcanoes like near Mt. Vesuvius in Italy – recall the story of Pompei. Microbes that thrive in the hot ocean are also found in environments less dramatic than deep sea vents, such as shallow volcanic outpourings on numerous beaches throughout the world in perhaps just a few feet of water - hard to find unless you know what to look for.

Hyperthermophiles (microbes that grow best at 75°C or higher) can be found in both **terrestrial** and **marine** locations, like undersea volcanoes or geothermal sediments on the beaches of places like Volcano Island, Italy.

3. The hotter it gets, the lower the biodiversity. Analysis of the species diversity, by sequencing of DNA from these environments, a mixture of DNA molecules from many microbes in a community, suggests that the diversity of microbial life narrows as the temperature goes up. That is, the variety of microbes decreases with increasing temperature.

Steam rising from fissures in Yellowstone National Park, USA – which sits on top of a large volcanic feature. Hyperthermophilic microbes can be isolated from these sites.

Isolating hyperthermophiles from a hot spring in Yellowstone National Park ca 1990. Regulations are enforced to minimize disruption to the hot springs and surroundings to preserve them.

Deep sea hydrothermal vent located thousands of meters below sea level. Fluids leaving vents contain metals which precipitate out as hot water meets cold ocean water – giving rise to the description of vents as 'black smokers'. Many animals, fish and microbes live in the surrounding waters and in ocean sediments.

Vulcano Island, Italy located in the Tyrrhenian Sea off the coast of Sicily. Natural hot springs can be found on the beaches around the island from which many hyperthermophiles have been isolated (including *Pyrococcus furiosus*!). The volcano can be seen in the upper right of the picture.

4. Hyperthermophiles do not like low temperatures. The microbes in hot places are not only surviving these extreme conditions – they need them to grow. These life forms cannot reproduce at the temperatures at which virtually all other life forms thrive – they need high temperatures, otherwise, they are inactive and "frozen" at the temperatures at which you are your most active. Indeed, some of these microbes are most active at temperatures above the normal boiling point of water (212°F, 100°C).

5. Are we descendants of hyperthermophiles? Many think that hyperthermophiles may be our ancestors and that evolution proceeded in the direction of life becoming more and more active at lower and lower temperatures. You and I are now so well adapted to the "cold" that we do not fare well at even slightly elevated body temperatures (above 98.6°F or 37°C). Present day high temperature microbes might well be relics of times long, long past on this planet and might even be representatives of current versions of life that thrive elsewhere in the universe (astrobiology).

6. While hyperthermophiles resemble their less thermophilic ('heat-loving') cousins that grow at moderate temperatures, there are some differences. Many hyperthermophiles belong to the Archaea, the third Domain of life, along with the domains Bacteria and Eukarya (including humans, plants and yeast – anything with cells that have a nucleus). The Archaea were recognized as this additional domain of life more than 50 years ago by the late Carl Woese, a renowned microbiologist at the University of Illinois, based on their membrane composition and differences in the DNA sequence of their ribosomal RNA (RNA in the ribosomes, the protein-production "machines" of the cell). While not all Archaea are hyperthermophiles, many are. Archaea look a lot like bacteria (they lack a nucleus) but have features that resemble eukaryotes (how they make DNA, RNA and proteins have more in common with how you do it rather than how bacteria do it). The relationship between the three Domains of life, as this relates to evolution, remains an interesting scientific puzzle.

7. Hyperthermophiles grow in ways that resemble less thermophilic microbes. Many hyperthermophiles are 'anaerobic', that is, they can grow only in the absence of oxygen. However, there are hyperthermophiles that are 'aerobic' and, like you, require oxygen to grow. The anaerobic hyperthermophiles produce gaseous products, such as hydrogen, carbon dioxide, methane (natural gas) and hydrogen sulfide ('rotten egg odor'), as well as liquid products like organic acids, such as acetate and lactate – which are typical products of fermentation. While many hyperthermophiles grow at neutral pH, others grow in hot acid (as low as pH 0). Some hyperthermophiles that grow in hot acid can use metals found in mineral ores (such as iron) or elemental sulfur as an energy source.

8. Proteins from hyperthermophiles are intrinsically robust, although the basis for this is not well understood. Most proteins of most cells are sensitive to heat: they are inactivated/denature – their folded structures unfold – as can be seen when we cook an egg and the proteinaceous egg white which is clear at first turns white (this is the basis of pasteurization of milk and the cooking of food, which are used to kill off microbes that cause these products to deteriorate or that make us ill). However, it should be no surprise that proteins found in hyperthermophiles are thermally stable – a requirement for their function in microbes growing at high temperatures. However, the basis for this high level of thermostability is not well understood, despite an enormous amount of effort to sort this enigma out. The proteins are made up of the same 20 amino acids found in "normal" mesophilic (moderate temperature) microbes. The prevailing wisdom is that there are thousands of subtle adjustments in structure (e.g., more stabilizing interactions between amino acids) that add up to confer the thermal robustness. While early on there was hope that clues from the study of hyperthermophilic proteins would lead to ways to make any protein more robust, this has not been the case. But, we have learned much about protein stability from the study of hyperthermophiles.

9. Hyperthermophiles have had a major impact in biotechnology. To assess the impact of the technological impact of hyperthermophiles, one needs to look no further than the Polymerase Chain Reaction, often referred to as PCR, the basis of current "gold standard" tests for COVID-19 infection. The capability to use PCR to produce large amounts of DNA from minute amounts of starting material gave a tremendous boost to biotechnology more than 50 years ago. It is a technique that remains at the core of molecular biotechnology. Nobel Laurate Kary Mullis recognized that a highly thermostable DNA polymerase (an enzyme that the cell uses to make copies of its DNA) was needed to withstand the heating and cooling (annealing) cycles for DNA replication outside of the cell ('in vitro') for DNA amplification. He turned to a thermophile (*Thermus aquaticus*) discovered in the hot springs of Yellowstone National Park as a source for a thermostable DNA polymerase and the rest is history. Beyond PCR, hyperthermophiles have been the source of enzymes used in many industrial applications, including starch processing to produce biofuels (corn ethanol). In essence, the discovery of hyperthermophiles extended the temperature range for which bioprocesses could be operated.

Relevance for Sustainable Development Goals and Grand Challenges

Hyperthermophiles and their enzymes impact several SDGs, including

- **Goal 3. Ensure healthy lives and promote well-being for all at all ages**. The polymerase chain reaction is widely used in medical diagnostics, witness the PCR test for COVID-

19, but also for detection of susceptibility to genetic disease, and epidemiology the distribution and transmission of disease.

- **Goal 7. Ensure access to affordable, reliable, sustainable and modern energy for all**. Sustainability requires the increasing use of renewables, including the production of fuels from plant materials - biofuels. Enzymes from hyperthermophiles are more stable than enzymes from other organisms and enable such conversions at high temperatures.
- **Goal 8. Promote sustained, inclusive and sustainable economic growth, full and productive employment and decent work for all**. The range of applications of biotechnology, especially that based on microbes, which benefit humanity, is enormous and rivals or even exceeds that of chemistry. Moreover, microbial biotechnology is highly entrepreneurial and rapidly growing, and hence a significant driver of the creation of new enterprises and employment.

Pupil Participation

1. Class discussion of whether hyperthermophiles could be found on other solar bodies (i.e., planets, moons, asteroids). What conditions would be needed for this to be the case?

2. ***Student awareness about hyperthermophiles***
    - **a.** Hyperthermophiles (so far as we know) do not cause human disease (they are not pathogenic).
    - **b.** Some predict that hyperthermophiles may be found in other places in our solar system (e.g., volcanic moons of Jupiter) and were the earliest life forms on earth. Do you agree or disagree?
    - **c.** Hyperthermophiles were the source of enzymes (DNA polymerases) to make PCR work, thereby kicking off the development of modern biotechnology.

Exercises

1. Find examples of volcanoes and other prominent geothermal features around the globe (places like Yellowstone in the US; for example, Rotorua in New Zealand). Has any hyperthermophile been isolated from these places? What are the microorganisms' names and what are their best conditions for growth?
2. Take temperatures around your house of hottest and coldest running tap water, best temperature for taking a shower, and water temperature for cooking pasta. What was the hottest and coldest temperature for the weather near your home last year? How do these compare to the temperatures that hyperthermophiles grow at?
3. What are the reasons that organisms (so far) do not grow much above 100°C or 212 °F (except in a few cases)? What would be the lowest temperatures (**Hint:** these organisms are called 'psychrophiles' or 'cold-loving').
4. Read about Dr. Kary Mullis, Nobel Laureate, who invented PCR.

The Evidence Base, Further Reading and Teaching Aids

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Glossary

Anaerobes – Life forms that grow in the absence of oxygen
Archaea – Single-celled microorganisms that resemble bacteria but have different cell membranes and molecular mechanisms for making DNA, RNA and proteins
Astrobiology – The study of living systems beyond earth.
Enzymes – Special type of proteins that catalyze the chemical reactions that enable cells to breakdown food, to obtain energy, and to make new cells
Eukarya – Life forms that are made up of cells in which their DNA is contained in a nucleus
Hyperthermophile – Microorganisms that grow best above 75°C, and up to 120°C.
Mesophile – Microorganisms that thrive at ambient temperatures, typically 20-45°C.
Polymerase Chain Reaction (PCR) – Used for making large number of copies of DNA
Proteins – Biomolecules based on amino acids that contribute to the structures of cells
Psychrophile – 'Cold-loving' microorganisms that thrive at cold temperatures, typically 15°C or lower.
Thermophile – Microorganisms that grow best between 45-75°C.