Some like it hot

I.A.L. Diamond and Billy Wilder

Chapter 6
Life in boiling water

The press most likely did not highlight the report of Thomas Brock (Bloomington, Indiana; later Madison, Wisconsin, USA) in 1969 on the isolation of a bacterium from a pond in Yellowstone National Park. This bacterium, *Thermus aquaticus*, grows at 70 °C (nearly 160 °F). Every molecular biologist or microbiologist is familiar with the enzyme Taq polymerase, which was isolated from *T. aquaticus* and has proved essential for the PCR technique used for analysis of trace amounts of DNA (see Chapter 25).

Growth at 70 °C sounds pretty exciting, but it's far from the 100 °C (212 °F) at which water boils!

Just wait a few minutes. Brock then isolated an organism that grows at 80 °C, *Sulfolobus acidocaldarius*. Among microbiologists, these reports aroused an interest in microorganisms growing at high temperatures, so sites in the immediate vicinity of volcanoes or geysers, where it spits and sputters, became the hunting grounds of microbiologists. Two German microbiologists from Munich, Wolfgang Zillig (1925–2005) and Karl Otto Stetter, were especially eager to isolate and characterize microorganisms growing in boiling water. First, they studied the sulfur-oxidizing *Sulfolobus* species from the Solfatara volcanic crater near Naples, Italy, then they concentrated on hot springs in Iceland. It is no easy task to take samples at such hostile sites and to then demonstrate in the samples the presence of living organisms that can be grown in cultures and are able to yield a sufficient cell mass for detailed studies. Zillig and Stetter obtained spectacular results, especially the realization that solely organisms of the *Archaea* domain are able to exist in boiling water. As for the members of the domain *Bacteria*, 80 °C is already quite hot. Nevertheless, there are species such as *Aquifex pyrophilus* and *A. aeolicus* that make it up to 95 °C, but boiling water is wholly and solely the habitat of some species of archaea.

The first "catches" of Zillig and Stetter were, for instance, *Thermoproteus tenax* and *Methanothermus fervidus*. These organisms and related ones grow at up to 97 °C. Ultimately it was Karl Otto Stetter, who really likes it hot when it comes to sampling sites, who dived to the hot seabed near the island of Vulcano, one of the Lipari Islands south of Naples. The fascinating result was the isolation of *Pyrodictium occultum*, which grows at the incredible temperature of 110 °C. But

Discover the World of Microbes: Bacteria, Archaea, and Viruses, First Edition. Gerhard Gottschalk.

let's have Karl Stetter (Regensburg, Germany) tell us how he took the sample and how he finally isolated *P. occultum*:

> "The question of life beyond the conventional sterilization of water by boiling had really fascinated me for some time. Of course, any habitat of organisms growing above 100 °C had to be under an elevated pressure in order to remain a liquid. To search for organisms of this sort, I took advantage of a family vacation in 1981 on the island of Vulcano. I took samples from the sea floor close to Porto di Levante at a depth of 5 to10 meters where temperatures were up to 110 °C. Back at my laboratory at the University of Regensburg, we performed growth experiments using various energy sources and temperatures. In experiment PL-19, I used artificial sea water, a volcanic gas mixture (molecular hydrogen, carbon dioxide, and hydrogen sulfide) as well as sulfur inoculated with material from the sampling site and incubated under pressure at 105 °C. After two days, I already saw with the naked eye some unusual, hazy material covering the sulfur granules. Under the microscope I saw novel disc-shaped cells that were connected by very thin threads. It took us a year to show that this isolate was able to grow at 110 °C. It was named the "hidden fire network" (*Pyrodictium occultum*) and it is shown in Figure 11. This was the first report that life was possible above 100 °C, which was completely unexpected, especially in the presence of high salt concentrations in the ocean."

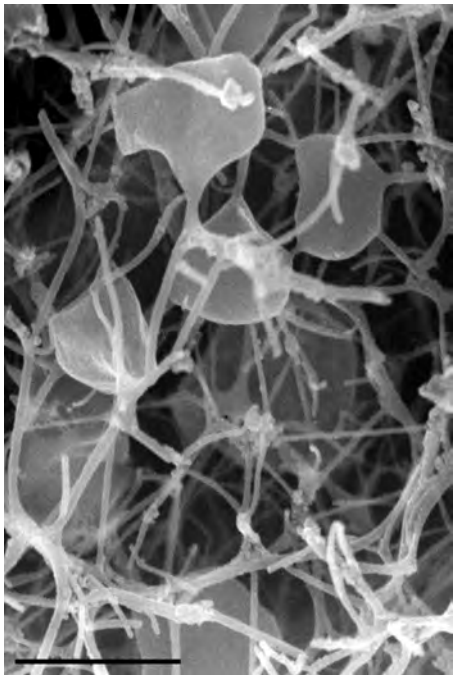

Figure 11 *Pyrodictium occultum*, scanning electron micrograph. The flat, irregularly shaped cells are connected by a network of protein filaments. (Reinhard Rachel and Karl Stetter, Regensburg, Germany.)

So, a specially equipped laboratory was established in the microbiology department at Regensburg to allow isolation and investigation of heat-loving archaea, now called hyperthermophilic archaea. It is fascinating to watch a growing culture of archaea in a vessel surrounded by boiling water. These microorganisms are so adapted to high temperatures that they usually don't even grow at temperatures below 80 °C. The world record in terms of growth temperature is held by *Pyrolobus fumarii*, which grows at 113 °C but is unable to grow below 90 °C; where it's simply too "cold."

It certainly wasn't easy to obtain these breathtaking results. Where else have microbe hunters been successful?

It really became a "hot" issue. Microbiologists got in touch with geologist colleagues because they were interested in hot sampling sites other than Yellowstone and the geysers in Iceland. Other extreme sites, those that were very acidic, alkaline, or salty, also became very attractive. One example is the Russian peninsula Kamchatka with its twenty-eight active volcanoes and hot springs. Several expeditions of microbiologists to Kamchatka came up with a rich variety of "new" organisms. The hot springs of Japan and the Azores were also visited by microbiologists, as well as the Wadi El Natrun in Egypt, an alkaline habitat with salt lakes and lagoons. The list of spectacular isolates of archaea is long, only a few will be mentioned here. *Picrophilus torridus* was isolated from hot acidic soil near Kawayu, on the Japanese island of Hokkaido (Figure 12). This archaeon is one of the most unusual living creatures on Earth. It grows at 60 °C and at a pH value of 0.7. In other words, in hot dilute sulfuric acid, caustic enough to burn our hands. The circular chromosome of *P. torridus* was sequenced in our laboratory. It consists of 1.5 million base pairs and is only one-third the size of the *Escherichia coli* genome.

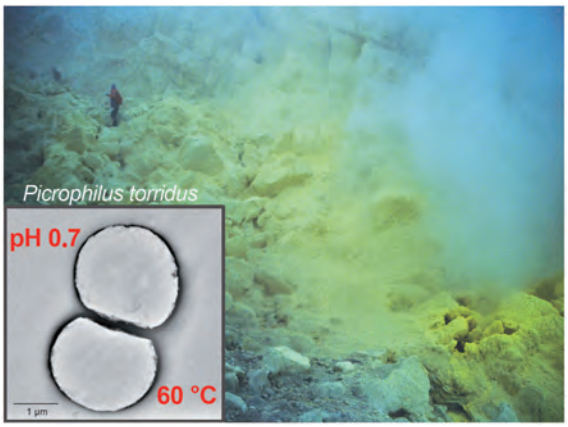

Figure 12 *Picrophilus torridus* and its habitat. The organism was isolated from a sample taken close to the volcano of Kawayu (Hokkaido, Japan). Christa Schleper (University of Vienna) is in the left background. (Photograph: Gabriela Puehler, Munich, Germany.)

Genes on the *P. torridus* chromosome can be identified with the conventional methods of molecular biology, but it proved very difficult to use this information for making enzymes. Obviously, *P. torridus* is an eccentric result of evolution.

A fascinating archaeal isolate was fished out of the Wadi El Natrun by microbiologists. It was named *Natronomonas pharaonis*; its habitat is a warm 45 °C, soda-containing brine with a pH of 9.5–10. Finally, an isolate from the Great Salt Lake in Utah (USA) should be mentioned, *Haloraptus utahensis*, which grows at 55 °C in saturated sodium chloride solution. Lagoons with a high salt content attract attention from the air because of their bright red color, for instance, the southern part of San Francisco Bay. These lagoons contain dense populations of haloarchaea, which have high concentrations of carotinoid pigments to protect themselves from the sun. Several of them also contain a fascinating purple compound, bacteriorhodopsin (see Chapter 8).

It is hard to believe: on the one hand, there are hard-boiled eggs with completely denatured protein, and, on the other hand, there are living creatures that grow vigorously under such conditions. Does anyone know why there are such stark differences? Why are archaea but no bacteria found in boiling water?

Those are good questions. For one thing, the composition of the cytoplasmic membranes differs between bacteria and archaea. The importance of this membrane for all living organisms was pointed out in Chapter 2. In bacteria and in all higher living organisms, the membrane is composed of so-called phospholipids, which are fat-like compounds. In archaea, however, they consist of special ether lipids that are more stable and better adapted to higher temperatures than phospholipids. At high temperatures, the DNA needs to be stabilized as well, which is achieved by binding proteins. In addition, the enzymes must be so engineered that they are active at temperatures at which we normally boil eggs, broil fish, or kill bacteria in the pasteurization process. Their existence can be considered a crowning achievement of evolution. The question, of course, is why these enzymes are so heat-resistant. Let's see what an expert, Gregory Zeikus (East Lansing, Michigan, USA), has to say about this:

> "Enzymes from hyperthermophilic microbes have very subtle structural differences from those of mesophiles or psychrophiles. These subtle structural differences allow hyperthermophilic enzymes (also called thermozymes) to be stable and active at very high temperatures (i.e. at 80 °C to 110 °C), but are generally less active at low temperatures (i.e. at 5 °C to 40 °C). A thermozyme shares the same general architecture and fold as that of catalytically similar mesozymes or psychrozymes. Thermozymes differ because they are more rigid, compact, and have fewer water accessible amino acids like asparagine and glutamine, which deaminate at high temperatures. Different metabolic types of thermozymes achieve a more rigid and compact structure by different means. The addition of a single salt bridge/ion pair at the enzyme's terminus can greatly enhance thermal

stability and prevent unfolding. Some thermozymes contain more hydrogen bonds than catalytically similar mesozymes. Enzymes that are stabilized by metal binding, like calcium-containing alpha amylases, come with an additional tightly-bound zinc in *Pyrococcus furiosus*. Dimeric enzymes from hyperthermophiles have evolved unique ways to enhance active dimer stability at high temperature. For example, xylose isomerase from *Thermotoga neopolitana* contains an additional proline in the dimer interface. Here, proline puts a bend in the protein, which rigidifies it and also exposes a surface aromatic amino acid for an additional hydrophobic bond to further enhance dimer stability."

This area still requires a great deal of research. Another question is why don't we find archaea that compete with bacteria in milk, in juices, or during the degradation of starch in soil. It cannot be ruled out that they once existed in such habitats but were superseded by bacteria. Only the rims of the domain of Archaea remain, much like the crater rim forming a circle of islands in Santorini (Greece). In their habitats, however, they are the masters.