

Geothermal Habitats and Adaptations of Thermophilic Microbes



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Abstract In this chapter, the main habitats of thermophiles, their discovery, and ecology are discussed. The focus of the discussion is on natural habitats associated with geothermal activity, their geological origin, and characteristics of different geothermal surface manifestations, including mud pools, solfatara fields, alkaline hot springs, and warm springs. The ecological discussion is primarily focused on strategies that thermophiles utilize to obtain energy.

1 Introduction: Brief History of Scientific Exploration of the Upper Thermal Boundary of Life

From the early nineteenth century, microorganisms were known to inhabit high-temperature environments and the first thermophilic bacterial strains were isolated toward the end of the century (reviewed in Allen 1953 and Brock 2001). By 1920, scientific interest in thermophiles had dwindled and was largely confined to moderate thermophiles studied in the context of food microbiology. It was the pioneering work of Thomas D. Brock in the 1960s and onward that sparked a new wave of scientific interest in thermophiles that has continued to this date. In the early 1960s, Brock was involved in microbiology research, including studies on the sulfur-oxidizing bacterium *Thiothrix mucor* and on cyanobacteria, and visited Yellowstone National Park in search of possible habitats for these organisms. During these visits in 1964–1965, Brock noted that hot springs not only gave rise to diverse and dense microbial life in efflux channels where temperatures were moderately high but noticed evidence of microbial life at elevated temperatures, previously thought to be devoid of life. Specifically, he noticed pink filaments in the geothermal water at 82 °C that he strongly suspected were of biological nature (Brock 1995). He returned to Yellowstone in 1966 and attempted to obtain a culture of the organism by

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inoculating a sample of the pink filaments into media and cultivating the enrichment at 70 °C under aerobic conditions. Instead of the desired pink organism, Brock was searching for, the culture became dense with yellow-pigmented cells. From the culture, the strain *Thermus aquaticus* YT-1 was first isolated, and subsequently, Brock and his colleagues isolated several other bacterial strains. They showed that the species grows optimally at 70 °C and has a maximal growth temperature of 79 °C (Brock and Freeze 1969). This work was the first to report an organism that grows at a temperature above 70 °C. The publication of these findings along with the deposition of strain YT-1 in the American Type Culture Collection was hugely influential, not only leading to an interest in the exploration of life at elevated temperatures but also to influential innovations in biotechnology. Taq polymerase, derived from *T. aquaticus*, became the standard enzyme for the polymerase chain reaction (PCR). Brock continued his studies in Yellowstone for several years. During that period, he showed that when microscope slides were immersed in geothermal hot springs at high temperatures, even at or around 100 °C, they became covered in cells that could be observed under a microscope (Brock 1967; Bott and Brock 1969). He also isolated the first representatives of thermoacidophilic aerobic archaea *Sulfolobus acidocaldarius* and *Thermoplasma acidophilum* (Brock et al. 1972; Darland et al. 1970).

In the 1980s, Karl Stetter and his colleague Wolfram Zillig became interested in microbial life at high temperatures (Stetter 2006) and their studies would lead to further leaps in knowledge about thermophiles, the discovery of the great diversity of anaerobes in geothermal sites, and the corresponding ecological and metabolic variety of these organisms. At that time, the aerobic archaeon, *Sulfolobus acidocaldarius*, was the most extreme thermophile known, with an optimal growth rate at 75 °C and an upper-temperature limit for growth at 85 °C. On a trip to Iceland in 1980, Stetter and Zillig sampled multiple boiling hot springs and, under the microscope, observed that the water teemed with what appeared to be microorganisms. Stetter noted that when the blue redox indicator resazurin was incubated in the hot-spring water, it immediately turned pink, an indication that the water was reducing—i.e., an anaerobic environment. This turned out to be a highly important observation since most strains that were later isolated from comparable environments are indeed strict anaerobes, and hence, their isolation requires careful handling under conditions devoid of oxygen. From samples collected in Kerlingarfjöll in central Iceland, Stetter and Zillig isolated the methanogen *Methanothermus fervidus* and a strictly anaerobic species of *Thermoproteales*, both of which grew at a maximum temperature of 97 °C (Zillig et al. 1981; Stetter et al. 1981)—far beyond the maximal growth temperature of *S. acidocaldarius*. Searching for still more extreme thermophiles, Stetter sampled submarine hydrothermal vents off the coast of Italy in 1981. From these samples, strictly anaerobic *Pyrodictium* strains were isolated with optimal growth at 105 °C and an upper limit for growth at 110 °C (Stetter 1982). Later, Stetter and colleagues isolated *Pyrolobus fumarii* from a black smoker in the Atlantic Ocean, which has an optimal growth temperature of 106 °C, an upper growth limit of 113 °C, and can survive in an autoclave for an hour at 121 °C (Blöchl et al. 1997). The term hyperthermophile has been coined for

microorganisms with optimum growth temperature above 80 °C and most of the new species isolated by Stetter and coworkers, at that time and in the following decades were anaerobic hyperthermophilic Archaea. These new species were chemolithotrophic, chemolithoautotrophic, or organotrophic, which harnessed energy by anaerobic respiration. Extremely thermophilic bacteria ($T_{\text{opt}} > 80$ °C) were also discovered such as the aerobic marine hyperthermophile *Aquifex pyrophilus* of the phylum *Aquificota* and hyperthermophilic fermentative marine species *Thermotoga maritima* of the phylum *Thermotogae*.

Research efforts on thermophilic microbiology and ecology increased extensively in the early 1990s when large international research projects (thermophiles and extremophiles) in the field were funded by the European Union. This helped to establish in Europe important research groups in the field and to advance collaborative research in the microbiology of terrestrial and marine geothermal habitats.

Geothermal areas are largely reduced and anaerobic habitats, with various adaptations to energy sources, physicochemical conditions, and scarcity of oxygen. Due to the high novelty and the extremophilic adaptations to both temperatures and pH, early research focused largely on the microbiology of Archaea, but early work on the microbiology and ecology of anaerobic and fermentative bacteria was carried out by Jurgen Wiegel in Yellowstone Park in the USA and by Birgitte Ahring (Denmark) in Iceland. Fermentative, anaerobic bacteria are important consumers of organic matter in microbial mats and sediments, and a number of thermophilic adaptations belonging to novel genera such as *Thermoanaerobacterium*, *Thermoanaerobacter*, *Thermotoga*, and *Caldicellulosiruptor* have been discovered and described from these biotopes.

Early work on aerobic Bacteria was mainly carried out by the effort of KO. Stetter, and R. Huber in Germany; RAD. Williams, R. Sharp, and NDH. Raven in Britain; JK. Kristjansson, G. Alfredsson, S. Hjörleifsdottir, GO. Hreggvidsson in Iceland; Da. Costa and H. Santos in Portugal; T. Oshima in Japan; and HW. Morgan and RM. Daniel in New Zealand. This included work on the heterotrophic genera *Thermus*, *Rhodothermus*, and *Geobacillus* and the autotrophic hydrogen oxidizing genera *Hydrogenobacter* and *Aquifex*.

Colorful photosynthetic microbial mats are a conspicuous feature of alkaline hot spring effluents composed of photosynthetic bacteria. They are abundant in summer and near disappearing in winter. Pioneering work on the microbial ecology of these microbial mats was done by DM. Ward, R. Castenholz, and SR. Miller who isolated and described novel species belonging to the phyla *Cyanobacteria* and *Chloroflexi*. Consequently, the photosynthetic temperature boundary at 70–74 °C was established, above which photosynthetic bacteria are not found.

2 Underlying Geology and Global Distribution of Geothermal Areas

Geothermal areas are the primary natural habitats of thermophiles. They are diverse in terms of temperature, water abundance, pH levels, and availability of various molecules that microorganisms utilize for growth—a result of different underlying geology, which is briefly discussed here.

Geothermal areas are primarily found at plate-tectonic margins, in regions of active volcanism, where recent plutonism has occurred, and at intracontinental rifts (Fig. 1, Nukman and Moeck 2013; Bogie et al. 2005; Faulds et al. 2009), due to the presence of a heat source in the form of magma, pluton, or mantle close to earth's surface (Moeck 2014).

These geothermal sites harbor biotopes of only thermophilic microbes completely different from those of the surrounding area. They have sporadic distribution, and they are far apart on a global scale, which gives these confined ecosystems distinct island characteristics.

Geothermal features arise because of different sub-surface geological phenomena that provide a heat source. These heat sources result in temperatures exceeding 200 °C within a depth of 3.000 m (Moeck and Beardsmore 2014). The features are formed as a result of heat being transferred from the heat source to the surface via convection—i.e., through the movement of fluids. The source of the fluids is generally meteoric water (Deon et al. 2012). As the fluids are exposed to the heat source, their composition changes when salts, minerals, acids, and other chemicals are dissolved. In their “journey” from heat source to the surface, the fluids may be further altered chemically as they pass through different geological layers. On breaking the surface, the hot fluids are manifested in geothermal features including fumaroles, geysers, hot springs, mud pools, or solfatara fields, depending on the water availability and resultant chemical composition, pH, and temperature of the fluids when they reach the surface. Further alterations then occur at the surface when encountering atmospheric oxygen due to abiotic oxidation, mainly of H₂S.

A shallow, active magma chamber provides an intense heat source that can give rise to geothermal features. Such geothermal systems can arise in association with active volcanism, for example, in Iceland, Java, the South American Andes, and Taiwan (Moeck and Beardsmore 2014). Surface features may arise directly above the heat source, atop a respective volcano, in the so-called upflow zone, which is generally characterized by high temperatures, water scarcity, and acidity. Fluids may also move horizontally after exposure to the heat source and surface at the roots of a volcano, in the so-called outflow zone. During the horizontal movement, the fluid cools down and approaches near neutral pH (Hochstein 1988). Magmatic geothermal systems can also arise due to the presence of an active magma chamber without active volcanism, for example, in the Taupo Volcanic Zone in New Zealand (Bogie et al. 2005; Moeck and Beardsmore 2014).

Further, geothermal features can be formed in areas where recent plutonism has occurred—i.e., where magma has risen through the crust without reaching the

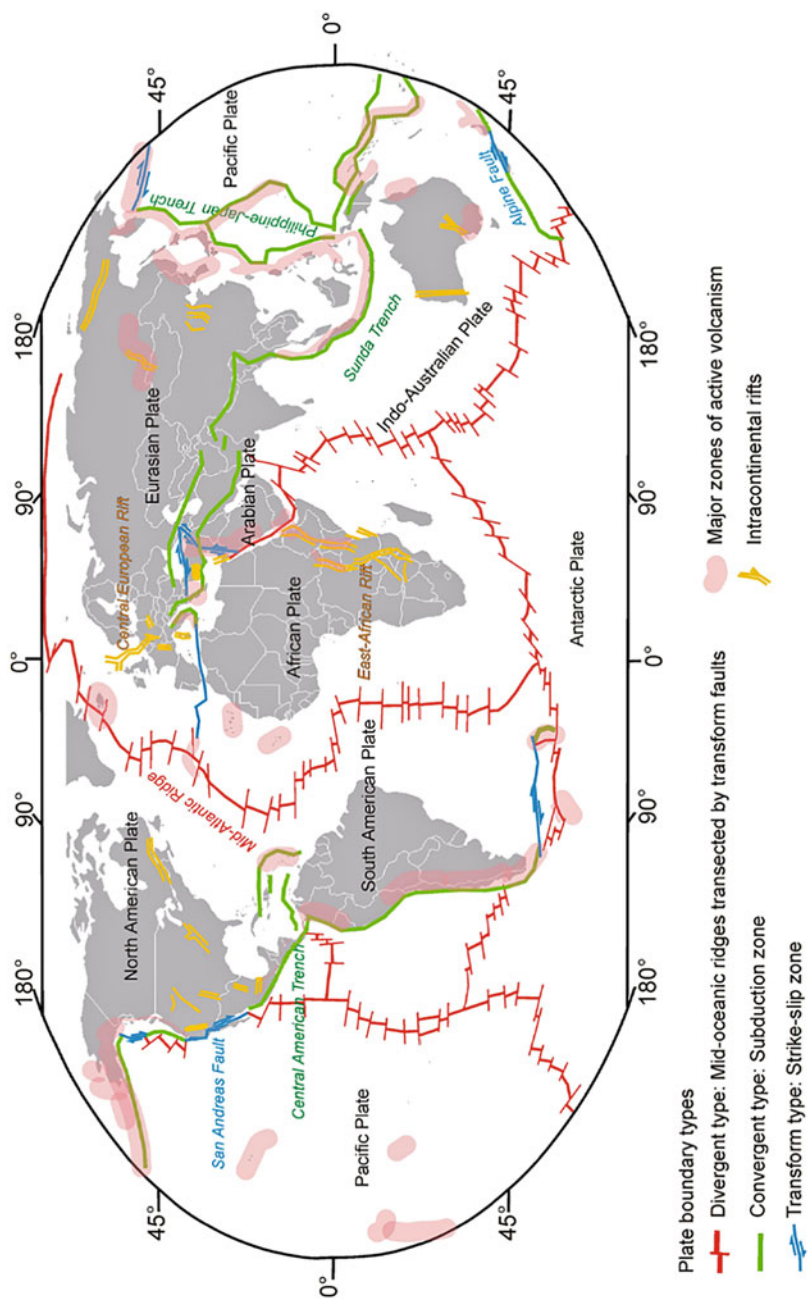


Fig. 1 Global distribution of plate boundaries, volcanism, and rifts. Geothermal surface features are found at plate-tectonic margins, in regions of active volcanism, where recent plutonism has occurred, and at intracontinental rifts. Adapted from Moeck (2014)

surface, is crystallizing, and radiates heat. They are generally formed at convergent continent–continent margins surrounded by mountains that provide a rich source of meteoric water to sustain convection (Moeck and Beardsmore 2014). Examples include the Geysers geothermal field in California and the Larderello geothermal system in Italy (Argus and Gordon 2001; Bertini et al. 2006).

Finally, geothermal features can be observed in areas where extension has caused crustal thinning such that the mantle is elevated to levels close to the surface, providing a heat source. Geothermal features form in this context where meteoric water can seep deep into the crust to interact with the heat source via faults or otherwise permeable layers. Geothermal systems of this type are found in western Turkey, East African rift, Upper Rhine graben in central Europe, and the Great Basin in the USA (Moeck and Beardsmore 2014).

2.1 High- and Low-Temperature Geothermal Areas

Geothermal areas with surface features can broadly be classified as high- or low-temperature fields.

In high-temperature fields, which usually coincide with active volcanic areas at high altitudes, temperatures exceed 200 °C at a depth of 1.000 m and groundwater levels are usually low. On the surface, they are characterized by the presence of steam, transformed and colorful soils, mud pools, and the release of gases, particularly N₂, CO₂, H₂S, and H₂. Below the surface, pH levels in these fields are circumneutral due to the presence of CO₂ and H₂S (pK_a = 6.3 and 7.2, respectively). However, as hydrogen sulfide reaches the surface, it is oxidized chemically due to exposure to atmospheric O₂ and biologically due to microbial respiration. This leads to the formation of H₂SO₄ (H₂S + O₂ → H₂SO₄) that lowers the pH level at the surface and transforms surface rocks into mud. Most of the surface of the respective geothermal area is turned into an acidic solfatara field with white and yellow sulfur precipitations, generally with mud pools scattered about where some water is present. These areas are further characterized by instability, in that water levels may change dramatically over a short time span, and surface features may be “lost” and others may form at regular intervals.

In low-temperature fields, temperatures are lower than 150 °C at 1.000 m depth. On the surface, they are characterized by pools of liquid water at neutral or slightly alkaline pH. Water influx is relatively generous, as compared with high-temperature fields, giving rise to effluent streams with temperature gradients that sustain diverse microbial communities, often providing colorful layers to the otherwise largely white or off-white geothermal field. Sulfide levels are low, and with high efflux rates, H₂SO₄ does not accumulate. The water contains dissolved minerals and silica (SiO₂) when the fluids interact with silicate rocks and bicarbonate when they interact with carbonate rocks and gases of varying levels. The silica precipitates as waters cool down at the surface of the hot springs, forming silica sinters, and accordingly, the sinters form low, broad deposits that extend several meters from the respective

hot spring. In contrast, calcium carbonate precipitates rapidly when CO_2 escapes from bicarbonate-rich fluids, causing supersaturation with respect to bicarbonate, and the formation of travertine. The rapid precipitation results in the formation of deposits near the edge of the respective hot spring, and as a result, the deposits form high-relief structures. In low-temperature fields, temperatures and water flow are generally stable.

2.2 *Surface Features in Terrestrial Geothermal Areas*

Solfatara fields are characterized by large surface areas covered in soft soil of varying hues of light brown—rock transformed by sulfuric acid (Fig. 2). Adding to the richness of color, the fields are generally scattered with tones of yellow due to



Fig. 2 Solfatara field. (*Top*): The hill Námafjall, east of Mývatn, Iceland, showing hues of *brown*, *yellow*, and *red* characteristic of solfatara fields. (*Bottom*): Close-up images from the same field, showing *red* (*left*) and *yellow* (*right*) deposits in more detail

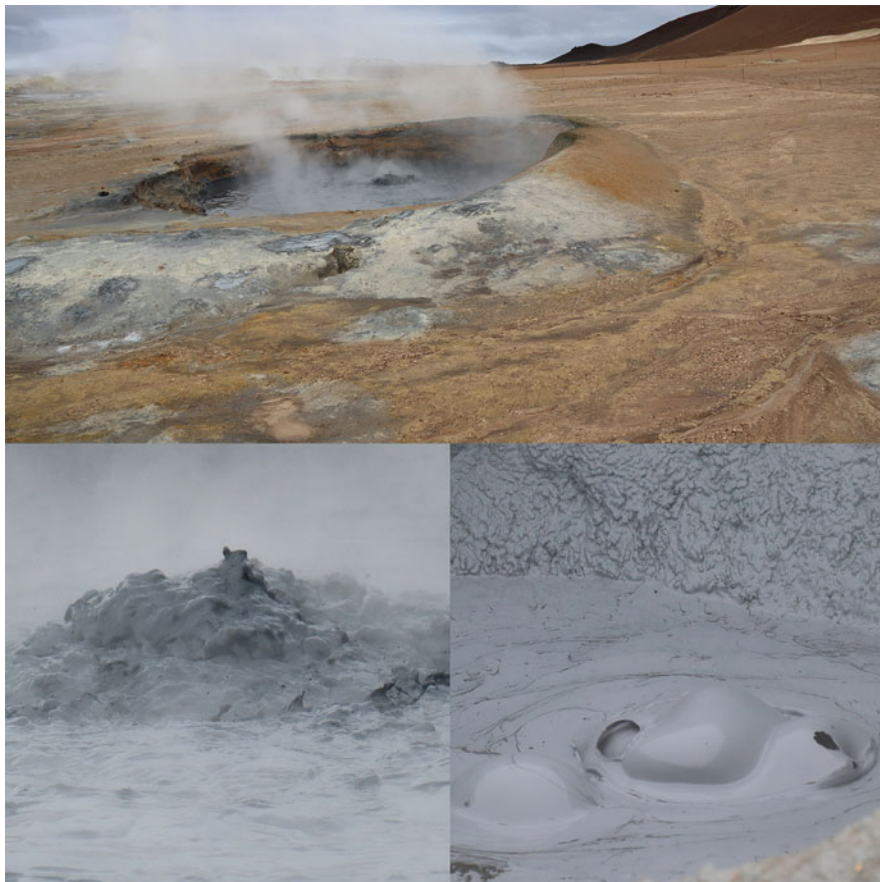


Fig. 3 Mud pools. (*Top*): A mud pool in the Hverarönd geothermal area in the slopes of Námafjall, Mývatn, Iceland. The pool is about 5 m in diameter (rough estimate by the photographer). (*Bottom*): Closeup images of the thick bubbling fluids of two mud pools. The *bottom left* image is from the same pool as that on *top*, and the *bottom right* image is from a separate pool with thicker fluids. The images were taken after a period of heavy rainfall, which likely reduced the thickness of the pool fluids

the deposition of sulfur, tones of red due to the deposition of iron-containing compounds (e.g., hematite Fe_2O_3), and hues of dark gray due to the deposition of ferrous sulfide (FeS). The acidic soil harbors communities of strictly anaerobic archaea and at lower temperatures of *Thermoplasma* and *Picrophilus* archaea and *Thiomonas*, *Thiobacillus*, *Geobacillus*, and *Deinococcus* bacteria (Hreggvidsson et al. 2017).

Mud pools are formed in solfatar fields where the field's mud and liquid water mix in varying proportions (Fig. 3). The pools have no efflux channel, water is generally scarce, and depending on the water influx rate, the mud pot can be thick or thin. Often, bubbling is observed in the pool, a result of gases and steam passing to



Fig. 4 Fumaroles. Both images are from Hverarönd, east of Mývatn in Iceland. Surrounding the fumarole in the top image is a mound has formed of about half a meter in height. The opening of the fumarole in the bottom image is very small, about 0.5–1 cm in diameter

the surface. Microorganisms frequently encountered in mud pools include *Stygiolobus* and *Sulfolobus* archaea, and *Hydrogenobaculum* bacteria (Hreggvidsson et al. 2017)

Fumaroles, often observed in solfataras fields, are small openings through which steam and volcanic gases (including CO_2 , H_2S , and SO_2) are released (Fig. 4). The flow rate of the gases is generally quite high, and as it passes through the surrounding rocks and mud at the surface, a rather loud sound is often emitted, reminiscent of a violent storm

Boiling pits are small, shallow depressions in the surface with bubbling water. They can be found in high-temperature areas, in which case the water bubbles as a result of volcanic gases streaming through the water, and they can be found in low-temperature areas, in which case the bubbling is caused by boiling of the water

Alkaline hot springs ($>50\text{ }^\circ\text{C}$) and warm springs ($<50\text{ }^\circ\text{C}$) are common in low-temperature fields. They are pools of mineral water, circular in form, and of varying sizes, often 2–5 m^2 . They generally have outlets where water from the pools

flows out into the colder, surrounding environment, creating a temperature gradient that can sustain diverse life. In alkaline hot springs, pH levels are in the range of 7–10. These habitats often give rise to colorful microbial mats, with distinct “bands” of different colors corresponding to particular microorganisms. The boundaries reflect gradients in physicochemical parameters—pH, temperature, and fluid composition—and the ability or competence of respective microorganisms to thrive therein. Commonly observed colors and associated microorganisms include green from cyanobacteria; hues of orange and/or red from phototrophic *Chloroflexus* and *Roseiflexus*; and white, gray, gray with hues of blue, and black from hydrogen oxidizing *Sulfurihydrogenibium* and *Thermocrinis albus* (Hreggvidsson et al. 2017).

Sulfide-rich hot springs (65–85 °C) are rare but are occasionally found in low-temperature geothermal fields and in high-temperature fields where water is abundant. Like alkaline hot springs, they are water-rich, approximately circular in form, and often 2–5 m² in size. They differ in that the water contains high concentrations of sulfide. The springs have relatively high water flow rates and outlets, and therefore, sulfuric acid does not accumulate, and the pH level is around 5.5–6.5. They are often associated with microbial mats containing sulfide-utilizing species, in particular dominated by *Sulfurihydrogenobium* species (Hreggvidsson et al. 2017).

Steam vents are sometimes found in low-temperature fields, often in the absence of any other nearby surface features (Fig. 5). They form where steam from hot groundwater rises through porous layers such as a young lava field and rises to the surface. From a distance, they may therefore give the appearance that the lava itself is fuming. On closer inspection, one can identify discrete openings where the steam escapes, in which temperatures are in the range of 55–85 °C and pH of the surrounding soil is 7–8. Commonly, members of *Thermus*, *Chloroflexus*, *Actinobacteria*, and *Acidobacteria* are found in these environments (Hreggvidsson et al. 2017).

3 Other High-Temperature Environments, Natural and Anthropogenic

Various other thermal environments, natural and anthropogenic (man-made), sustain thermophilic microbial communities. They are, however, generally less extensively studied than those discussed above—in some cases a result of their rarity and/or inaccessibility—and will therefore only be briefly discussed here.

Intertidal and submarine hot springs: Geothermal fluids can in principle surface through earth’s crust anywhere on the globe, given the presence of a geological heat source as discussed above—i.e., they can surface on dry land, on shore, or underwater. Intertidal hot springs are formed when geothermal fluids surface on an ocean’s shore. They are quite unique environments in that temperatures fluctuate greatly with tidal movements. At high tide, the pools are covered in seawater, and temperatures match that of the respective ocean, except right at the hot-spring source. At low tide, the pool is quickly heated by the geothermal fluids. The temperature shift at low and



Fig. 5 Steam vents. *Top* image shows steam arising from multiple steam vents on and under the slopes of Jarðbaðshólar, east of Mývatn in Iceland. Note the larger plume in the foreground and the smaller plumes on the hill. The *bottom* image shows a steam vent opening. Note the lack of fluids and the transformed vegetation

high tide depends on the temperature of the geothermal fluids and that of the ocean water. These types of environments are for example found at various locations around the coast of Iceland (Bjornsdottir et al. 2021; Kale et al. 2013; Hobel et al. 2005), in Italy, New Zealand, Fiji (Burgess et al. 2007), and others. Submarine hot springs are formed when geothermal fluids discharge underwater. They can be located at depths from a few meters to a few kilometers and include vents off the coast of Milos Island, Greece (Sievert et al. 2000), off the northern coast of Iceland (Marteinsson et al. 2001), and deep-sea vents located at a great depth near Galápagos islands (Corliss et al. 1979). The hydrothermal fluids that emanate from the vents differ significantly in chemical composition and hence the associated microbial communities. In some systems, they contain high concentrations of sulfides (Kelley et al. 2002), while in others they are enriched in hydrogen and methane (Kelley et al. 2005). Heat at the source can be extremely high, but mixing with seawater causes a steep temperature gradient, thus sustaining communities of thermophiles, mesophiles, and psychrophiles.

Anthropogenic thermal environments: Various human activities have resulted in the formation of “non-natural” warm habitats that are conducive for the growth of

thermophiles (Pask-Hughes and Williams 1975; Brock and Boyle 1973). Examples of such habitats include heat exchangers and pipelines in homes that carry hot water, e.g., in the context of delivering hot water from a boiler to a radiator; district heating systems that distribute hot water to entire towns or cities; thermophilic waste treatment plants; burning coal waste piles; and various industrial processes that involve heating, e.g., in the context of reducing microbial content in foods.

Transient natural thermal habitats can result from the self-heating of composts; of hay, straw, or other similar agricultural products that are stored in large quantities; or of manure. Though these types of environments may form in the absence of humans, they exist at a larger scale due to human activities.

4 Ecology in Geothermal Habitats

Temperature strongly affects the physiology of organisms and hence their ability to thrive in a given environment. Some multicellular organisms can regulate body heat and their cells are therefore partially protected from the surrounding heat, while in microorganisms, which are unicellular, cytoplasmic temperature directly follows that of the environment. Many cellular macromolecules like DNA and enzymes are quite vulnerable to loss of structure (and hence function) due to elevated heat since their structure is largely the result of weak chemical interactions (hydrogen bonds, etc.). Many thermophilic microorganisms have evolved with genomes that have high GC content and encode rigid enzymes/proteins to withstand thermal disruption (Feller 2010; Radestock and Gohlke 2011; Hu et al. 2022). These adaptations, however, render the organism's incapable of growth at lower temperatures. Each microorganism is adapted for growth at a particular temperature, the T_{opt} , where its growth is fastest. Growth rates generally reduce linearly some 15–25 °C from the T_{opt} to the lowest temperature that will sustain growth, the T_{min} . Above the T_{opt} , the growth rate is reduced more rapidly to a temperature, T_{max} , above which no growth is observed. There is no single accepted consensus for the defining growth temperature that would classify an organism as a thermophile. Brock proposed that this temperature should be 55–60 °C since habitats with temperatures below 55 °C are common in nature, while habitats with higher temperatures are rare. Further, he noted that no eukaryotes grow at temperatures beyond this limit, while certain bacteria and archaea thrive. According to this definition, the thermophiles are therefore exclusively prokaryotes. The terms moderate thermophile, thermophile, extreme thermophile, and hyperthermophile are now used in microbiology of thermophiles to describe different temperature adaptations. The demarcations are not clear, but the following criteria have been proposed for defining more accurately both thermophiles and hyperthermophiles, the former having $T_{\text{max}} \geq 65$ °C and the latter having $T_{\text{opt}} > 80$ °C (Hreggvidsson et al. 2017; Kristjánsson and Stetter 1991). Taking into account the definition of Brock, a moderate thermophile would then have T_{max} higher than 55 °C and lower than 65 °C.

Thermophiles have diverse chemotrophic catabolic processes for harnessing energy, both organotrophic and lithotrophic, and they use both autotrophic and

organotrophic processes for supplying carbon to anabolic pathways. Autotrophic thermophiles include both photoautotrophs and chemolithoautotrophy. Photoautotrophs are, however, not found at temperatures beyond 70–74 °C, the photosynthetic boundary, while diverse chemolithoautotrophs that utilize various inorganic electron acceptors and donors thrive at more extreme temperatures. In addition to the primary production of organic chemicals that occurs in geothermal habitats, the organic material may be introduced into the habitat from outside sources—e.g., leaves or other plant material that are blown in a gust of wind into a hot spring. Overall, geothermal habitats are nevertheless generally oligotrophic. Sudden changes in temperature in hot springs can, however, lead to a rapid increase in nutritional availability. After a period of stable temperature, in which certain organisms can thrive at or close to their T_{opt} , with a corresponding accumulation of biomass, a sudden increase in temperature can result in their death and hence elevated nutritional level in the habitat that other more thermophilic species then utilize. Some chemolithoautotrophic thermophiles utilize oxygen as an electron acceptor in their metabolism. Oxygen concentration in geothermal fluids is, however, relatively low, as compared with concentrations found in lakes or seawater, since oxygen solubility in water decreases with increased temperature (Geng and Duan 2010).

Among archaea, the most extensively studied thermophiles are methanogens and a broad group of sulfur-metabolizing species. Methanogens obtain energy via an anaerobic respiratory pathway called methanogenesis that is uniquely found in archaea. They can be further classified as hydrogenotrophs, which comprise five orders, and methylotrophs that comprise a single order. Hydrogenotrophs obtain energy primarily by the reduction of CO_2 into CH_4 using H_2 as an electron donor, though a few other small organic molecules can act as electron donors as well. Methylotrophs are similarly capable of the reduction of CO_2 into CH_4 , but are characterized by their ability to convert various methyl group-containing compounds—methanol, methyl amines, and methyl sulfides—and acetate into CH_4 (Costa and Leigh 2014). Examples of thermophilic methanogens are *Methanothermus fervidus* ($T_{\text{opt}} = 83$ °C, $\text{pH}_{\text{opt}} = 6.5$) that is found in anaerobic mud and soil and *Methanobacterium thermoautotrophicum* ($T_{\text{opt}} = 65$, $\text{pH}_{\text{opt}} = 7.4$) found in alkaline hot springs and sewage sludge (Stetter et al. 1981; Zeikus and Wolfe 1972).

Archaea use diverse aerobic and anaerobic metabolic pathways for energy conservation using sulfur-containing compounds as electron donors and acceptors (reviewed in Liu et al. 2012 and Hreggvidsson et al. 2017). The compounds include elemental sulfur S^0 , sulfate, sulfite, thiosulfate, sulfide, and others. In aerobic sulfur oxidation, S^0 is the electron donor and oxygen is the electron acceptor. The process is, e.g., utilized by the thermoacidophilic *Sulfolobus acidocaldarius* ($T_{\text{opt}} = 75$ °C, $\text{pH}_{\text{opt}} = 2.5$) and *Acidianus infernus* ($T_{\text{opt}} = 90$ °C, $\text{pH}_{\text{opt}} = 2$) that inhabit acidic solfatara fields. Under anaerobic conditions, S^0 can be reduced for energy conservation by at least three mechanisms, all of which are commonly found in thermophiles that inhabit anaerobic geothermal soil. First, this can occur by autotrophic respiration using H_2 as an electron donor—e.g., in *A. infernus* and in *Thermoproteus tenax* ($T_{\text{opt}} = 90$ °C, $\text{pH}_{\text{opt}} = 5$) and *Pyrodictium occultum* ($T_{\text{opt}} = 105$ °C,

pH_{opt} = 6.5); second, by heterotrophic respiration with organic chemicals as electron donors—e.g., in *Thermoproteus tenax* ($T_{\text{opt}} = 90\text{ }^{\circ}\text{C}$, pH_{opt} = 5); and third, by fermentation of organic chemicals—for example, *Pyrococcus furiosus* ($T_{\text{opt}} = 100\text{ }^{\circ}\text{C}$, pH_{opt} = 6). Under anaerobic conditions, sulfate and sulfite can also be reduced with organic compounds or H₂ as electron donors—e.g., in *Archaeoglobus fulgidus* ($T_{\text{opt}} = 83\text{ }^{\circ}\text{C}$, pH_{opt} = 7) and *A. profundus* ($T_{\text{opt}} = 82\text{ }^{\circ}\text{C}$, pH_{opt} = 6). Other thermophilic archaea found in geothermal areas include the heterotrophs *Thermoplasma volcanium* ($T_{\text{opt}} = 60\text{ }^{\circ}\text{C}$, pH_{opt} = 2) and *Sulfolobus acidocaldarius* ($T_{\text{opt}} = 75\text{ }^{\circ}\text{C}$, pH_{opt} = 2.5) that can obtain energy through aerobic respiration of organic matter and *Pyrococcus furiosus* ($T_{\text{opt}} = 100\text{ }^{\circ}\text{C}$, pH_{opt} = 6) that obtain energy through anaerobic fermentation of organic matter.

Some metabolic pathways for energy conservation in archaea are also found in bacteria, such as the Embden–Meyerhof and Entner–Doudoroff glycolytic pathways. The pathways are, however, partially different in the two domains, in that reactants are converted to products via different enzymes and hence through different intermediates (Bräsen et al. 2014). Methanogenesis is not found in bacteria, and, conversely, photo-autotrophy based on the electron transport chain is found in bacteria but not in archaea—e.g., in *Synechococcus lividus* ($T_{\text{opt}} = 65\text{ }^{\circ}\text{C}$, pH_{opt} = 8) and *Chloroflexus aurantiacus* ($T_{\text{opt}} = 56\text{ }^{\circ}\text{C}$, pH_{opt} = 8). Photoautotrophic thermophilic bacteria do, however, not survive at very high temperatures—the highest T_{max} observed is in the range of 70–75 °C. Other autotrophic thermophilic bacteria oxidize inorganic molecules (reviewed in Kristjansson et al. 2000), including hydrogen and hydrogen sulfide—e.g., aerobic species of the phylum *Aquificae*, the extreme thermophile, *Hydrogenobacter thermophilus* ($T_{\text{opt}} = 72\text{ }^{\circ}\text{C}$, pH_{opt} = 6.8) and the hyperthermophile, *Thermocrinis ruber* ($T_{\text{opt}} = 80\text{ }^{\circ}\text{C}$, pH_{opt} = 7–8.5), respectively, that inhabit alkaline hot springs. Other thermophilic autotrophic bacteria in the same habitat utilize sulfate as an electron acceptor and hydrogen as electron donor for energy conservation—e.g., *Thermodesulfobacterium thermophilum* ($T_{\text{opt}} = 65\text{ }^{\circ}\text{C}$, pH_{opt} = 7.5).

Thermophilic aerobic chemoorganotrophic bacteria such as species belonging to the genera *Thermus*, *Geobacillus*, and *Rhodothermus* oxidize organic matter and thrive in circumneutral and alkaline environments with growth optima $\leq 80\text{ }^{\circ}\text{C}$.

Anaerobic thermophilic and hyperthermophilic fermentative bacteria are isolated from microbial mats and geothermal anaerobic mud generally in the range of pH 5.5–7.5. Identified species are usually strict anaerobes, and fermentation is their predominant catabolic process. A great variety of these species belong to anaerobic genera of the phylum *Firmicutes*, e.g., *Clostridium*, *Thermoanaerobacter*, *Thermoanaerobacterium*, and the hyperthermophilic genus *Caldicellulosiruptor* (Scully and Orlygsson 2015; Willquist and van Niel 2012). Whereas *Firmicutes* has both aerobic and anaerobic members, *Thermotogae* has only the latter, glycolytic fermentative catabolism being the predominant catabolic process of the phylum. Temperature growth optima of the members range from 55° to 80 °C, and the optimum pH for growth ranges from 5.5 to 7.5. Different genera have been isolated from terrestrial and marine habitats, e.g., respectively, the genera *Fervidobacterium* and *Thermotoga*. Hyperthermophiles belonging to the phylum have only been

identified in marine habitats, by species such as *Thermotoga maritima* ($T_{\text{opt}} = 80\text{ }^{\circ}\text{C}$, $\text{pH}_{\text{opt}} = 6.5$) and *Thermotoga neapolitana* ($T_{\text{opt}} = 80\text{ }^{\circ}\text{C}$, $\text{pH}_{\text{opt}} = 7$), the latter of which uses S^0 as an electron acceptor and have only been found in marine geothermal sites (Frock et al. 2010).

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