

Life in High-Temperature Environments

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DIVERSITY OF HIGH-TEMPERATURE ENVIRONMENTS

High-temperature biotopes colonized by thermophilic and hyperthermophilic microorganisms are distributed on all continents and oceans and span a huge diversity of physicochemical niches, with pH values in high-temperature spring sources spanning >7 units (1–3) and aqueous concentrations of biologically relevant elements spanning several orders of magnitude (1, 2). Studies of high-temperature environments and the thermophiles inhabiting them are motivated by a number of factors, including basic scientific foci such as the molecular bases of thermophily and thermal limits of life, biodiversity exploration, and the origin and early evolution of life. Although the setting for the origin of life is debated (4, 5), a high-temperature origin of life is strongly favored in the literature (6–9), and there is an abundance of evidence for high-temperature aquatic environments following the Late Heavy Bombardment, the time when fossil and isotopic evidence suggest that life first evolved (10–12). The study of thermophiles is also of acute interest for applied science, including microbially enhanced oil recovery and inhibition of oil souring (13), isolation of novel molecules (14), and the discovery and development of thermostable enzymes for biotechnology (15–18).

Continental Subsurface

The subsurface is a vastly underexplored thermal habitat. Although geothermal gradients vary greatly due to local and regional geology, gradients away from plate boundaries average about 25°C per km, fueled primarily by latent heat from planetary accretion and radioactive decay in the mantle, particularly ^{40}K , ^{235}U , ^{238}U , and ^{232}Th (19). Subsurface water reservoirs reach temperatures of at least 340°C (20) under geologic pressure. Estimates of the habitable volume of the subsurface and biomass inhabiting the subsurface are still rough, due primarily to uncertainty in the extent of liquid water in the subsurface; however, some estimates have been made that more than half of Earth's biomass resides in the subsurface (21, 22). Most sampled subsurface environments have yielded microbial cells or signatures of microbial life, although the low biomass of these habitats and the engineering challenges associated with subsurface exploration mandate strict contamination controls (23), which have been variably applied. Recently, deep fracture fluids isolated since

the Precambrian have been sampled and lacked detectable biomarkers or cultivable microorganisms (24). Deep subsurface microbial communities may obtain energy from chemical disequilibria produced through radiolysis of water (25), serpentinization (26), or reaction of water with subsurface basalts and other ferrous rocks (27, 28). Many subsurface microbial environments host very low amounts of biomass and very limited diversity, despite concentrations of potential electron donors and acceptors being many orders of magnitude out of equilibrium in some continental systems (e.g., mM levels of H_2 and SO_4^{2-} [29]). Thus, microbial communities in these systems may be limited by the availability of nutrients by mass transfer or other factors.

Terrestrial Geothermal Systems

Many terrestrial geothermal systems occur in volcanically active areas, including along tectonic boundaries, spreading centers, or “hot spots,” where magma bodies may reach within a few kilometers of the Earth's surface. The composition of thermal fluids is ultimately controlled by geologic drivers (climate, tectonics, heat input) that manifest as proximal factors, such as water–rock interaction, boiling, evaporation, microbial metabolism, and mixing (1, 30, 31). Terrestrial geothermal springs form a bimodal distribution with regard to pH, with the majority of springs falling between pH 1.5 to 3.5 and pH 7 to 9 (32). This pattern is driven primarily by subsurface boiling, leading to three major types of geothermal features (Fig. 1; Fig. 2). Circumneutral to alkaline springs, also known as “water-dominated systems” (1), are directly sourced with geothermal fluids (Fig. 1c; Fig. 2a–f). In most regions, the carbonate/bicarbonate system ($\text{H}_2\text{CO}_3 \leftrightarrow \text{H}^+ + \text{HCO}_3^- \leftrightarrow 2\text{H}^+ + \text{CO}_3^{2-}$) is the dominant buffering system, and typically chloride and sodium are the other most abundant ions. Hydrothermal reservoir temperatures for these systems, estimated by a variety of geothermometers, can range from 180 to 270°C (3, 38). Important inorganic electron donors sourced by subsurface fluids include the reduced endmembers hydrogen, hydrogen sulfide, ammonia, and reduced metals (e.g., Fe^{2+} , As^{3+}). However, if the fluids have undergone subsurface boiling, then the remaining liquid water may be stripped of gases, including hydrogen sulfide and ammonia. Depending on volumetric flow rates, concentrations of reductants, and other factors, dissolved oxygen may or may not be measurable in the source waters of geothermal systems. However, due to the thermodynamic

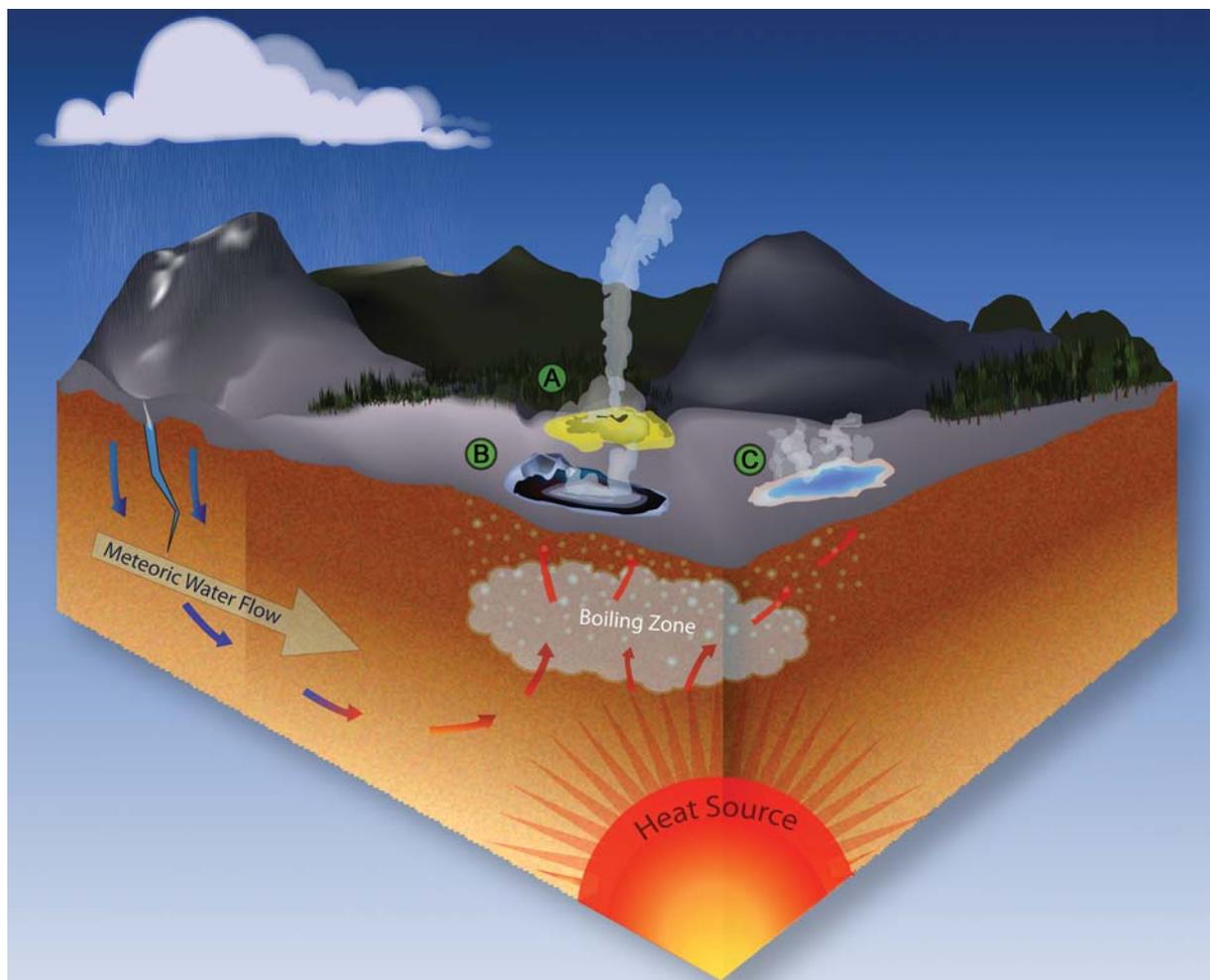


FIGURE 1 Generalized schematic of terrestrial geothermal systems such as those in Yellowstone National Park. Local or regional aquifers sourced by meteoric water follow fault lines or permeable layers and become heated by proximity to shallow magma bodies or heated rocks. Heated water may boil in the subsurface due to adiabatic decompression as fluids rise. Dissolved gases such as H_2 , H_2S , and NH_3 become enriched in vapor phases, which source either fumaroles (a) or acidic pools or mudpots (b). These so-called vapor-dominated systems become acidified to pH 1.5–3.5 by biotic and abiotic oxidation of H_2S to sulfuric acid at or near the surface; acid weathers the host rock and solubilizes clays. The liquid phase, typically with lower concentrations of volatiles, but enriched in soluble ions such as Na^+ and Cl^- , is buffered to pH 7–9 by the carbonate buffering system and may source large, clear pools or streams with substantial discharge (c).
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favorability of aerobic respiration, oxygen is likely to be an important terminal electron acceptor where it is present. In addition, the presence of many alternative terminal electron acceptors in geothermal systems depends directly or indirectly on the aerobic oxidation of reduced compounds issuing from the geothermal water. Exceptions are sulfate and bicarbonate/carbon dioxide, both of which are often present at high-temperature spring sources at millimolar concentrations.

Acidic springs, pools, and mudpots are sourced with geothermal vapor that is formed during adiabatic decompression of geothermal fluids as a result of changes in pressure or volume as geothermal fluids rise in geothermal plumbing systems (Fig. 1b; Fig. 2g–i). As a result of this hydrologic origin, these systems are often called “vapor-dominated” systems (1). The vapor becomes enriched in volatiles such as hydrogen sulfide (H_2S), ammonia (NH_3), carbon dioxide (CO_2), and other gases. Upon condensation of the vapor

at or near the surface, and contact with molecular oxygen from the atmosphere, hydrogen sulfide becomes oxidized to sulfuric acid either abiotically or biotically via the action of sulfide- and sulfur-oxidizing chemolithotrophs such as *Sulfolobus* or *Hydrogenobaculum*. Sulfuric acid then becomes the dominant buffer for these systems ($H_2SO_4 \leftrightarrow H^+ + HSO_4^- \leftrightarrow 2H^+ + SO_4^{2-}$) (1,30,31). Acid solutions contribute to rock weathering and often solubilize clays, leading to the production of erosional features and mudpots of varying mineral composition (Fig. 2g–i). The acid also serves as a trap for ammonia that is sourced as vapor to the spring, leading to concentrations of ammonium of up to 57 mM (39). Depending on the amount of water in these types of systems and the local topography along the flow path of the vapor, the vapor may not condense into pools of water, instead forming steam fumaroles (Fig. 1a). Fumaroles tend to vent the same gases as acidic pools; limited work done on these systems suggests that vapor condensing on rocks is similar in

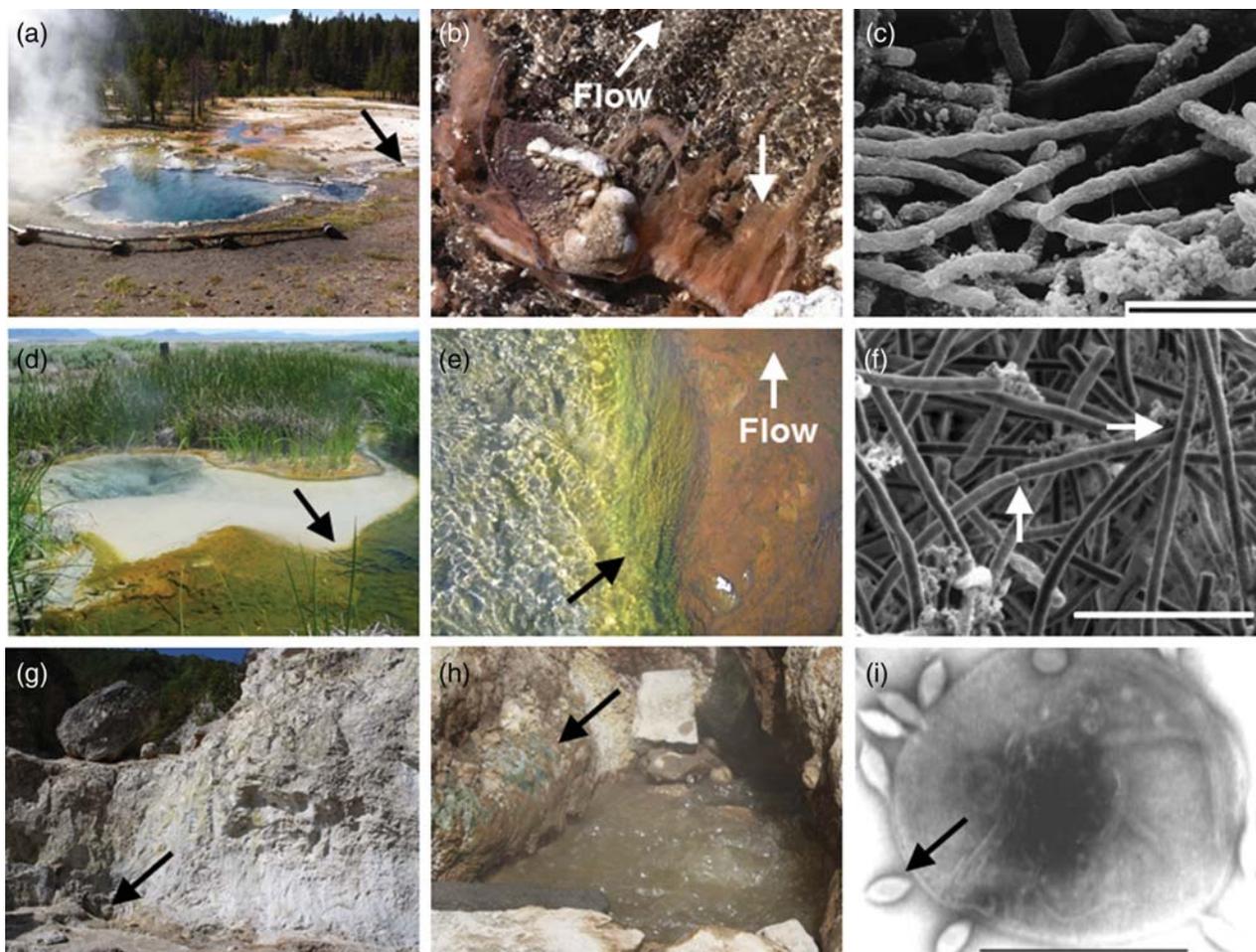


FIGURE 2 Terrestrial geothermal systems at a variety of scales. (a–c) Abundant microbial growth in Octopus Spring, a high-temperature, alkaline spring in Yellowstone National Park’s Lower Geyser Basin. (a) View of the source pool ($\sim 92^{\circ}\text{C}$; $\text{pH} \sim 8.0$) from above the “head” of the Octopus, with outflow streams (“tentacles”) flowing away and to the right (arrow, site of pink streamer community). (b) Pink streamer community in the outflow of Octopus Spring at ~ 84 to 88°C , with abundant growth of *Thermocrinis ruber* and yet-uncultivated lineages (33) (width of view, ~ 0.3 m). (c) Scanning electron micrograph (SEM) of a pure culture of *Thermocrinis ruber* isolated from Octopus Spring showing flow-dependent filamentous morphology (bar, $2\ \mu\text{m}$; used with permission from [34]). (d–f) Sharp transition of phototrophic biofilm growth in Mud Hot Spring (Sandy’s Spring West) in the U.S. Great Basin. (d) View of the source pool ($\sim 86^{\circ}\text{C}$; $\text{pH} \sim 7.2$) with the upper temperature limit of photosynthesis clearly visible (arrow, $\sim 73^{\circ}\text{C}$). (e) Close up of the outflow channel showing the upper temperature limit of photosynthesis (arrow; width of view, ~ 0.5 m). (f) SEM of a pure culture of *Thermoflexus hugenholzii*, an abundant resident of sediments in Mud Hot Spring and nearby Great Boiling Spring (35, 36) (bar, arrow, $\sim 2\ \mu\text{m}$; arrows, septa between individual cells in filaments). (g–i) Direitayanqu (“Experimental Site”), an acidic system in Tengchong, China, and abundant microorganisms. (g) Large, geothermally altered erosional feature typical of many acidic geothermal systems (arrow, area in focus in H). (h) One of several hot, acidic pools ($\sim 86^{\circ}\text{C}$; $\text{pH} \sim 2.6$) actively gassing and with thermoacidophilic algae (*Cyanidiales*) visible in vapor condensate (arrow; width of view, ~ 0.5 m). (i) Transmission electron micrograph of *Sulfolobus tengchongensis*, the dominant microorganism at this site (2) with *Sulfolobus tengchongensis* spindle-shaped virus (STSV1) (arrow; bar, $1\ \mu\text{m}$; used with permission from [37]). doi:10.1128/9781555818821.ch4.3.4.f2

chemistry and microbial community composition to acidic pools (40). Acidic pools and fumaroles often form in volcanically active areas. Since they are formed by gas condensate, acidic features tend to be small and have low volumetric flow rate compared to alkaline systems, although large acidic features can form in erosional basins if the gas source is diffuse and partially sourced with fresh meteoric water or local groundwater (41).

Terrestrial geothermal systems also occur in areas where geophysical forces allow deep penetration of meteoric water, typically by deep faulting within an extensional setting. For example, in the U.S. Great Basin, most geothermal activity is found in regions with the highest strain rates, such as the

region northeast of the Walker Lane fault system and, to a lesser extent, the Wasatch Front (42). These systems are distinguished from geothermal systems in volcanic settings by generally lower subterranean reservoir temperatures ($<220^{\circ}\text{C}$), lower concentrations of magmatic gases, and absence or near absence of acidic systems and fumaroles.

Marine Hydrothermal Systems

Marine hydrothermal systems form along mid-ocean ridges and back-arc spreading centers (on-axis systems) where Earth’s tectonic plates are separating, allowing hydrothermal systems to form (43), and at off-axis locations supported by mantle cooling or hot spots (44, 45). The dynamics of

4.3.4-4 ■ EXTREME ENVIRONMENTS

the speed at which the plates spread, the process of submarine hydrothermal recharge, and host rock composition create a diversity of high-temperature geochemical environments with a frequency of occurrence correlated to the rate of seafloor spreading (46, 47). The diversity of geochemical environments and broad geographic distribution leads to a large diversity of microbial communities associated with marine hydrothermal systems (48, 49). There are currently more than 630 active hydrothermal fields (confirmed or inferred) in both shallow and deep-sea marine settings (50). Due to the abundance and distribution of hydrothermal fields and the abundant energy sources provided by them, it has been suggested that as much as 25% of the deep-marine organic carbon inventory is supplied by chemoautotrophy supported by hydrothermal activity (47, 48, 51). Although not restricted to deep-sea locations, the high pressures at deep-marine vent systems allows for water temperatures well over 100°C, with some fields venting supercritical water (52), making them ideal locations for hyperthermophiles, including the most thermophilic organism known (122°C, *Methanopyrus kandleri* [53]). The extent to which microbial communities can be found actively metabolizing above 122°C is still under debate and is complicated by steep and dynamic temperature gradients of hydrothermal systems and the cost and technical difficulties associated with sampling the deep-sea environment.

Although there is a wide variety of marine hydrothermal vents, three main types exist in proximity to divergent plate margins: (a) basalt-hosted, high-temperature systems (~400°C) that typically exhibit low concentrations of H₂ and CH₄, and high concentrations of metals; (b) serpentinite and gabbro-hosted, high-temperature systems (~360°C) that typically exhibit high concentrations of H₂ and CH₄, and metals; and (c) serpentinite-hosted, low-temperature systems (~40–90°C) that typically exhibit high concentrations of H₂ and CH₄ and low concentrations of metals (45, 48, 54). Although, it should be noted that there is likely a continuum of diversity of hydrothermal systems where specific discharge locations may be a hybrid of these broad categories (e.g., [55, 56]).

The first confirmed deep-sea hydrothermal vent systems were basalt-hosted on-axis systems (57). These systems are found in areas of fast plate spreading, display relatively high vent fluid temperatures, low pH, high metal and H₂S concentrations, and low amounts of H₂ and CH₄ (45, 54). Some basalt-hosted systems have relatively oxidized fluids, compared with other hydrothermal systems (48). The low H₂ concentrations and less reduced fluid leads to the dominance of microaerophilic hyperthermophiles and few if any methanogens (48, and sources therein). Archaeal lineages found at this vent type and at serpentinite and gabbro-hosted vents include *Desulfurococcales*, *Thermococcales*, *Thermoproteales* (*Thermofilaceae*), *Archaeoglobales*, “*Aciduliprofundales*,” and *Nanoarchaeota* (48). Bacterial lineages found at this vent type and at serpentinite and gabbro-hosted vents are largely dominated by moderately thermophilic *Epsilonproteobacteria* and contain sulfur-oxidizing and/or hydrogenotrophic autotrophs (48, and sources therein). Other bacterial lineages include *Gamma*proteobacteria and *Thermotogae*.

In contrast to the basalt-hosted vent systems, serpentinite and gabbro-hosted systems occur predominantly in areas of slow plate spreading and display high vent fluid temperatures (although typically slightly lower than basalt-hosted), highly reduced geochemistry, low pH and H₂S concentration, relatively high salinity, and high metal, H₂, and CH₄ concentrations (54). At these sites, methanogens are highly

abundant due to high concentrations of H₂ (48). Hydrogenotrophic sulfate reduction has also been implicated as an important metabolism at these sites (e.g., *Thermodesulfator*, *Thermodesulfobacterium*, and *Desulfonauticus*; [48]).

Serpentinite-hosted hydrothermal vent systems are typically alkaline and highly reduced, forming porous carbonate chimneys, where high levels of H₂ and CH₄ formed through serpentinization of peridotite at relatively low temperatures (~150°C) support chemosynthetic communities through anaerobic oxidation of CH₄, generating H₂S, which is subsequently an energy source for sulfide-oxidizing bacteria (45, 58). The Lost City is probably the most well-known hydrothermal system of this type (44, 58), where H₂ and CH₄ are the most abundant energy source for microbial oxidation and metal concentrations are low, in contrast to abundant H₂S and metals at systems derived from high-temperature basalt interactions. These differences result in distinct microbial communities, where Archaea in the order *Methanosarcinales*, responsible for both methane consumption and production, dominate high-temperature, high-pH, end-member fluids (58). In lower temperature fluids, anaerobic methane-oxidizing (ANME-1) Archaea were found to be abundant. Bacteria from the Firmicutes were present at high temperature and high pH, with *Chloroflexi*, *Epsilonproteobacteria*, and *Gamma*proteobacteria occurring at cooler temperatures. It is suggested that archaeal methanogens consume hydrogen from vent fluids and use seawater dissolved inorganic carbon as a substrate (58), contributing to the overall production of methane from these systems.

Other Systems Supporting Thermophiles

A wide variety of other natural and engineered habitats support populations of thermophiles. These habitats are heated by solar radiation, industrial processes, radioactive decay, or combustion reactions. For example, dark soils and rock surfaces can exceed 60°C, particularly in hot desert regions, where heating to these temperatures occurs on a daily basis in the summer (59, 60) and may even reach 80°C (D. Devitt, personal communication). Yet even soils in cooler regions have been shown to contain populations of thermophiles, particularly *Bacillales*, including vegetative forms (61, 62). A wide variety of high-temperature engineered systems are habitats for thermophiles. These include domestic and industrial heating systems, which have long been known to harbor thermophiles (63), high-temperature industrial digesters, and power plants and associated cooling ponds. Natural and engineered combustion systems include compost piles and self-heating coal refuse piles, some of which reach temperatures of 150°C (64).

DIVERSITY OF MODERATELY AND EXTREMELY THERMOPHILIC MICROORGANISMS

Hyperthermophiles and Thermophiles: Definitions

A variety of different definitions have been used to describe microorganisms that grow at high temperature, but the most widely used definitions are based on optimal growth temperature, with thermophiles having an optimal growth temperature ranging from 45 to <80°C and hyperthermophiles having an optimal growth temperature of ≥80°C (65). Since many thermophiles and hyperthermophiles inhabit environments that are extreme with regard to other factors, additional terms are commonly used to describe polyextremophiles, such as thermoacidophiles, capable of growth at high temperature and low pH, halophilic thermoalkaliphiles, capable of

growth at high temperature, high salt, and high pH (66), and thermophilic piezophiles, capable of growth under high temperature and pressure.

Although these terms can apply to all types of life, the phylogenetic distribution of thermophily is not evenly distributed. For example, the known upper temperature limits of growth of Archaea, Bacteria, and Eukarya are very different at 122°C (*Methanopyrus*; [53]), 100°C (“*Geothermobacterium*”; [67]), and 60°C (*Thermomyces*; [68]), respectively. The pattern of thermophily within the domains is also nonrandom; for example, Table 1 lists some general characteristics, including growth temperature data, for several taxonomically valid orders of Bacteria and Archaea, many of which belong to phyla that are found exclusively in high-temperature habitats. In fact, a large body of literature shows that the bacterial phyla that dominate most nonthermal environments, such as *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, and *Cyanobacteria* are almost completely replaced by thermophilic specialists above ~80°C. These thermophilic specialists include but are not limited to members of the bacterial phyla *Aquificae*, *Thermi*, *Thermotogae*, *Thermodesulfobacteria*, and *Dictyoglomi* and thermophilic *Crenarchaeota* and *Euryarchaeota*. Many thermal systems also host abundant populations of yet-uncultivated “microbial dark matter” lineages of Bacteria and Archaea; for example, a meta-analysis of 16S rRNA gene censuses in terrestrial geothermal systems globally estimated high relative abundances of yet-uncultivated phyla (\bar{x} = 16.1%), classes (\bar{x} = 34.0%), orders (\bar{x} = 42.1%), and families (\bar{x} = 46.9%) (89).

It is useful to recognize that upper temperature growth limits and the terms *thermophile* and *hyperthermophile* refer to growth parameters of axenic cultures in the laboratory and these characteristics may or may not be fully relevant in nature. There are several cases where laboratory cultivation data seem to be discordant with observations in natural environments. For example, mixed cultures of “*Candidatus Nitrosocaldus yellowstonii*” and related ammonia-oxidizing Archaea (AOA) have only been grown in the laboratory up to ~74°C (90, 91); however, several researchers have documented abundant and active populations of “*Candidatus Nitrosocaldus*” species at temperatures above 80°C (35, 92). Another example is *Thermoflexus hugenholtzii*, which is the most abundant bacterium in 79–87°C sediments from Great Boiling Spring, Nevada (76), but has a very restricted growth range in laboratory axenic cultures (67.5–75°C) (93). A third example is the reciprocal dependence of the monocot *Dichanthelium lanuginosum* (panic grass), a common inhabitant of geothermal soils in Yellowstone National Park, and its fungal endosymbiont *Curvularia protuberata*, for thermal tolerance (94). Although this last example features eukaryotes, rather than Bacteria or Archaea, and the molecular basis of symbiont-mediated thermal tolerance is not understood, it does provide a well-documented precedent for a role of species–species interactions in thermophily. This is also a strong justification for microbial ecology studies in thermal environments to complement the study of axenic cultures.

Physiological Diversity of Thermophiles and Hyperthermophiles

The co-occurrence of reduced geothermal fluids and cold, oxygenated waters (for hydrothermal systems) or atmospheric gases (for terrestrial geothermal systems) provides a wide variety of inorganic chemical disequilibria that can be used by

microorganisms (1, 95, 96). This is particularly important in light of the limited distribution of phototrophs in geothermal systems; most marine hydrothermal systems are located in the aphotic zone and thermophilic phototrophs are limited to temperatures of $\leq 73^\circ\text{C}$ at circumneutral to alkaline pH and $\leq 50^\circ\text{C}$ in acidic systems below pH 5 (97, 98). Indeed, studies of pure cultures of thermophiles and hyperthermophiles have shown that various thermophiles are capable of a wide variety of metabolic lifestyles (Table 1). Collectively, known thermophiles can use as electron donors a variety of simple (e.g., methane, formate, and acetate) and complex organic compounds as well as inorganic compounds, such as hydrogen (H_2), carbon monoxide (CO), ammonia (NH_3); reduced sulfur compounds (H_2S , S^0 , $\text{S}_2\text{O}_3^{2-}$); and reduced metals (Fe^{2+} , As^{3+}) (95). Of these metabolisms, hydrogenotrophy, sulfur oxidations, organic respirations, and fermentations are especially widely distributed (Table 1).

Many studies have quantified the chemical energy potential based on nonequilibrium concentrations of various biologically relevant redox couples in geothermal fluids (1, 95, 96). In some cases, these calculations, along with other lines of evidence, have been used to infer the dominant metabolisms present in an ecosystem. For example, Spear and colleagues (99) used the relatively high concentrations of hydrogen gas, the energetic favorability of hydrogenotrophy, and the high abundance of *Aquificales* to infer the importance of hydrogen as an electron donor in Yellowstone National Park. However, experiments in some Yellowstone springs have suggested that other electron donors might also be important. For example, experiments in Dragon Spring, Yellowstone National Park, showed that microbial mats dominated by *Hydrogenobaculum* oxidize hydrogen sulfide at rates that are orders of magnitude higher than that of hydrogen; additionally, isolates from this site use both hydrogen sulfide and hydrogen or only hydrogen sulfide as electron donors for chemolithotrophy (100). Experiments in two hot springs in the Great Basin suggested that microbial communities have diversified metabolically to take advantage of the diversity of geochemical energy present, since addition of hydrogen, ammonia, thiosulfate, methane, organic acids, and complex organics each stimulated oxygen consumption; however, some of these activities were spatially segregated, occurring only in sediments or in the bulk water (101).

Within terrestrial geothermal systems, many genera partition between “water-dominated” alkaline systems and “vapor-dominated” acidic systems. For example, a variety of cultivation-independent studies covering all major geothermal features in the Rehai Geothermal Field in Yunnan Province, China, showed that pH had a dominant control on microbial community composition (2, 102). The pH dependence of orders of *Thermoprotei* (*Crenarchaeota*) and genera of *Aquificales* were particularly clear, with high-temperature acidic systems (pH < 5) hosting abundant populations of *Sulfolobales* and lower-temperature acidic systems hosting *Hydrogenobaculum*. Alkaline systems (pH > 7) hosted *Thermoproteales*, *Desulfurococcales*, *Hydrogenobacter*, and *Hydrogenobaculum* (102). However, some genera of terrestrial *Thermoprotei* from other locations have pH optima in the mildly acidic range (e.g., *Desulfurococcus* and *Ignisphaera* [103]), between the two major spring types, which is consistent with observations of these organisms in springs with intermediate pH, such as Obsidian Pool in Yellowstone National Park (104). Similarly, the *Korarchaeota*, which was originally discovered by cultivation-independent surveys in Obsidian Pool (105), has been shown by cultivation-independent surveys and ecological niche modeling to be

TABLE 1 Some characteristics of major orders that consist primarily or exclusively of (hyper)thermophiles

Thermophilic order (phylum)	# of species	T _{opt} range (°C)	Metabolism	Electron donors	Inorganic electron acceptors	Autotrophy (pathway) ^a	Habitats	Refs.
<i>Aquificales (Aquificae)</i>	21	65–90	Respiratory	H ₂ , S ₂ O ₃ ²⁻ , SO ₃ ²⁻ , S ⁰ , Fe ²⁺ , simple org.	O ₂ , NO ₃ ⁻ , SO ₃ ²⁻ , S ⁰ , Fe ³⁺ , As ⁵⁺ , Se ⁴ ₊₆₊	Yes (rTCA)	Terrestrial, marine	69
<i>Desulfurobacteriales (Aquificae)</i>	8	70–77	Respiratory	H ₂	NO ₃ ⁻ , S ₂ O ₃ ²⁻ , SO ₃ ²⁻ , S ⁰	Yes (rTCA)	Marine	69
<i>Dictyoglomales (Dictyoglomi)</i>	2	70–78	Fermentative	Carbohydrates, complex org.	None	No	Terrestrial	70
<i>Thermales (Deinococcus-Thermus)</i>	31	50–75	Respiratory	H ₂ ^c , S ₂ O ₃ ²⁻ ^c , carbohydrates, complex org.	O ₂ , NO ₃ ⁻ , NO ₂ ⁻ , S ⁰ , Fe ³⁺	No	Terrestrial, marine	71, 72
<i>Thermodesulfobacteriales (Thermodesulfobacteria)</i>	8	65–85	Respiratory	H ₂ , simple org. acids, ethanol	SO ₄ ²⁻ , S ₂ O ₃ ²⁻ , SO ₃ ²⁻ , S ⁰ , Fe ³⁺	Yes (Ac-CoA?)	Terrestrial, marine	73–75
<i>Thermoflexales (Chloroflexi)</i>	1	72.5–75	Respiratory, fermentative	Complex org.	O ₂	No	Terrestrial	76
<i>Thermotogales (Thermotogae)</i>	46	37 ^b –80	Fermentative	Carbohydrates, complex org.	SO ₄ ²⁻ , S ₂ O ₃ ²⁻ , SO ₃ ²⁻ , S ⁰	No	Terrestrial, marine	77
<i>Acidilobales (Crenarchaeota)</i>	3	70–85	Fermentative	Carbohydrates, complex org.	S ⁰	No	Terrestrial	78
<i>Desulfurococcales (Crenarchaeota)</i>	22	85–106	Respiratory, fermentative	H ₂ , amino acids, complex org.	O ₂ , NO ₃ ⁻ , NO ₂ ⁻ , S ₂ O ₃ ²⁻ , S ⁰	Yes (DC/4HB)	Terrestrial, marine	79
<i>Fervidicoccales (Crenarchaeota)</i>	1	65–70	Fermentative	Carbohydrates, complex org.	None	No	Terrestrial	80
<i>Sulfolobales (Crenarchaeota)</i>	19	65–90	Respiratory, fermentative	H ₂ , S ₂ O ₃ ²⁻ , S ⁰ , Fe ²⁺ , metal sulfides, simple/complex org.	O ₂ , S ⁰	Yes (3HP/4HB)	Terrestrial	81, 82
<i>Thermoproteales (Crenarchaeota)</i>	17	75–102	Respiratory, fermentative	H ₂ , S ₂ O ₃ ²⁻ , simple/complex org.	O ₂ , NO ₃ ⁻ , NO ₂ ⁻ , SO ₄ ²⁻ , S ⁰ , Fe ³⁺ , As ³ ₊₅₊ , Se ⁶⁺	Yes (DC/4HB)	Terrestrial, marine	83, 84
<i>Archaeoglobales (Euryarchaeota)</i>	8	70–88	Respiratory, fermentative	H ₂ , simple/complex org.	NO ₃ ⁻ , SO ₄ ²⁻ , S ₂ O ₃ ²⁻ , SO ₃ ²⁻ , Fe ³⁺	Yes (Ac-CoA)	Marine, terrestrial ^d	85
<i>Methanopyrales (Euryarchaeota)</i>	1	98	Respiratory	H ₂	CO ₂	Yes (Ac-CoA)	Marine	86
<i>Thermococcales (Euryarchaeota)</i>	40	80–100	Fermentative	Carbohydrates, complex org.	S ⁰	No	Marine, terrestrial	87

^aAutotrophic pathways: rTCA, reverse tricarboxylic cycle; Ac-CoA, reductive acetyl-CoA or Wood-Ljungdahl pathway; DC/4HB, dicarboxylate/4-hydroxybutyrate cycle; 3HP/4HB, 3-hydroxypropionate/4-hydroxybutyrate cycle (88). Genomic evidence suggests an Ac-CoA pathway for *Thermodesulfobacteriales*.

^bA few members, including *Mesotoga* species, are mesophilic.

^cFacultatively chemolithoheterotrophic.

^dAll isolates are from marine environments, but 16S rRNA genes have been detected in terrestrial geothermal systems.

most abundant in springs with pH 5.7 to 7.0 in Yellowstone National Park (106). High sulfate concentrations suggest these fluids are influenced by contributions from hydrothermal vapors that may be neutralized to some extent by mixing with water from deep geothermal sources or meteoric water.

BIOENERGETIC CHALLENGES OF LIFE AT HIGH TEMPERATURE AND LIPIDS OF THERMOPHILES AND HYPERTHERMOPHILES

Bioenergetic Challenges of Life at High Temperature

All organisms must meet minimal energy demands to maintain the biomolecular complexity that constitutes life and avoid “decay into the inert state of equilibrium” (107). Increasing temperature provides an increase in bioenergetic challenges life must face (108–112), most fundamentally due to increases in the rate of degradation of molecules through direct cleavage of bonds or through reactions with other chemicals in the environment (113, see references therein; 114). In this way, temperature likely provides a strong influence on the diversity of life inhabiting high-temperature ecosystems (76, 115); the dynamics of growth, maintenance, and survival (111, 116); and delineating the limits of habitability (113, 114, 117). Although there is ample evidence that temperature is a strong physical parameter influencing the distribution and diversity of life, we have yet to gain a clear picture of the fundamental consequences of temperature on the biomolecular complexity that constitutes life and the subsequent effect on ecosystem functions.

Maintenance energy (ME), or the more environmentally applicable term “basal power requirement,” are descriptors of the amount of “energy consumed for functions other than production of new cell material” (118) or the “energy turnover rate per cell or per unit biomass associated with the minimal complement of functions required to sustain a metabolically active state of the cell” (108). It has been shown in laboratory and environmental settings that the demand for ME increases with temperature and scales linearly with biomass quantity, regardless of metabolic strategy (111, 112), suggesting a fundamental energetic cost to life at high temperature. However, it is useful to point out that the observed demand for ME in laboratory high-energy settings (which typically measure growing cells [112]) is several orders of magnitude greater than that predicted for natural environments (108, 111). Hoehler and Jørgensen (108) suggest this difference is due to energy-spilling reactions and biological actions (e.g., motility) that may or may not be employed in the natural environment where energy is often limiting, and strongly urge caution when applying laboratory-determined values to the natural environment. In either case, the lab or the environment, the energetic cost of performing functions not directly related to growth increases with temperature. Interestingly, a reduction in genome size has been shown to correlate with increasing temperature, perhaps reflecting the increase in demand for ME associated with biomass (119).

A high demand for ME would have the effect of increasing ecosystem metabolic rates and dynamics in high-temperature ecosystems. This bioenergetic cost stands to limit the amount of energy that can be supplied to anabolic processes, challenging a cell’s ability to produce enough biomass to replicate. This reasoning provides greater insight into the challenges of growth at high temperatures and perhaps fundamental logic for the upper temperature limit for life both within a single organism and across all life, beyond simple disintegration of essential physiological processes as a direct cause of

temperature (e.g., thermal degradation of essential enzymes) (117). If cells are simply maintaining, that is, sustaining metabolic activity but not replicating cellular components, they may still be highly metabolically active without increases in biomass, and this has implications on biogeochemical cycles in high-temperature ecosystems. For example, this bioenergetic principle of life at high temperature could translate into fast rates of microbially catalyzed redox reactions (e.g., ammonia oxidation) without substantial increases in biomass (e.g., carbon fixation). For heterotrophs, a high demand for ME could result in a greater proportion of organic carbon being mineralized, resulting in lower amounts of organic carbon left in the system. Even survival may be energetically challenging in high-temperature environments due to increased rates of amino acid racemization and nucleic acid depurination with increasing temperature (111). If a cell is in a survival state (e.g., spore) and has a finite pool of ATP, NADPH, or other bioenergy currency for repair, then the time until the cell is rendered unviable will be less in high-temperature settings (116).

ME demands directly related to maintaining physiological integrity are likely the dominant energy sinks in natural nongrowth settings (120, 121), and examples of physiological adaptations by thermophiles and hyperthermophiles that may help reduce ME demands at high temperature can be found. Of multiple broad nongrowth-associated categories associated with demands for ME, half of which are related to physiological maintenance (121), one category in particular seems to be highly represented in thermophiles: proofreading, repair, and turnover of macromolecular compounds.

The degree to which process influences the demands on ME will depend on the environment in question, with polyextremophiles facing a greater diversity of energetic challenges, increasing the demands for ME and providing an intriguing area for comparative analysis. One challenge for investigators is that ME is rarely measured with the resolution needed to quantitatively address individual processes (121). Future experimental designs should attempt to encompass and delineate the contributions of individual processes to ME (121). Further investigation is needed to qualitatively address the contributions to ME under the variety of conditions found in the natural environment and link the consequences to biogeochemical cycles, diversity patterns, and our evolving definition of “habitable.”

Lipids Found in Hyperthermophiles and Thermophiles

A major bioenergetic challenge of life at high temperature is maintenance of an energized membrane (116). Many hyperthermophiles and thermophiles have membrane-spanning lipids called glycerol dialkyl glycerol tetraethers (GDGTs; Fig. 3), which resist delamination, reduce proton permeability, and increase overall stability at high temperature and low pH (123). In fact, the core lipids of GDGTs are so stable that they can accumulate in sediments and remain intact for hundreds of millions of years, leading to their use as environmental paleoproxies (124–126).

The best-known tetraether lipids, the isoprenoid GDGTs (iGDGTs), are composed of membrane-spanning lipids composed of isoprenoidal chains joined by four ether bonds to two glycerol backbones. iGDGTs are found in a variety of Archaea, including all known thermophilic and hyperthermophilic *Thermoprotei* (*Crenarchaeota*), and at least eight orders of *Euryarchaeota*, including hyperthermophilic and thermophilic members of the orders *Methanopyrales*, *Methanococcales*, *Methanobacteriales*, *Archaeoglobales*, and *Thermococcales* (reviewed in 127). iGDGT structures vary

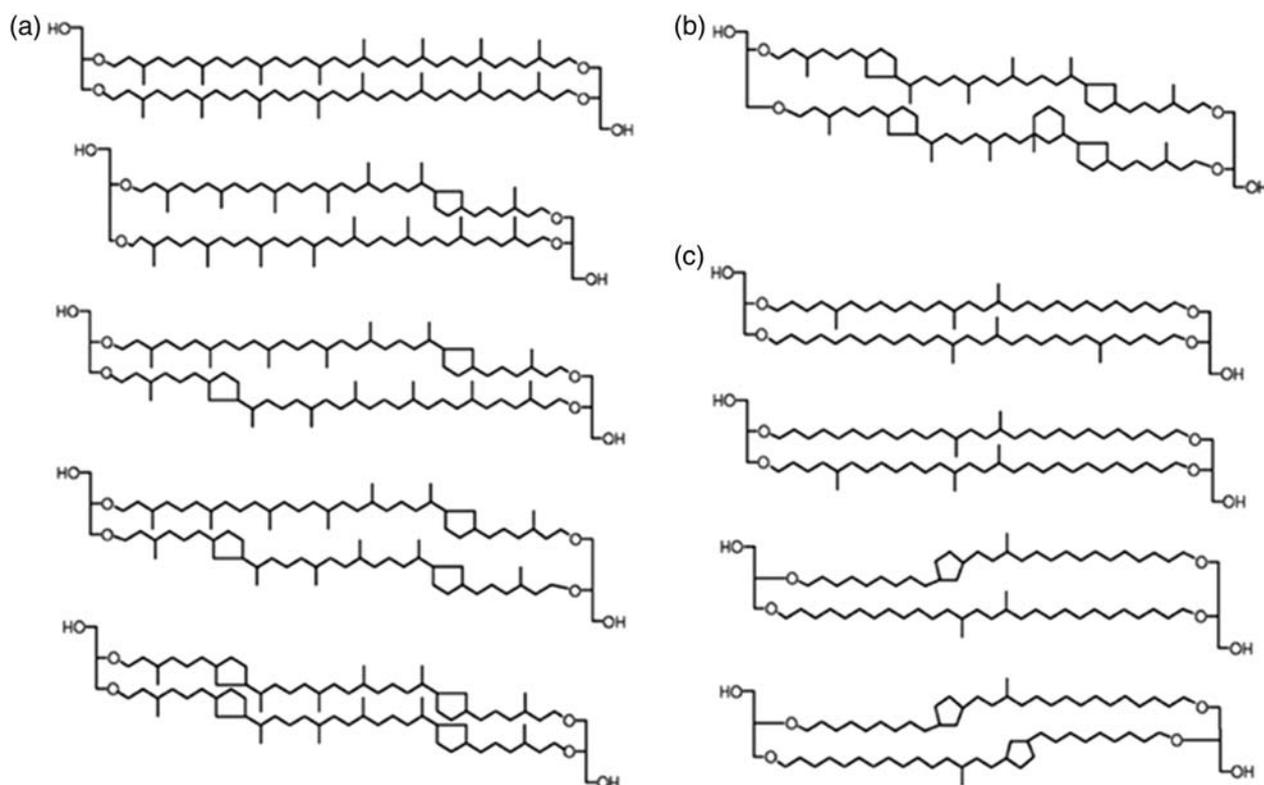


FIGURE 3 Example core tetraether lipid structures. (a) Example isoprenoid dialkyl glycerol tetraethers (iGDGTs) found in thermophilic *Crenarchaeota* and *Euryarchaeota*; increasing cyclization is associated with increased temperature and/or decreased pH (top to bottom). (b) Thaumarchaeol (formerly crenarchaeol), a distinctive lipid of both thermophilic and mesophilic *Thaumarchaeota*. (c) Example branched GDGTs (bGDGTs) produced by mesophilic *Acidobacteria* and likely produced by unknown moderate thermophiles; methylation is inversely proportional to temperature in terrestrial geothermal systems. Figure modified from (122). doi:10.1128/9781555818821.ch4.3.4.8

according to the degree of cyclization of isoprenoid groups into cyclopentyl rings (Fig. 3), ranging from 0 to 8 per GDGT. Studies of microbial pure cultures have shown that Archaea increase the ratio of tetraether lipids to diether lipids in response to increases in growth temperature or decreases in pH (128–131). In addition, archaeal pure cultures also increase the degree of cyclization in response to higher temperature and lower pH (132, 133). Similarly, environmental surveys of iGDGTs in geothermal spring sediments and mats have shown positive correlations between temperature and the degree of cyclization of iGDGTs (134). Molecular modeling studies have shown that increased cyclization stabilizes membranes by tightening membrane packing in the hydrophobic core and strengthening hydrogen bonding at the membrane surface (132).

Another type of tetraether lipid, the branched GDGTs (bGDGTs), have been identified in lipid extracts prepared from a wide variety of environments, including terrestrial geothermal systems (122, 135–137). The source of bGDGTs in geothermal systems is not completely understood since no thermophiles producing them have ever been isolated; however, they are known to be produced by some mesophilic *Acidobacteria* (138) and are almost certainly produced only by Bacteria because they are not isoprenoidal and the stereochemical configuration of the second carbon position of the glycerol backbone has been only found in *Bacteria* (139). Originally, bGDGTs in geothermal environments were thought to be contamination from soil mesophiles (136), but more recent studies have established that bGDGTs

are likely to be synthesized by moderate thermophiles with growth temperature optima $<70^{\circ}\text{C}$ (135) and may even be produced at higher temperatures (137). bGDGT types vary according to the degree of methylation of the alkyl chains, with four to six methyl groups per GDGT, and the number of cyclopentyl moieties, with zero to three rings per GDGT (Fig. 3). The distribution of various bGDGTs in environmental samples shows a negative correlation between the degree of methylation and temperature, suggesting a role for less methylated bGDGTs in thermostability (135).

EFFECT OF HIGH TEMPERATURE ON MICROBIAL COMMUNITY DIVERSITY, STRUCTURE, AND ECOSYSTEM FUNCTIONING

Relationships between High Temperature and Microbial Diversity and Composition

Despite decades of study on the physiology and biotechnological applications of thermophilic microbes, the ecology of geothermal ecosystems is still poorly understood.

An emerging body of literature demonstrates a quantitative decrease in microbial diversity as temperature increases in terrestrial geothermal environments (76, 115). While it might be expected that this relationship could impact ecosystem function in a general sense, temperature can also affect specific biogeochemical processes due to inhibition of key enzymes or pathways. Alternatively, as discussed, the high

ME required for life at high temperature might also limit temperatures at which energetically marginal metabolisms can occur.

One example of a thermally limited metabolism is phototrophy, which has an upper temperature limit of $\sim 73^\circ\text{C}$ at circumneutral to alkaline pH and $\leq 50^\circ\text{C}$ in acidic systems below pH 5 (97, 98). This limit is clearly visible in many terrestrial geothermal systems (e.g., Fig. 2d, e) and is particularly important because it limits primary production to chemoautotrophic pathways above this temperature (Table 1) and likely places energetic constraints on these ecosystems. While this limit on phototrophy can be obvious to the casual observer based on the presence/absence of photosynthetic pigments, temperature limits on other important biogeochemical processes are much less obvious. One key caveat to studying physicochemical limits for any microbial process is that it necessarily involves making inferences based on the absence of particular microorganisms, biomarkers, or ecosystem process rates. This concern is particularly significant given the difficulty growing and studying many thermophiles in the laboratory, where conditions can be precisely controlled. Nevertheless, with enough observations in diverse systems it should be possible to delineate upper temperature limits for any phylogenetic group, guild, or ecosystem process.

Carbon Cycle

A wide variety of thermophilic Bacteria and Archaea can use soluble cellulose, such as carboxymethylcellulose, as sole carbon and energy sources, including members of the archaeal orders *Thermococcales* and *Sulfolobales* and the bacterial orders *Thermotogales* and *Clostridiales* (140). These microorganisms include hyperthermophiles that can grow optimally at 100°C (T_{opt}) and thermoacidophiles that grow at T_{opt} 85°C and pH_{opt} 2–3. However, the enzymes made by most of these organisms have low catalytic efficiency and are not active on crystalline cellulose. Among thermophilic Bacteria and Archaea, only a few organisms are known to depolymerize crystalline cellulose, the most thermophilic being *Desulfurococcus fermentans* (T_{opt} $80\text{--}82^\circ\text{C}$; [141]), *Caldicellulosiruptor kristjanssonii* (T_{opt} 78°C ; [142]), *Caldicellulosiruptor bescii* (T_{opt} 75°C ; [143]), *Clostridium thermocellum* (T_{opt} 60°C ; [144]), and *Kallotenua papulyticum* (T_{opt} 55°C ; [145]). *Caldicellulosiruptor* and *Clostridium* are closely related and initiate cellulose degradation under strictly anaerobic conditions; however, highly efficient, membrane-bound cellulosome complexes may be limited to organisms with $T_{\text{opt}} \leq 60^\circ\text{C}$. *Kallotenua* depolymerizes crystalline cellulose under aerobic conditions and does not use a cellulosome (145, 146). Analysis of the *D. fermentans* genome failed to yield homologs of known cellulases (147) so the mechanism of cellulose hydrolysis by that organism remains unknown. However, a multidomain cellulase from the TIM barrel glycosyl hydrolase superfamily, originating from a yet-uncultivated member of the *Desulfurococcales*, has been identified and expressed from a functional metagenomic library and shown to degrade crystalline cellulose at much higher temperature (T_{opt} 109°C ; [148]).

Some other, simpler metabolisms also appear to be constrained in high-temperature environments, although it is not entirely clear that temperature is the only factor delimiting these metabolisms. For example, the most thermophilic organism known, *Methanopyrus*, is a marine, hydrogenotrophic methanogen (53); however, enrichment culture studies and ecosystem rate measurements (149–152) suggest methanogenesis may be limited to temperatures $\leq 75^\circ\text{C}$ in terrestrial geothermal systems. If this limit is true, then this limit

has important implications for both biomass processing and autotrophy in high-temperature terrestrial systems. In this case, the dramatic difference in thermophily between marine and terrestrial methanogens cannot be attributed to biochemical limitations and instead might be tied to bioenergetic constraints. Dissolved hydrogen concentrations reach 16 mM in serpentine/gabbro-hosted marine hydrothermal systems (54), whereas hydrogen concentrations rarely exceed 300 nM in terrestrial geothermal systems (98). Similarly, the highest known temperature for aerobic methanotrophs is 72°C (153).

Nitrogen Cycle

Until recently, very little was known about the nitrogen biogeochemical cycle at high temperatures. Research concentrated over the last decade suggests the nitrogen cycle is active in geothermal systems; however, some energetically marginal processes such as chemolithotrophic nitrite oxidation may be severely thermally inhibited. Studies of N_2 -fixation in Yellowstone springs showed that diazotrophy is nearly ubiquitous, occurring in very wide temperature and pH range ($48\text{--}89^\circ\text{C}$; pH 1.9–7.0), even in the presence of elevated ammonium concentrations in acidic systems (92, 154). Similarly, diazotrophy has been documented in a pure culture of *Methanocaldococcus jannaschii* up to 92°C (155). Thus, diazotrophy may be an important process at extreme temperatures.

In contrast, the oxidative nitrogen cycle may be restricted at high temperatures. Early hints of thermophilic AOA came when the tetraether lipid thaumarchaeol (Fig. 3) was discovered in terrestrial geothermal systems (156) and this hint was confirmed when “*Candidatus Nitrosocaldus yellowstonii*” was enriched from Heart Lake hot springs (Yellowstone) and shown to oxidize ammonia at 74°C (90). Since those discoveries, a variety of biomarkers for AOA have been recovered from temperatures up to 97°C (90, 157–159), although ammonia oxidation activity has only been measured up to $\sim 86^\circ\text{C}$ (91, 92, 158) and studies of the abundance of “*Candidatus Nitrosocaldus*” have shown dramatic declines in natural systems above $\sim 81^\circ\text{C}$ (35). No strong evidence of ammonia-oxidizing bacteria above $\sim 50^\circ\text{C}$ exists.

Fewer studies have focused on chemolithotrophic nitrite oxidation; however, those that have been done have failed to uncover evidence of nitrite oxidation above $\sim 63^\circ\text{C}$ (160). Enrichment cultures with *Nitrospira* have been studied from a variety of locations (161, 162). The pure culture showing nitrite oxidation activity at $\sim 63^\circ\text{C}$, *Nitrolanceus hollandicus*, has a high K_s (1 mM); since millimolar concentrations of nitrite are unlikely to occur in thermal environments, the environmental relevance of this organism is uncertain (160). If a substantial difference in upper temperature limits for AOA and NOB does exist, this may decouple the two steps of nitrification in high-temperature ecosystems, in contrast to most ecosystems where ammonia oxidation is typically rate-limiting.

The reductive, dissimilatory nitrogen cycle operates at very high temperatures. For example, the archaea *Pyrolobus fumarii* and *Thermoproteus aerophilum* respire nitrate to ammonium in marine systems at 113°C (163) and nitrate to dinitrogen (with N_2O as the dominant product) in terrestrial systems at 100°C (164). Much less is known about anaerobic ammonium oxidation (anammox); however, isotope pairing experiments have shown anammox activity up to 85°C in marine systems (165) and DNA and lipid biomarkers for anammox *Planctomycetes* have been recovered from terrestrial geothermal systems at temperatures up to 65°C (166).

RECENT DEVELOPMENTS AND FUTURE DIRECTIONS

Traditional microbiology focuses heavily on studies of axenic laboratory cultures; however, the limitations on pure culture studies are well documented (167, 168), particularly for terrestrial geothermal systems (68). To address these limitations and explore still uncultivated lineages of thermophiles, microbiologists have turned to a rapidly developing arsenal of cultivation-independent approaches. Among the most powerful of these approaches are single-cell genomics and metagenomics, which have allowed expansion of the genomics revolution to uncultivated microbes (169). Both techniques have the potential to yield partial or complete genomes from individual cells or from composite genomes from clonal or closely related populations, thereby yielding insight into the physiological potential of uncultivated lineages and providing a foundation for application of other “omics” approaches to probe their functions (e.g., 170). Although each of these techniques has its own strengths and weaknesses, there is much potential for synergistic application of single-cell genomics and metagenomics (171). These techniques have yielded genomic data from dozens of major lineages of microorganisms (172), including several that contain (hyper)thermophiles (173–179). Several of these candidate phyla (“Acetothermia,” “Aigarchaeota,” “Calesmantes,” “Fervidibacteria,” and “Korarchaeota”) appear to be restricted to thermal environments and thus likely add to the list of lineages composed primarily or exclusively of thermophilic specialists. As mentioned, it will be important to couple the rapidly expanding knowledge base provided by cultivation-independent genomics techniques with functional studies that can test the predicted capabilities of members of these lineages, such as the potential for autotrophy in the “Acetothermia” (179) and “Aigarchaeota” (177).

Another very important direction is to more deeply understand the ecological implications of life at high temperature. The study of thermophiles has focused very much on pure culture microbiology and biochemistry; however, there is a lot to learn about the roles of individual thermophiles in nature and how they work together as functional guilds and communities. Although some important ecosystem functions have been studied in a variety of geothermal systems, these studies are relatively uncommon and fragmented, providing excellent opportunities for microbial ecologists to gain deeper insights into the fundamentals of life at high temperature.

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