

11 Hyperthermophilic Microorganisms

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The first traces of life on Earth date back to the Early Archaean age. Microfossils of prokaryotes demonstrate the existence of life already 3.5 to 3.9 billion years ago [1, 2]. Although nothing is known about the original growth temperature requirements of these organisms, the Earth is generally assumed to having been much hotter at that time than today [3]. Recently, fossil-remains of thread-like microorganisms have been discovered in a 3.2 billion year old deep sea volcanogenic hydrothermal deposit, indicating the existence of hyperthermophiles already at Early Archaean times [4].

Today, most life forms known are mesophiles adapted to ambient temperatures within a range from 15 to 45 °C. Among Bacteria, thermophiles (heat-lovers) have been recognized for some time which grow optimally (fastest) between 45 and 70 °C. They thrive within sun-heated soils, self-heated waste dumps and thermal waters and are closely related to mesophiles. During the last years, hyperthermophilic Bacteria and Archaea (formerly the Archaeobacteria) with unprecedented properties have been isolated mostly from areas of volcanic activity [5-7]. They grow between 80 and 113 °C and represent the organisms at the upper temperature border of life. At ambient temperatures, although unable to grow, hyperthermophiles can survive for many years in a kind of "frozen" state. Within eukaryotes, the upper temperature of growth known is about 60 °C and, therefore much lower than in Bacteria and Archaea [8]. Here, an overview will be presented about the biotopes, modes of life, and phylogeny of hyperthermophiles. Due to their likely presence already during the impact bombardment at the Early Archaean, they may have even been able to spread between the young planets of our solar system.

11.1 Biotopes of Hyperthermophiles

Hyperthermophiles are found in water-containing volcanically and geothermally heated environments situated mainly along terrestrial and submarine tectonic fracture zones where plates are colliding (subduction) or moving away from each other (spreading). Within saturated or superheated steam, life is not possible and liquid water is a fundamental prerequisite. Several hyperthermophiles exhibit growth temperatures exceeding 100 °C at an increased boiling point of water (e.g., by elevated atmospheric, hydrostatic or osmotic pressure).

Due to the presence of reducing gasses and the low solubility of oxygen at high temperatures, biotopes of hyperthermophiles are essentially anaerobic (Table 11.1.). Hyperthermophiles have been isolated from terrestrial and marine environments.

11.1.1 Terrestrial Biotopes

Terrestrial biotopes of hyperthermophiles are mainly sulfur-containing solfataric fields, named after the Solfatara Crater at Italy. Solfataric fields consist of soils, mud holes and surface waters heated by volcanic exhalations from magma chambers, a few kilometers below. Very often, solfataric fields are situated at or in the close neighborhood of active volcanoes and activity is greatly increased during eruption phases. Depending on the altitude above sea level, the maximum water temperatures are up to 100 °C. The salinity of solfataric fields is usually low. However, there are exceptions if they are situated at the beach (e.g., Faraglione, Vulcano, Italy).

The chemical composition of solfataric fields is very variable and depends on the site. Steam within the solfataric exhalations is mainly responsible for the heat transfer. CO₂ keeps the soils anaerobic and prevents penetration of oxygen into greater depths. In addition, H₂S reduces oxygen to water yielding elemental sulfur. An important gaseous energy source for hyperthermophiles is hydrogen, which may be formed either pyrolytically from water or chemically from FeS and H₂S [9]. Many solfataric fields are rich in iron minerals like ferric hydroxides, pyrite, and other ferrous sulfides.

Less usual compounds may be enriched at some sites like magnetite or arsenic minerals auripigment and realgar in Geysiriraja Valley, Kamchatka. Sometimes, solfataric fields contain silicate-rich neutral to slightly alkaline (pH 7-10) hot springs originating from the depth. Their content of sulfur compounds is usually low.

Table 11.1. Biotopes of hyperthermophiles

Characteristics	Type of thermal area	
	Terrestrial	Marine
Locations	Solfataric fields Deeply originating hot springs; Subterranean oil stratifications	Submarine hot vents and hot sediments "Black smokers"; Active sea-mounts
Temperatures	Surface: up to 100 °C *); Depth: above 100 °C	Up to about 400 °C
Salinity	Usually low (0.1 to 0.5% salt)	Usually about 3% salt
PH	0 to 10	5 to 8.5 (rarely: 3)
Major life-supporting gasses and nutrients	H ₂ O (steam) CO ₂ , CO, CH ₄ , H ₂ , H ₂ S, S ⁰ , S ₂ O ₃ ²⁻ , SO ₃ ²⁻ , SO ₄ ²⁻ ***) NH ₄ ⁺ , N ₂ , NO ₃ ⁻ , Fe ²⁺ , Fe ³⁺ , O ₂ (surface)	
*)	Depending on the altitude	
***)	Sea water contains about 30 mmol/l of sulfate	

Active volcanoes may harbor hot crater lakes which are heated by fumaroles (e.g., Askja, Iceland). Usually, those abound in sulfur and are very acidic and represent a further biotope of hyperthermophiles. Nothing is known about possible microbial life in the interior of active volcanoes. These mountains are assumed to be "hot sponges" which may contain a lot of aquifers like cracks and holes, possibly providing so far unexplored biotopes for hyperthermophiles. First evidence for the presence of communities of hyperthermophiles within geothermally heated rocks 3500 meters below the surface had been recently demonstrated [10]. Soils on the flanks of volcanoes, depending on the interior heat flow may harbor hyperthermophiles, too. For example, at the "Tramway Ridge" and southern crater on top of Mount Erebus, and on top of Mount Melbourne, both Antarctica, there are wet soils with temperatures between 60 and 65 °C and pH 5-6 at an altitude of 3500 meters. They represent "islands" of thermophilic life within a deep-frozen continent.

11.1.2 Marine Biotopes

Marine biotopes of hyperthermophiles consist of various hydrothermal systems situated at shallow to abyssal depths. Similar to ambient sea water, submarine hydrothermal systems usually contain high concentrations of NaCl and sulfate and exhibit a slightly acidic to alkaline pH (5-8.5). Otherwise, the major gasses and life-supporting mineral nutrients may be similar to that in terrestrial thermal areas. Shallow submarine hydrothermal systems are found in many parts of the world, mainly on beaches with active volcanism, like at Vulcano island, Italy with temperatures of 80 to 105 °C.

Most impressive are the deep sea "smoker" vents (Fig. 11.1) where mineral-laden hydrothermal fluids with temperatures up to about 400 °C escape into the cold (2.8 °C), surrounding deep sea water and build up huge rock chimneys. Although these hot fluids are sterile, the surrounding porous smoker rock material appears to contain very steep temperature gradients which provide zones of suitable growth temperatures for hyperthermophiles. Some smoker rocks are teeming with hyperthermophiles (for example 10^8 cells of *Methanopyrus* per gram of rock inside a Mid Atlantic Snake Pit hot vent chimney). Deep sea vents are located along submarine tectonic fracture zones for example the "TAG" and "Snake Pit" sites situated at the Mid Atlantic Ridge in a depth of about 4000 meters.

A further type of submarine high temperature environments is provided by active sea mounts. Close to Tahiti, there is a huge abyssal volcano, Macdonald Seamount (28°58.7' S, 140°15.5' W), the summit of which is situated approximately 40 meters below the sea surface. Samples taken during an active phase from the submarine eruption plume and rocks from the active crater contained high concentrations of viable hyperthermophiles [11].

11.2 Phylogeny of Hyperthermophiles

During recent years, powerful molecular techniques had been developed in order to investigate phylogenetic relationships of living organisms [12-14]. Based on the pio-

neering work of Carl Woese, 16S rRNA (harbored by the small ribosomal subunit) is widely used in phylogenetic studies of prokaryotes [13]. It consists of about 1500 bases and is homologous to the eukaryotic 18S rRNA. Based on 16/18S rRNA sequence comparisons, a universal phylogenetic tree is now available [15]. It exhibits a tripartite division of the living world into the bacterial (former: "eubacterial"), archaeal (former: "archaeobacterial") and eukaryal (former: "eukaryotic") domains (Fig. 11.2) [16, 15]. The root was inferred from phylogenetic trees of duplicated genes of ATPase subunits and elongation factors Tu and G [17, 18]. Within the 16S rRNA phylogenetic tree, deep branches are evidence for early separation. For example, the separation of the Bacteria from the stem common to Archaea and Eucarya represents the deepest and earliest branching point. Short phylogenetic branches indicate a rather slow rate of evolution. In contrast to the Eucarya, the bacterial and archaeal domains within the universal phylogenetic tree exhibit some extremely deep and short branches. Based on the unique phylogenetic position of *Thermotoga*, a thermophilic ancestry of the Bacteria had been taken into consideration [19]. Surprisingly, hyperthermophiles are represented by all short and deep phylogenetic branches, which form a cluster around the phylogenetic root (Fig. 11.2, bold lines). The deepest and shortest phylogenetic branches are represented by *Aquifex* and *Thermotoga* within the Bacteria and *Methanopyrus*, *Pyrodictium*, and *Pyrolobus* within the Archaea. On the other

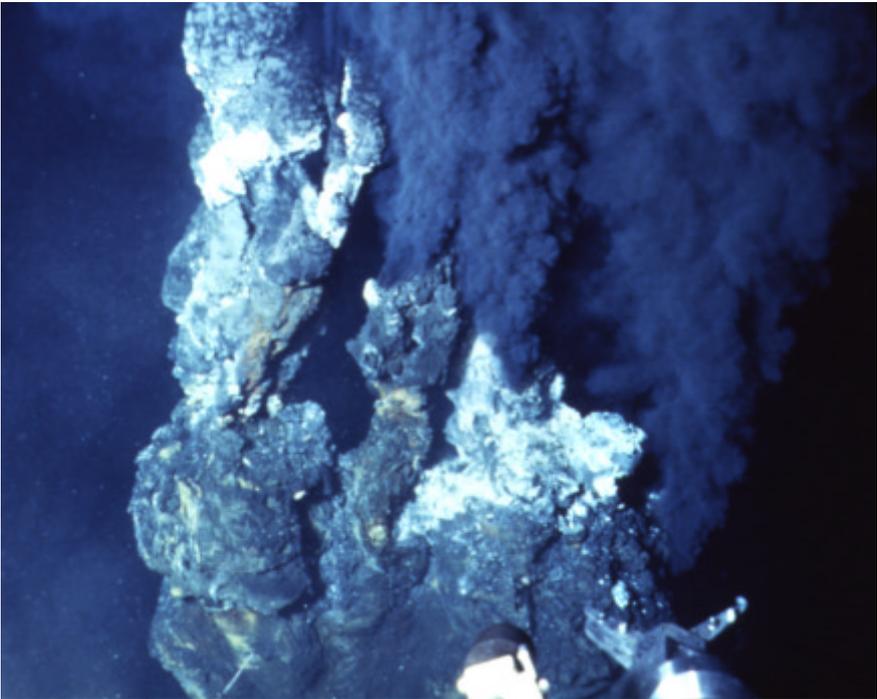


Fig. 11.1 Active deep sea vent chimney, a "Black Smoker" spewing superheated mineral-laden black water into the cold deep sea environment. The plume appears like black "smoke".

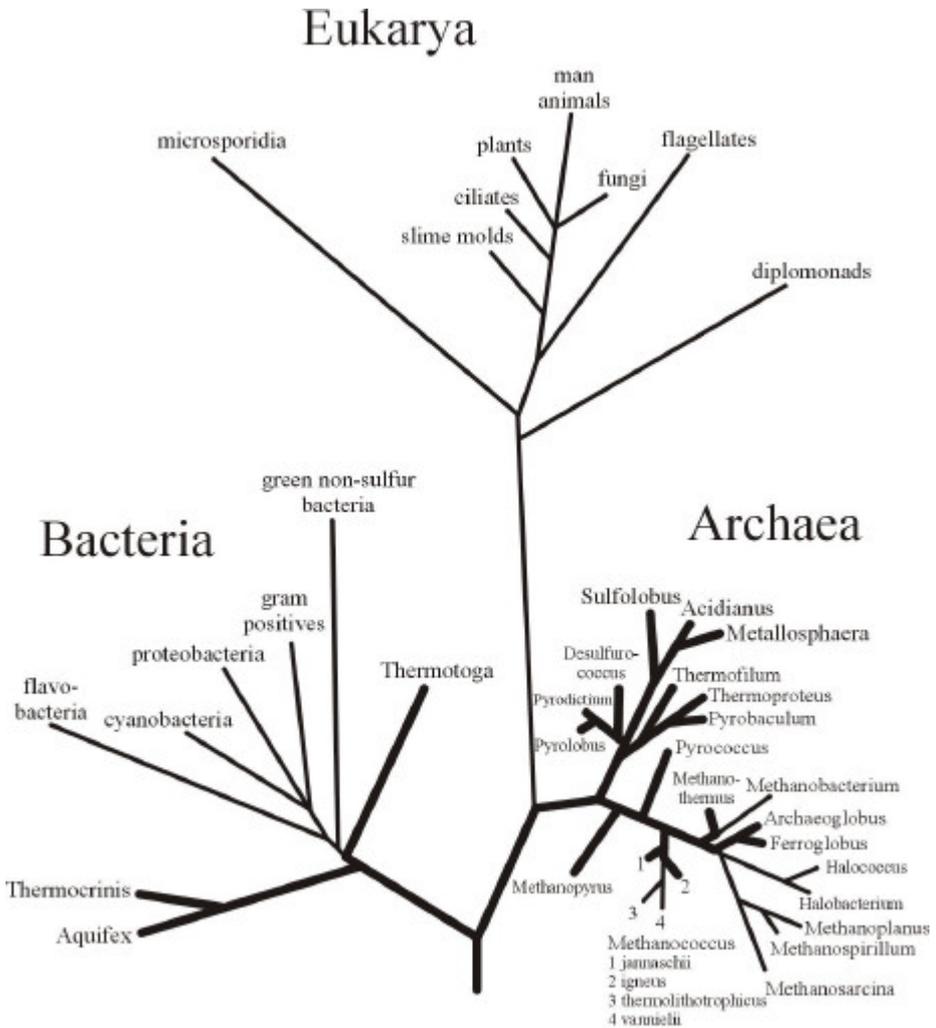


Fig. 11.2 16S rRNA-based phylogenetic tree, [6], modified. Small subunit ribosomal RNA sequences were compared according to Woese [15]. Schematic drawing. Bold lines represent hyperthermophiles.

hand, mesophilic and moderately thermophilic Bacteria and Archaea, as a rule represent long lineages within the phylogenetic tree and had a fast rate of evolution (e.g., Gram-positives; Proteobacteria; *Halobacterium*; *Methanosarcina*; Fig. 11.2). Total genome sequencing makes it possible to determine phylogenetic relationships of many other genes homologous at different organisms. Comparison of genes involved in gene expression confirm the 16S rRNA phylogenetic tree. Within the genes of metabolism, however, based on frequent lateral gene transfer rather a network than a tree may reflect their phylogenetic relations [20].

11.3 Taxonomy of Hyperthermophiles

So far, about 70 species of hyperthermophilic Bacteria and Archaea are known, which had been isolated from different terrestrial and marine thermal areas in the world. Hyperthermophiles are very divergent, both in terms of their phylogeny and physiological properties, and are grouped into 29 genera in 10 orders (Table 11.2.). At present, 16S rRNA sequence-based classification of prokaryotes appears to be imperative for the recognition and characterization of novel taxonomic groups. In addition, more traditional taxonomic features such as GC-contents of DNA, DNA-DNA homology, morphology, and physiological features may be used as separating characters in order to obtain high resolution of taxonomy within members of a phylogenetic lineage (e.g., description of different species and strains).

11.4 Strategies of Life and Environmental Adaptations of Hyperthermophiles

Hyperthermophiles are adapted to distinct environmental factors including composition of minerals and gasses, pH, redox potential, salinity and temperature.

11.4.1 General Metabolic Potentialities

Most hyperthermophiles exhibit a chemolithoautotrophic mode of nutrition: inorganic redox reactions serve as energy sources (chemolithotrophic), and CO₂ is the only carbon source required to build up organic cell material (autotrophic). Therefore, these organisms are fixing CO₂ by chemosynthesis and are designated chemolithoautotrophs. The energy-yielding reactions in chemolithoautotrophic hyperthermophiles are anaerobic and aerobic types of respiration (Table 11.3.).

Molecular hydrogen serves as an important electron donor. Other electron donors are sulfide, sulfur, and ferrous iron. Like in mesophilic respiratory organisms, in some hyperthermophiles oxygen may serve as an electron acceptor. In contrast, however, oxygen-respiring hyperthermophiles are usually microaerophilic and, therefore, grow only at reduced oxygen concentrations (even as low as 10 ppm; [21]). Anaerobic respiration types are the nitrate-, sulfate-, sulfur- and carbon dioxide-respirations. While chemolithoautotrophic hyperthermophiles produce organic matter, there are some hyperthermophiles, which depend on organic material as energy- and carbon sources. They are designated as chemoorganoheterotrophs (or, shortly: heterotrophs). Several chemolithoautotrophic hyperthermophiles are facultative heterotrophs (Table 11.3.). Those are able to use organic material alternatively to inorganic nutrients whenever it is provided by the environment (e.g., by decaying cells). Heterotrophic hyperthermophiles gain energy either by aerobic or different types of anaerobic respiration, using organic material as electron donors, or by fermentation.

11.4.2 Physiological Properties

11.4.2.1 Terrestrial Hyperthermophiles

The acidic hot oxygen-exposed surface of terrestrial solfataric fields almost exclusively harbours extremely acidophilic hyperthermophiles. They consist of lobed coccoid-shaped (Fig. 11.3a) aerobes and facultative aerobes, growing between pH 1 and 5 with an optimum around pH 3 (Table 11.4.). Phylogenetically they belong to the archaeal genera *Sulfolobus*, *Metallosphaera* and *Acidianus*. Together with the strictly anaerobic *Stygiolobus* these genera form the *Sulfolobales* order (Fig. 11.2, Table 11.2.). Members of *Sulfolobus* are strict aerobes. During autotrophic growth, S^0 , S^{2-} , and H_2 [22] may be oxidized, yielding sulfuric acid or water as end products (Table 11.3.). During heterotrophic growth, sugars, yeast extract, and peptone may serve as energy sources [8]. Members of *Acidianus* are able to grow by anaerobic and aerobic oxidation of H_2 , using S^0 and O_2 as electron acceptors, respectively [23]. Alternatively, *Acidianus* is able to grow by S^0 -oxidation (Table 11.3.).

Terrestrial hot springs with low salinity and the depth of solfataric fields harbour slightly acidophilic and neutrophilic hyperthermophiles, which are usually strict anaerobes. They are members of the genera *Pyrobaculum*, *Thermoproteus*, *Thermofilum*, *Desulfurococcus*, *Sulfophobococcus*, and *Thermosphaera* (Table 11.2.). Cells of *Pyrobaculum*, *Thermoproteus*, and *Thermofilum* are stiff, regular rods with almost rectangular edges (Fig. 11.3b). During the exponential growth phase, under the light microscope, spheres become visible at the ends ("golf clubs"). Cells of *Pyrobaculum* and *Thermoproteus* are about 0.50 μm in diameter whereas those of *Thermofilum* ("the hot thread") are only about 0.17 - 0.35 μm . *Thermoproteus tenax*, *Thermoproteus neutrophilus* and *Pyrobaculum islandicum* are able to gain energy by formation of H_2S from H_2 and S^0 [24] (Table 11.4.). *Thermoproteus tenax* and *Pyrobaculum islandicum* are facultative, *Pyrobaculum organotrophum*, *Thermoproteus uzoniensis*, *Thermofilum librum*, and *Thermofilum pendens* obligate heterotrophs. Members of *Desulfurococcus* are coccoid-shaped, strictly heterotrophic sulfur-respirers, while *Thermosphaera* and *Sulfophobococcus* are purely fermentative coccoid organisms. *Thermosphaera aggregans* grows in grape-shaped aggregates. Exclusively from the depth of solfataric fields in the southwest of Iceland (Kerlingarfjöll mountains), members of the genus *Methanothermus* have been isolated which appear to be endemic species of this area. They are highly oxygen-sensitive strictly chemolithoautotrophic methanogens, gaining energy by reduction of CO_2 by H_2 (Table 11.3.) and growing at temperatures between 65 and 97 $^\circ C$ (Table 11.4.).

Table 11.2. Taxonomy and upper growth temperatures of hyperthermophiles (Examples)

Order	Genus	Species	T max ($^\circ C$)
Domain: BACTERIA			

Thermotogales	Thermotoga	T. maritima	90
		T. thermarum	84
	Thermosipho	T. africanus	77 *
Aquificales	Fervidobacterium	F. nodosum	80 *
		Aquifex	A. pyrophilus
	Calderobacterium	A. aeolicus	93
		Thermocrinis	C. hydrogenophilum
		T. ruber	89

Domain: ARCHAEA

I. Kingdom: Crenarchaeota

Sulfolobales	Sulfolobus	S. acidocaldarius	85
		S. solfataricus	87
	Metallosphaera	M. sedula	80 *
	Acidianus	A. infernus	95
	Stygiolobus	S. azoricus	89
	Sulfurococcus	S. mirabilis	86
	Sulfurisphaera	S. ohwakuensis	92
Thermoproteales	Thermoproteus	T. tenax	97
		Pyrobaculum	P. islandicum
	Thermofilum	P. aerophilum	104
		T. pendens	95
	Thermocladium	T. librum	95
		Caldivirga	T. modestius
		C. maquilingsensis	92
Desulfurococcales	Desulfurococcus	D. mucosus	97
		Staphylothermus	S. marinus
	Sulfophobococcus	S. zilligii	95
	Stetteria	S. hydrogenophila	102
	Aeropyrum	A. pernix	100
	Ignicoccus	I. islandicus	100
	Thermosphaera	T. aggregans	90
	Thermodiscus	T. maritimus	98
	Pyrodictium	P. occultum	110
	Hyperthermus	H. butylicus	108
	Pyrolobus	P. fumarii	113

*) extreme thermophiles related to hyperthermophiles

Table 11.2. Continuation: Taxonomy and upper growth temperatures of hyperthermophiles (Examples)

Order	Genus	Species	T max (°C)
Domain: ARCHAEA			
II. Kingdom: Euryarchaeota			
Thermococcales	Thermococcus	T. celer	93
		Pyrococcus	P. furiosus
		P. abyssi	102
		P. horikoshii	102

Archaeoglobales	Archaeoglobus	A. fulgidus	92
		A. veneficus	88
	Ferroglobus	F. placidus	95
Methanobacteriales	Methanothermus	M. fervidus	97
Methanococcales	Methanococcus	M. jannaschii	86
		M. igneus	91
Methanopyrales	Methanopyrus	M. kandleri	110

*) extreme thermophiles related to hyperthermophiles

Table 11.3. Energy-yielding reactions in chemolithoautotrophic hyperthermophiles

Energy-yielding reaction	Genera
$2S^0 + 3O_2 + 2H_2O \rightarrow 2H_2SO_4$ $(2FeS_2 + 7O_2 + 2H_2O \rightarrow 2FeSO_4 + 2H_2SO_4)$ = "metal leaching"	<i>Sulfolobus</i> ^a , <i>Acidianus</i> ^a , <i>Metallosphaera</i> ^a , <i>Aquifex</i>
$H_2 + \frac{1}{2} O_2 \rightarrow H_2O$	<i>Aquifex</i> , <i>Acidianus</i> ^a , <i>Metallosphaera</i> ^a , <i>Pyrobaculum</i> ^a , <i>Sulfolobus</i> ^a
$H_2 + HNO_3 \rightarrow HNO_2 + H_2O$	<i>Aquifex</i> , <i>Pyrobaculum</i> ^a
$4H_2 + HNO_3 \rightarrow NH_4OH + 2H_2O$	<i>Pyrolobus</i>
$2FeCO_3 + HNO_3 + 5H_2O$ $\rightarrow 2Fe(OH)_3 + HNO_2 + 2H_2CO_3$	<i>Ferroglobus</i>
$4H_2 + H_2SO_4 \rightarrow H_2S + 4H_2O$	<i>Archaeoglobus</i> ^a
$H_2 + S^0 \rightarrow H_2S$	<i>Acidianus</i> , <i>Stygiolobus</i> , <i>Pyrobaculum</i> ^a , <i>Ignicoccus</i> , <i>Pyrodictium</i> ^a
$H_2 + 6FeO(OH) \rightarrow 2Fe_3O_4 + 4H_2O$	<i>Pyrobaculum</i>
$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$	<i>Methanopyrus</i> , <i>Methanothermus</i> , <i>Methanococcus</i>

^a facultatively heterotrophic

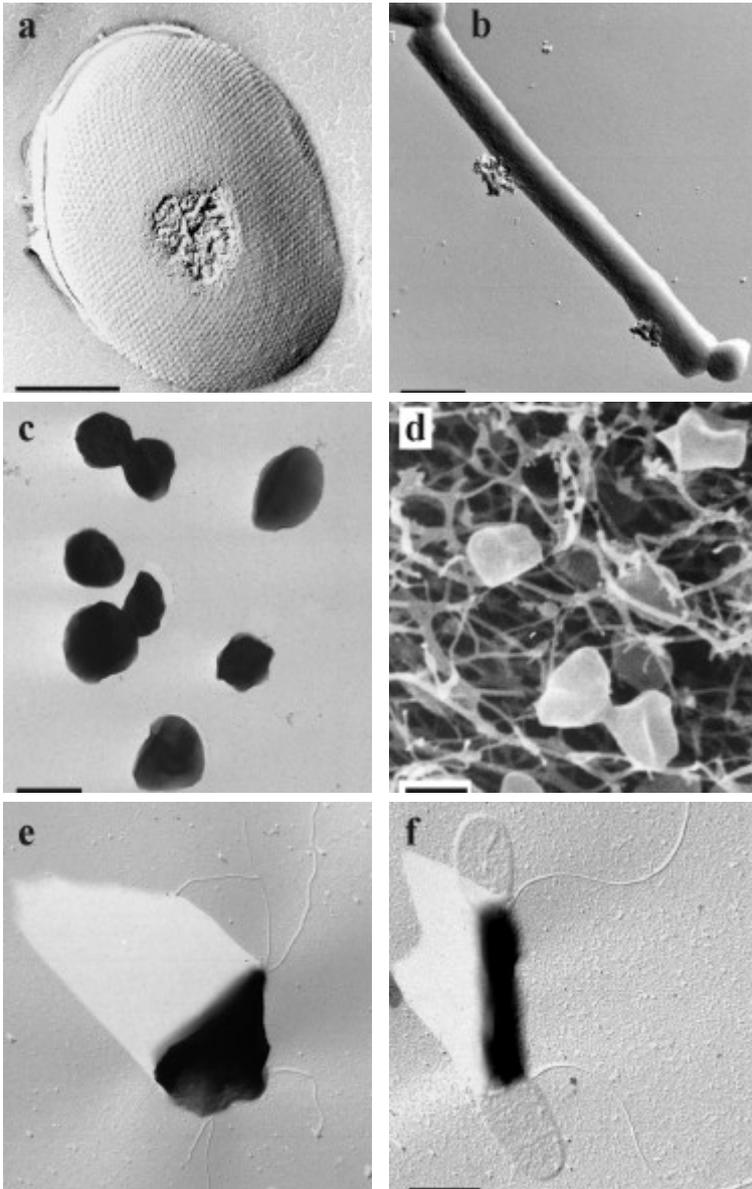


Fig. 11.3 Electron micrographs of cells of hyperthermophilic Archaea and Bacteria
a. *Metallosphaera sedula*, freeze fracturing, bar, 0.2 μm ;
b. *Thermoproteus tenax*, Pt-shadowing, bar, 1.0 μm ;
c. *Pyrolobus fumarii*, UAc-staining, bar, 1.0 μm ;
d. *Pyrodictium abyssi*, scanning micrograph, bar, 1.0 μm ;
e. *Archaeoglobus fulgidus*, Pt-shadowing, bar, 1.0 μm ;
f. *Thermotoga maritima*, Pt-shadowing, bar, 1.0 μm .

Table 11.4. Growth conditions and morphology of hyperthermophiles

Species	Growth conditions					
	Min. Temp. (°C)	Opt. Temp. (°C)	Max. Temp. (°C)	pH	Aerobic (ae) versus anaerobic (an)	Morphology
<i>Sulfolobus acidocaldarius</i>	60	75	85	1-5	ae	Lobed cocci
<i>Metallosphaera sedula</i>	50	75	80	1-4.5	ae	Cocci
<i>Acidianus infernus</i>	60	88	95	1.5-5	ae/an	Lobed cocci
<i>Stygiolobus azoricus</i>	57	80	89	1-5.5	an	Lobed cocci
<i>Thermoproteus tenax</i>	70	88	97	2.5-6	an	Regular rods
<i>Pyrobaculum islandicum</i>	74	100	103	5-7	an	Regular rods
<i>Pyrobaculum aerophilum</i>	75	100	104	5.8-9	ae/an	Regular rods
<i>Thermofilum pendens</i>	70	88	95	4-6.5	an	Slender regular rods
<i>Desulfurococcus mobilis</i>	70	85	95	4.5-7	an	Cocci
<i>Thermosphaera aggregans</i>	67	85	90	5-7	an	Cocci in aggregates
<i>Sulfophobococcus zilligii</i>	70	85	95	6.5-8.5	an	Cocci
<i>Staphylothermus marinus</i>	65	92	98	4.5-8.5	an	Cocci in aggregates
<i>Thermoplasma maritimum</i>	75	88	98	5-7	an	Disks
<i>Aeropyrum pernix</i>	70	90	100	5-9	ae	Irregular cocci
<i>Stetteria hydrogenophila</i>	70	95	102	4.5-7	an	Irregular disks
<i>Ignicoccus islandicus</i>	65	90	100	3.9-6.3	an	Irregular cocci
<i>Pyrodictium occultum</i>	82	105	110	5-7	an	Disks with cannulae
<i>Hyperthermus butylicus</i>	80	101	108	7	an	Lobed cocci
<i>Pyrolobus fumarii</i>	90	106	113	4.0-6.5	ae/an	Lobed cocci
<i>Thermococcus celer</i>	75	87	93	4-7	an	Cocci
<i>Pyrococcus furiosus</i>	70	100	105	5-9	an	Cocci
<i>Archaeoglobus fulgidus</i>	60	83	95	5.5-7.5	an	Irregular cocci
<i>Ferroplasma placidus</i>	65	85	95	6-8.5	an	Irregular cocci
<i>Methanothermobacter sociabilis</i>	65	88	97	5.5-7.5	an	Rods in clusters
<i>Methanopyrus kandleri</i>	84	98	110	5.5-7	an	Rods in chains
<i>Methanococcus igneus</i>	45	88	91	5-7.5	an	Irregular cocci
<i>Thermotoga maritima</i>	55	80	90	5.5-9	an	Rods with sheath
<i>Aquifex pyrophilus</i>	67	85	95	5.4-7.5	ae	Rods

11.4.2.2 Marine Hyperthermophiles

A variety of hyperthermophiles are adapted to the high salinity of sea water of about 3% salt. They are represented by members of the archaeal genera *Pyrolobus*, *Pyrodictium*, *Hyperthermus*, *Stetteria*, *Thermoplasma*, *Igneococcus*, *Staphylothermus*, *Aeropyrum*, *Pyrobaculum*, *Methanopyrus*, *Pyrococcus*, *Thermococcus*, *Archaeoglobus* and *Ferroglobus*, and of the bacterial genera *Aquifex* and *Thermotoga* (Table 11.2.). The organism with the highest growth temperature is *Pyrolobus fumarii*, exhibiting an upper temperature border of growth above 113 °C (Table 11.4.) [21]. *Pyrolobus* had been isolated from the walls of an active deep sea smoker chimney at the Mid Atlantic Ridge. It is so dependent on high temperatures that it is unable to grow below 90 °C. Cells of *Pyrolobus* are lobed cocci about 0.7 - 2.5 µm in diameter (Fig. 11.3c). *Pyrolobus fumarii* gains energy by chemolithoautotrophic nitrate reduction, forming ammonia as an end product. Alternatively, under microaerophilic conditions, it shows weak but significant growth on H₂ and traces of oxygen (0.05%). Close relatives to *Pyrolobus* are members of *Pyrodictium*, cells of which are disks (0.2 µm thick and up to 3 µm in diameter) which are usually connected by networks of hollow cannulae, about 25 nm in diameter (Fig. 11.3d) [25, 26]. As a rule, members of *Pyrodictium* are chemolithoautotrophs, gaining energy by reduction of S⁰ by H₂. In addition, some isolates are able to grow by reduction of sulfite and thiosulfate [27]. A very close relative to *Pyrodictium* is *Hyperthermus*. However, in contrast to *Pyrodictium*, it is a purely fermentative hyperthermophile which does not form cannulae and does not grow at 110 °C [28]. *Stetteria hydrogenophila* represents a group of disk-shaped hyperthermophiles which gains energy on a combination of H₂ and oxidized sulfur compounds, and peptides [29]. *Igneococcus islandicus* is a coccoid-shaped strictly chemolithoautotrophic member of the *Desulfurococcaceae* which gains energy by sulfur respiration [30]. *Pyrobaculum aerophilum* so far is the only marine member of the *Thermoproteales*. It is a rod-shaped strictly chemolithoautotrophic hyperthermophile which gains energy either anaerobically by nitrate reduction or microaerobically by reduction of O₂ (traces). Molecular hydrogen serves as electron donor. The rod-shaped *Methanopyrus kandleri* occurs the same within shallow as abyssal submarine hot vents and exhibits the highest growth temperatures in methanogens (up to 110 °C). Further marine hyperthermophilic methanogens are the coccoid-shaped *Methanococcus jannaschii* and *Methanococcus igneus* within the *Methanococcales* (Fig. 11.2) where they represent the shortest phylogenetic branch-offs. Archaeal sulfate reducers are represented by members of *Archaeoglobus* [31, 32]. *Archaeoglobus fulgidus* and *Archaeoglobus lithotrophicus* are chemolithoautotrophs able to grow by reduction of SO₄²⁻ and S₂O₃²⁻ by H₂ (Table 11.3.), while *Archaeoglobus veneficus* is only able to reduce SO₃²⁻ and S₂O₃²⁻. *Archaeoglobus fulgidus* is a facultative heterotroph growing on a variety of organic substrates like formate, sugars, starch, proteins, and cell extracts [33]. Sequencing of the total genome of this organism revealed the presence of genes for the fatty acid β-oxidation pathway, indicating that its physiological properties may be even broader than initially described [33]. Cells of members of *Archaeoglobus* are coccoid to triangular-shaped (Fig. 11.3e). Like methanogens, they show a blue-green fluorescence at 420 nm under the UV-microscope. In agreement, *Archaeoglobus* possesses several coenzymes that had been thought to be unique for methanogens (e.g.,

F₄₂₀, methanopterin, tetrahydromethanopterin, methanofurane). Usually, members of *Archaeoglobus* are found in shallow and abyssal submarine hydrothermal systems. In addition, the same species have been detected in deep geothermally heated subterranean oil reservoirs and may be responsible for some of the H₂S formation there ("reservoir souring", [31, 10]). *Ferroglobus* represents a further genus within the *Archaeoglobales*. It gains energy anaerobically by reduction of NO₃⁻ with Fe²⁺ (Table 11.3.), H₂, and H₂S as electron donors. The unique metabolism of *Ferroglobus placidus* may offer a biological mechanism for the anaerobic formation of Fe³⁺ which may have operated during banded iron formations (BIFs) under the anoxic high-temperature conditions at the Hadean Earth [34]. Within the Bacteria domain, the deepest phylogenetic branch is represented by *Aquifex*. *Aquifex pyrophilus* is a motile rod-shaped strict chemolithoautotroph. It is a facultative aerobe. Under anaerobic conditions, *Aquifex pyrophilus* grows by nitrate reduction with H₂ and S⁰ as electron donors. Alternatively, at very low oxygen concentrations (up to 0.5%, after adaptation) it is able to gain energy by oxidation of H₂ and S⁰. Members of *Aquifex* are found in shallow submarine vents. *Aquifex pyrophilus* grows up to 95 °C, the highest growth temperature found so far within the bacterial domain (Table 11.4.).

Groups of strictly heterotrophic hyperthermophiles are thriving in submarine vents, too. *Thermotoga maritima* is a disk-shaped archaeal heterotroph growing by sulfur respiration on yeast extract and prokaryotic cell homogenates. In the absence of S⁰, it is able to gain energy by fermentation [5]. Cells of *Staphylothermus marinus* are coccoid and arranged in grape-like aggregates. They are highly variable in diameter from 0.5 to 15 μm. *Aeropyrum pernix* is a strictly aerobic spherical-shaped marine hyperthermophile, growing optimally at neutral pH. It gains energy in metabolizing complex compounds like yeast extract and peptone [35]. Members of *Pyrococcus* and *Thermococcus* are submarine motile coccoid species, cells of which occur frequently in pairs. They gain energy by fermentation of peptides, amino acids and sugars, forming fatty acids, CO₂ and H₂. Hydrogen may inhibit growth and can be removed by gassing with N₂ [36]. Alternatively, inhibition by H₂ can be prevented by addition of S⁰, whereupon H₂S is formed instead of H₂. *Pyrococcus furiosus* grows optimally at 100 °C (Table 11.4.). In addition to their marine environment, species of *Pyrococcus* and *Thermococcus* are found in geothermally heated oil reservoirs of high salinity (together with *Archaeoglobus*, see above) and are able to grow in the presence of crude oil [10]. Many submarine hydrothermal systems contain hyperthermophilic members of *Thermotoga* which represent the second deepest phylogenetic branch within the bacterial domain (Fig. 11.2). Cells of *Thermotoga* are rod-shaped, thriving together with archaeal hyperthermophiles within the same environment. They show a characteristic "toga", a sheath-like structure surrounding cells and overballooning at the ends (Fig. 11.3f). *Thermotoga* ferments various carbohydrates and proteins. As end products, H₂, CO₂, acetate and L(+)-lactate are formed. Hydrogen is inhibitory to growth. In the presence of S⁰, H₂S is formed instead of H₂, which does not inhibit growth. Other genera within the *Thermotogales* are members of *Thermosipho* and *Fervidobacterium* which are strict heterotrophs, too. Similar to *Thermotoga*, cells possess a "toga". However, they are less extreme thermophilic (Table 11.2.).

11.5 Conclusions: Hyperthermophiles in the History of Life

Although nothing is known yet about the prerequisites and mechanisms which had led to the first living cell, investigations on recent hyperthermophiles yield surprising results, enabling us to draw conclusions about possible features of the common ancestor of life. Within the (16SrRNA-based) universal phylogenetic tree of life, hyperthermophiles form a cluster around the root, occupying all the short and deep phylogenetic branches (Fig. 11.2, bold lines). This is true for both the archaeal and bacterial domains. As a rule, members of the deepest and shortest lineages exhibit the highest growth temperatures ("the shorter and deeper, the hotter"). By their 16SrRNA, these slowly evolving organisms appear to be still closest to the root (common ancestor) and, therefore the most primitive ones still existing. In general, the shortest and deepest lineages exhibit a chemolithoautotrophic mode of nutrition (e.g., *Pyrolobus*, *Pyrodictium*, *Methanopyrus*, *Aquifex*). In an ecological sense, they are primary producers of organic matter (mainly of their own cell material), which gives rise to complex hyperthermophilic microcosms. These chemolithoautotrophs are able to use various mixtures of oxidized and reduced minerals and gasses as energy sources and to assimilate carbon from CO₂. In addition, for growth they just need liquid water, trace minerals and heat. Hydrogen and sulfurous compounds are widely used inorganic energy sources which mainly occur within volcanic environments. Most hyperthermophiles do not require or even tolerate oxygen for growth. They are completely independent of sunlight. In view of a possible similarity to ancestral life, a chemolithoautotrophic hyperthermophilic common ancestor appears probable. In addition, growth conditions of recent hyperthermophiles fit well to our view of the primitive Earth about 3.9 Ga ago when life could have originated: the atmosphere was overall reducing and there was a much stronger volcanism [3]. In addition, Earth's oceans were continuously heated by heavy impacts of meteorites [37]. Therefore, within that scenario, early life had to be heat resistant to survive at all and ancestral hyperthermophiles should even have dominated in the Early Archaean age. Intense impacts, in addition, caused significant material exchange in between planets of our solar system. Based on their ability to survive for long times in the cold, even at -140 °C, hyperthermophiles could successfully have disseminated between the planets: when ejected into space by heavy impacts, insides of rocks in dormant state, they should have been able to pass long distances to other planets (see Chap. 4, Horneck et al.). After landing, they could have flourished on their new home. Since early planets like Mars are assumed to have harbored volcanic and hydrothermal activity [38], colonization by primitive hyperthermophiles appears rather probable. Concerning Mars, its surface at present is too cold and neither liquid water nor active volcanism are apparent. Therefore, hyperthermophiles could not grow there, anymore. However, the discovery of deep geothermally heated subterranean communities several kilometers below Earth's surface [10] makes it imaginable that similar active microcosms of hyper-thermophiles could still exist on Mars deep below its surface, assuming that water, heat and simple nutrients are present. In view of the early impact material exchange scenario between planets, it is uncertain if life had originated on Earth, at all. Therefore, hyperthermophiles could play a key role in search for life on Mars in the future.

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