

**Microorganisms in the Environment –
Unique Adaptations, Basic Metabolism,
and Evolution**

Hyperthermophiles – Life in a Hot and Inorganic Environment

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With 3 Figures

Abstract

Natural hot-water-containing environments harbor complex communities of hyperthermophilic archaea and bacteria (optimal growth: 80–106 °C), representing the upper temperature limit of life (maximal growth temperature: 113 °C). Most hyperthermophiles are anaerobic chemolithoautotrophs, which obtain energy from inorganic redox reactions. In line with the scenario of a hot early Earth, hyperthermophiles cover the deepest and shortest branches within the phylogenetic tree of life. The earliest archaeal phylogenetic lineage is represented by members with extremely tiny cells belonging to the novel kingdom of Nanoarchaeota.

Zusammenfassung

Wasserhaltige heiße Gebiete beinhalten komplexe Gemeinschaften hyperthermophiler Archaeen und Bakterien (optimales Wachstum: 80–106 °C), welche die oberste Temperaturgrenze von Leben repräsentieren (maximale Wachstumstemperatur: 113 °C). Die meisten Hyperthermophilen sind anaerobe Chemolithoautotrophe, die ihre Energie aus anaeroben Redoxreaktionen gewinnen. Alle kürzesten tiefsten Äste im phylogenetischen Baum sind von Hyperthermophilen besetzt, im Einklang mit dem Bild von einer heißen frühen Erde. Die früheste phylogenetische Linie innerhalb der Archaeen wird von den extrem kleinen Mitgliedern des neuen Reiches der Nanoarchaeota repräsentiert.

On Earth, most known life forms are adapted to ambient temperatures within the range of 15–45 °C. Among microorganisms, thermophiles growing optimally between 45 and 70 °C have been known for a long time. During the past decades, novel microbes exhibiting unprecedented optimal growth temperatures in excess of 80 °C have been cultivated. To distinguish them from the usual thermophiles, I designated these newly cultivated microorganisms as hyperthermophiles (STETTER 1992). As a rule, they grow fastest between 80 and 105 °C (upper temperature limit of *Pyrolobus fumarii*: 113 °C) and are unable to propagate at 50 °C or below. For some species of hyperthermophiles, 80 °C is still too low to support growth. Here, I give an overview of the modes of life, biotopes, and phylogeny of hyperthermophiles, and discuss evidence for their probable existence since the dawn of life in the early Archaean age.

Hyperthermophiles are found in natural and artificial environments. On land, volcanic emissions from deep magma chambers heat up soils and surface waters, forming sulfur-

containing solfataric fields and neutral to slightly alkaline hot springs. The surface of solfataric soils is rich in sulfate and exhibits low pH values (pH 0.5–6). Deeper down, solfataric fields are less acidic (pH 5–7) and are anaerobic. As a rule, solfataric fields contain large amounts of elemental sulfur. Deep subterranean, non-volcanic, geothermally heated biotopes were discovered about 3,500 m below the bottom of the North Sea and below the Alaskan North Slope permafrost, where *in situ* temperatures reach approximately 100 °C (STETTER et al. 1993). These Jurassic oil-bearing sandstone and limestone formations harbor hyperthermophilic communities, as indicated by hydrogen sulfide formation (“reservoir souring”) and a mixture of about 10^7 viable cells of various species of hyperthermophiles per liter of extracted fluids. These organisms are very similar to those found in submarine hydrothermal systems. Marine biotopes can be shallow (e.g. at the beach of Vulcano Island, Italy) or deep hot sediments and hydrothermal systems. Most impressive are the deep sea “smoker” vents, where mineral-laden hydrothermal fluids with temperatures of up to 400 °C build huge rock chimneys. Although these hot fluids are sterile, the surrounding rock material with a much lower temperature is teeming with hyperthermophiles (e.g. 10^8 cells of *Methanopyrus* spp. per g of rock at the Mid-Atlantic Snake Pit vent). A further type of submarine high temperature environment is provided by the active seamounts (e.g. Teahicya and Macdonald seamounts, close to Tahiti). When the Macdonald seamount erupted, about 10^4 viable cells of hyperthermophiles per liter were detected within its submarine plume (HUBER et al. 1990).

Volcanic emissions usually contain large amounts of steam, carbon dioxide, and hydrogen sulfide; variable quantities of carbon monoxide, hydrogen, methane, and nitrogen; and traces of ammonia, all of which could serve as energy sources and nutrients for hyperthermophiles. Although unable to grow at the low ambient temperatures, hyperthermophiles are able to survive under these conditions for several years. This is true even for strict anaerobes in the presence of oxygen, as long as they are kept cold in the laboratory.

During the past decades, powerful molecular techniques had been developed to investigate phylogenetic relationships of living organisms. Based on the pioneering work of Carl WOESE, small subunit (ss) rRNA is widely used in phylogenetic studies (WOESE and FOX 1977, WOESE et al. 1990). In bacteria and archaea, ss rRNA consists of about 1500 bases. ss rRNA sequence comparisons have been used to construct a universal phylogenetic tree (Fig. 1). The tree shows a tripartite division of the living world into the bacterial (formerly eubacterial), archaeal (formerly archaebacterial), and eukaryal (formerly eukaryotic) domains. Deep branches within the phylogenetic tree are evidence for early separation. The separation of the bacteria from the stem shared by archaea and eukarya represents the deepest and earliest branching point. Short phylogenetic branches indicate a rather low rate of evolution. In contrast to the eukaryal domain, the bacterial and archaeal domains within the universal phylogenetic tree exhibit some extremely short and deep branches. Surprisingly, these branches are covered exclusively by hyperthermophiles, which therefore form a cluster around the phylogenetic root. 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 hand, mesophilic and moderately thermophilic bacteria and archaea, as a rule, represent long lineages within the phylogenetic tree, and, therefore, had a high rate of evolution (e.g. Gram-positive bacteria, Proteobacteria, *Halobacterium*, and *Methanosarcina*).

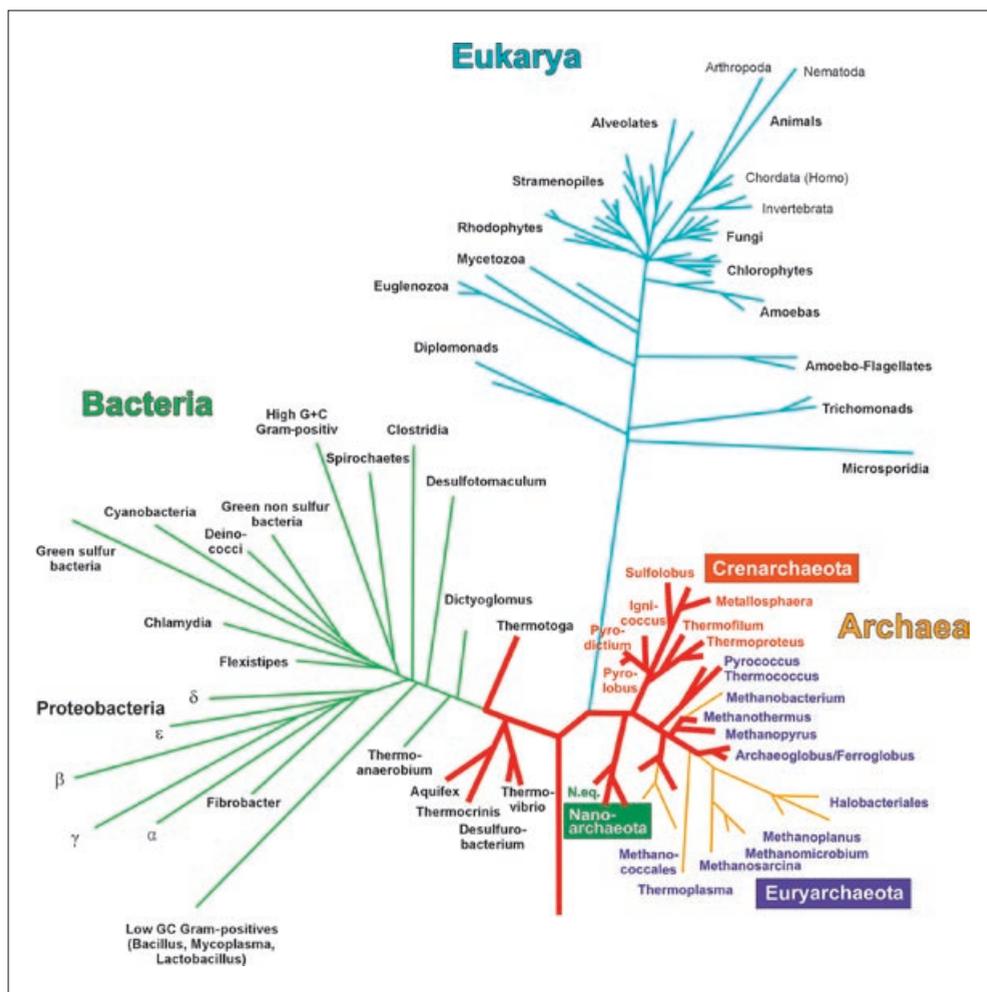


Fig. 1 Phylogenetic tree of life based on the small subunit ribosomal RNA (ss rRNA). Hyperthermophile lineages are represented by thick red lines.

To date, about 70 species of hyperthermophilic bacteria and archaea have been described. These are grouped into 29 genera in 10 taxonomic orders. Hyperthermophiles are well adapted to growing in extremes of temperature, pH, redox potential, and salinity (for a review, see STETTER 2005). The energy sources of hyperthermophiles are very simple. Most species have a chemolithoautotrophic mode of nutrition (Fig. 2). Within their biotopes, they form complex ecosystems that can consist of both primary producers and consumers of organic matter.

Members of the extremely acidophilic genera *Sulfolobus*, *Metallosphaera*, *Acidianus*, and *Stygiolobus* are lobed cocci and are found in acidic, hot, solfataric fields and coal refuse piles. They are aerobic, facultatively aerobic and anaerobic chemolithoautotrophs that gain energy through the use of H_2 , H_2S and S^0 as electron donors and S^0 and O_2 as electron acceptors.

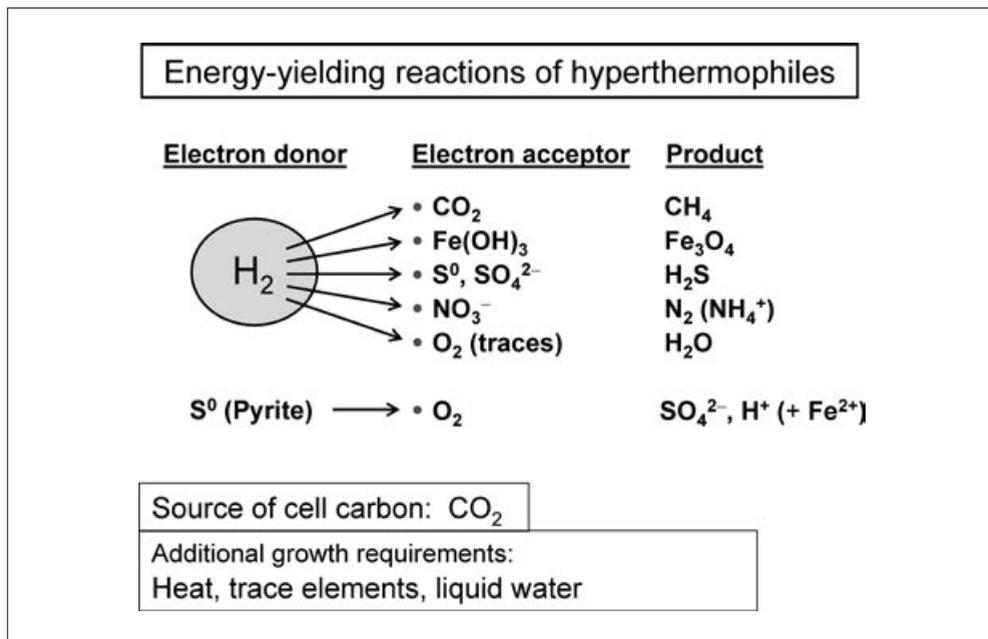


Fig. 2 Main energy-yielding reactions in chemolithoautotrophic hyperthermophiles.

Slightly acidic to alkaline terrestrial hydrothermal systems contain members of the rod-shaped archaeal genera *Thermoproteus*, *Thermofilum*, and *Pyrobaculum* and the coccoid-shaped archaeal genus *Desulfurococcus*; the species of these genera are either chemolithoautotrophs or facultative and obligate heterotrophs. Most of them are anaerobes and usually grow by sulfur respiration.

The highest growth temperatures observed occur among communities of marine hyperthermophiles consisting of members of the bacterial genera *Aquifex* and *Thermotoga* and the archaeal genera *Pyrobaculum*, *Staphylothermus*, *Pyrodictium*, *Thermodiscus*, *Thermococcus*, *Pyrococcus*, *Archaeoglobus*, *Methanopyrus*, and *Methanococcus*. They are found within shallow and deep submarine hydrothermal systems. Species of *Aquifex* are rod-shaped, obligate chemolithoautotrophs and grow at the highest growth temperature known among the bacteria (95 °C). They gain energy by oxidizing H₂ or S⁰ under microoxic conditions. In the absence of O₂, members of *Aquifex* can grow by nitrate reduction.

Within archaea, the organisms with the highest growth temperatures (110–113 °C) are of the genera *Pyrolobus*, *Pyrodictium*, and *Methanopyrus*. *Pyrodictium* is an anaerobic, facultatively chemolithoautotrophic sulfur respirer. It forms disk-shaped cells that connect to one another through unique networks of hollow tubules about 30 nm in diameter. Although resting forms, such as spores, have never been observed, cultures of *Pyrodictium occultum* grown at 110 °C are extraordinarily heat resistant and even survive autoclaving for 1 h at 121 °C. The temperature record of growth is held by *Pyrolobus fumarii*, a deep sea hyperthermophile that grows up to 113 °C (BLÖCHL et al. 1997). *Methanopyrus* is a rod-shaped methanogen that grows optimally at 100 °C, with a population doubling time of 50 min. It contains a primitive geranyl-ether lipid in its membrane; this lipid serves as a lipid precursor in other archaea.

Archaeoglobus spp. are coccoid, facultatively lithoautotrophic, archaeal sulfate reducers. They share several coenzymes with methanogens. In its genome, *Archaeoglobus* has acquired whole sets of genes from bacteria, e.g. those for fatty acid degradation.

A novel group of hyperthermophilic archaea had been completely overlooked until recently. From a submarine hydrothermal system at the Kolbeinsey Ridge, north of Iceland, we were able to isolate *Nanoarchaeum equitans*, which represents a novel kingdom of archaea (HUBER et al. 2002). In ecological studies based on the polymerase chain reaction, its ss rRNA gene was not detected using the commonly used primers that were thought to be universal. With a cell diameter of only 400 nm, *Nanoarchaeum equitans* is the smallest living organism known. Cells grow attached to the surface of a specific crenarchaeal host, a new member of the genus *Ignicoccus* (Fig. 3).

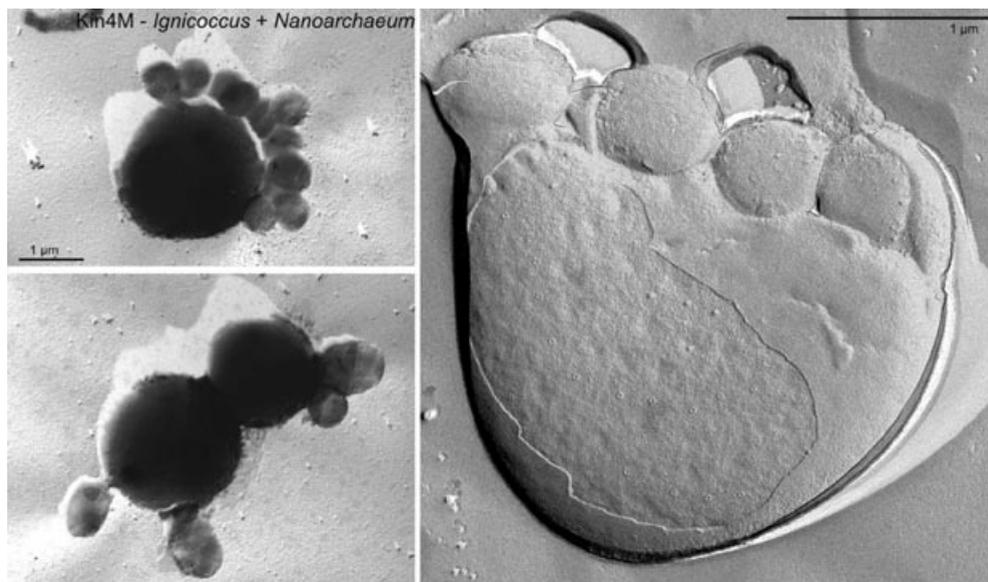


Fig. 3 Transmission electron micrographs of cells of *Nanoarchaeum equitans* (small cells) attached to *Ignicoccus* strain Kin 4M (large). Left: platinum shadowing. Right: freeze etching.

With only 490,885 base pairs, *N. equitans* harbours the smallest microbial genome known to date (WATERS et al. 2003). This genome encodes the complete machinery for information management and repair, but lacks genes for amino acid, nucleotide, lipid, and cofactor biosynthesis. The limited biosynthetic and catabolic capacity of *N. equitans* suggests that its symbiotic relationship to its host may be parasitic. However, unlike the small genomes of bacterial parasites that are undergoing reductive evolution, the small genome of *N. equitans* has a well-equipped DNA recombination system and very few pseudogenes. On the molecular level, *N. equitans* harbors further unexpected, most likely primitive features, such as separately encoded enzyme modules and tRNA gene fragments (WATERS et al. 2003, RANDAU et al. 2005). At present, we are still far from a deeper understanding of the *Nanoarchaeum*–*Ignicoccus* relationship and further investigations are required. The discovery of the *Nanoarchaeota* suggests that other groups of microbes with unusual ss rRNA genes that cannot be recognized by “universal” primers might still be out there, waiting to be discovered.

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