Hyperthermophiles in the history of life

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Today, hyperthermophilic ('superheat-loving') bacteria and archaea are found within high-temperature environments, representing the upper temperature border of life. They grow optimally above 80°C and exhibit an upper temperature border of growth up to 113°C. Members of the genera, *Pyrodictium* and *Pyrolobus*, survive at least 1 h of autoclaving. In their basically anaerobic environments, hyperthermophiles (HT) gain energy by inorganic redox reactions employing compounds like molecular hydrogen, carbon dioxide, sulphur and ferric and ferrous iron. Based on their growth requirements, HT could have existed already on the early Earth about 3.9 Gyr ago. In agreement, within the phylogenetic tree of life, they occupy all the short deep branches closest to the root. The earliest archaeal phylogenetic lineage is represented by the extremely tiny members of the novel kingdom of Nanoarchaeota, which thrive in submarine hot vents. HT are very tough survivors, even in deep-freezing at –140°C. Therefore, during impact ejecta, they could have been successfully transferred to other planets and moons through the coldness of space.

Keywords: archaea; hyperthermophiles; origin of life

1. INTRODUCTION

The first traces of life on Earth date back to the Early Archaean, between 3.5 and 3.9 Gyr ago. Although the recognition of ancient microfossils just by morphology turned out to be very difficult, there are chemical traces of life within the rocks from Precambrian deep-sea vents (Schopf & Packer 1987; Brasier *et al.* 2002; van Zullen *et al.* 2002). These had been the times at the end of the heavy meteorite bombardment. The surface of the early Earth must have been much hotter than today. Expelled by impacts, microbes could have spread in between the planets and moons of the early Solar System. Only superheat-loving microbes similar to the hyperthermophiles (HT; Stetter 1992) would have been able to thrive and survive in such an 'early times of life' scenario. Twenty-five years ago, the first HT had been isolated, which exhibited unprecedented optimal growth temperatures above 80°C, where usual mesophilic and thermophilic bacteria are killed within seconds (Brock 1978; Stetter *et al.* 1981; Zillig *et al.* 1981; Stetter 1982). HT turned out to be very common in hot terrestrial and submarine environments. Their position within the universal phylogenetic tree of life provides further strong evidence of a hyperthermophilic last common ancestor (see subsequently). In my research, I am looking for today's HT, their environments, properties and modes of living. So far, I have been collecting more than 1500 strains of HT. These include isolates that even survive autoclaving at 121°C. The question as to whether such organisms at 121°C are still slowly growing or just surviving is still unanswered (Blöchl *et al.* 1997; Kashefi & Lovley 2003; Cowan 2004).

2. PHYLOGENETIC RELATIONSHIPS OF HT

Based on the pioneering work of Carl Woese, the small subunit ribosomal RNA (ss rRNA) is widely used in phylogenetic studies (Woese & Fox 1977; Woese *et al.* 1990). In Bacteria and Archaea it consists of about 1500 bases. Owing to sequence comparisons, a phylogenetic tree is now available (figure 1). It shows a tripartite division of the living world into the bacterial, archaeal and eukaryal domains. Within this tree, deep branches are evidenced 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 slow rate of evolution. In contrast to the Eukarya, the bacterial and archaeal domains within the phylogenetic tree exhibit some extremely short and deep branches. Surprisingly, these are covered exclusively by HT, which therefore form a cluster around the phylogenetic root (figure 1, thick lineages). The deepest and shortest phylogenetic branches are represented by the Aquificales and Thermotogales within the bacteria and the Nanoarchaeota, Pyrodictiaceae and Methanopyraceae within the Archaea, indicating a slow rate of evolution. Now, several total genome sequences are already available. Within the sequences, as a rule, trees based on genes involved in information management (e.g. DNA replication, transcription, translation) parallel the ss rRNA tree. However, within the genes of metabolism, based on the frequent lateral gene transfer, a network rather than a tree may reflect their phylogenetic relations (Doolittle 1999).

At present, about 90 species of hyperthermophilic archaea and bacteria are known, which had been isolated from different terrestrial and marine thermal areas in the world. HT are very divergent, in terms of both their phylogeny and physiological properties and are grouped into 34 genera and 10 orders.

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Figure 1. Small subunit ribosomal RNA-based phylogenetic tree. The thick lineages represent HT.

Figure 2. Schematic of main energy-yielding reactions in chemolithoautotrophic HT.

3. ENERGY SOURCES AND LIFESTYLE

The energy sources of HT are very simple. Most species exhibit a chemolithoautotrophic mode of nutrition (figure 2). Anaerobic and aerobic types of respiration follow inorganic redox reactions (chemolithotrophic), and CO_2 is the only carbon source required to build-up organic cell material (autotrophic). Therefore, these organisms fix CO_2 by chemosynthesis and are designated chemolithoautotrophs. Molecular hydrogen serves as an

Figure 3. Krafla solfataric field (Iceland), 2 days before a volcanic fissure eruption. A huge fumarole throws up lumps of boiling mud that contained viable HT.

important electron donor. Other electron donors are sulphide, sulphur and ferrous iron. As in mesophilic respiratory organisms, in some HT, oxygen may serve as an electron acceptor. However, in contrast, oxygen-respiring HT are usually microaerophilic and therefore grow only at reduced oxygen concentrations. Anaerobic respiration types are the nitrate, sulphate, sulphur and carbon dioxide respirations. While chemolithoautotrophic HT produce organic matter, there are some HT that depend on organic material as energy and carbon

Figure 4. Antler-shaped cell of *Thermoproteus*, about 100 μm in length and 0.4 μm in diameter. Phase-contrast light micrograph.

Figure 5. Oil wells within the permafrost soil at Prudhoe Bay, North Alaska.

sources. They are designated as heterotrophs. Several chemolithoautotrophic HT are opportunistic heterotrophs. These are able to use organic material alternatively to inorganic nutrients, whenever it is provided by the environment (e.g. by decaying cells). Heterotrophic HT gain energy either by aerobic or different types of anaerobic respiration, using organic material as electron donor or by fermentation.

HT are adapted to distinct environmental factors including composition of minerals and gasses, pH, redox potential, salinity and temperature. Similar to mesophiles, they grow within a temperature range of about 25–30°C between the minimal and maximal growth temperature. Fastest growth is obtained at their optimal growth temperature, which may be up to 106°C. As a rule, HT do not propagate at 50°C or below. HT are very tough survivors. Although unable to grow at ambient temperatures and space temperatures of -140°C , they are able to survive there for many years. Based on their simple growth requirements, HT could grow on any hot water-containing site, even on other planets and moons like Mars and Europa. Today, the surface of Mars is too cold and contains no liquid water and is therefore hostile to life. However, in a depth of a few kilometres below the permafrost layer, there may be hot liquid water and nutrients to support the growth of HT even today. Microbial life could have colonized Mars via meteorites, about 4 Gyr ago. At that

Figure 6. Sampling at the submarine hydrothermal vents at Porto di Levante, Vulcano (Italy). Hot water and gas escape from cracks within hot volcanic rocks.

Figure 7. *Pyrodictium abyssi*, disk-shaped cells within a network of ultrathin tubules. Scanning electron micrograph.

Figure 8. Sampling of hot rocks from a 'Beehive Smoker' at the Mid-Atlantic Ridge (TAG site; 21° N; depth approx. 4000 m).

time, the surface of Mars contained liquid water and perhaps harboured hydrothermal systems, therefore being favourable to HT.

4. BIOTOPES

HT form complex communities within water-containing geothermally and volcanically heated

environments situated mainly along the terrestrial and submarine tectonic spreading and the subduction zones. The availability of liquid water is a fundamental prerequisite of life. At an increased boiling point of water (for example, by elevated atmospheric, hydrostatic or osmotic pressure), several HT exhibit growth temperatures exceeding 100°C. Owing to the presence of reducing gases like H₂S and the low solubility of O₂ at high temperatures, the biotopes of HT are virtually oxygen-free (anaerobic). HT have been isolated from terrestrial and submarine environments.

Natural terrestrial biotopes of HT are mainly hot springs and sulphur-containing solfataric fields, with a wide range of pH values (pH 0–9.0) and usually low salinity (0.1–0.5%). Solfataric fields consist of soils, mud holes and surface waters, heated by volcanic exhalations from magma chambers several kilometres below. Very often, solfataric fields are found close to active volcanoes and the activity is greatly increased during eruption phases (figure 3). Within the boiling mud, under our field microscope, we found up to 10⁷ per gram of antler-shaped giant cells, members of the novel genus *Thermoproteus* (figure 4; Zillig *et al.* 1981). The chemical composition of solfataric fields is very variable and depends on the site. Many of them are rich in iron minerals like ferric hydroxides and pyrite. Less common compounds like magnetite and the arsenic minerals, realgar and auripigment, may be enriched at some sites, for example in Caldera Uzon, Kamchatka. Steam within the solfataric exhalations is mainly responsible for the heat transfer. In addition, they may contain carbon dioxide, variable amounts of H₂, methane, nitrogen, carbon monoxide and traces of nitrate and ammonia. Hydrogen may be formed either pyrolytically from water or chemically from FeS and H₂S (Drobner *et al.* 1990). Other suitable biotopes for HT are deep subterranean, geothermally heated oil stratifications, about 3500 m below the bottom of the North Sea and the surface of the permafrost soil at the North Slope, Northern Alaska (figure 5; Stetter *et al.* 1993). The produced fluids contained up to 10⁷ viable cells per litre of different species of HT such as *Archaeoglobus* and *Pyrococcus*. Artificial biotopes include smouldering coal refuse piles and hot outflows from geothermal power plants.

Submarine biotopes of HT consist of various hydrothermal systems situated at shallow to abyssal depths. Similar to ambient seawater, submarine hydrothermal systems usually contain high concentrations of salt and exhibit a slightly acidic to alkaline pH (5–8.5). The major gasses and life-supporting mineral nutrients may be similar to those within the terrestrial thermal areas. Shallow submarine hydrothermal systems are found in many parts of the world, mainly on beaches with active volcanism. The hot vents at Vulcano, Italy, exhibit temperatures of 80–105°C (figure 6). Within these, I discovered life above 100°C (Stetter 1982): *Pyrodicticum occultum* grows fastest at 105°C and exhibits an upper temperature border of growth at 110°C. Members of *Pyrodicticum* form networks of disk-shaped cells connected by ultra thin tubules (figure 7). The finding of *Pyrodicticum* disproved the prejudice about marine thermophiles being less

Figure 9. *Pyrolobus fumarii*, lobed coccoid cell. Ultrathin section. Transmission electron micrograph. Scale bar, 0.5 µm.

thermophilic than those from terrestrial environments (Castenholz 1979).

Most impressive are the deep sea ‘smoker’ vents, where mineral-laden hydrothermal fluids with temperatures up to about 400°C escape into the cold (2.8°C), surrounding deep seawater 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 HT. Some smoker rocks are teeming with HT. For example, 10⁸ cells of *Methanopyrus* per gram of rock were found in a mid-Atlantic ‘beehive’ hot vent chimney (figure 8). Another type of submarine high-temperature environment is provided by active sea mounts. Close to Tahiti, there is a huge abyssal volcano, Macdonald Seamount (28°58.7′, 140°15.5′ W), the summit of which is situated approximately 40 m below the sea surface. The samples taken during an active phase from the submarine eruption plume and rocks from the active crater contained high concentrations of viable HT (Huber *et al.* 1990).

5. EXAMPLES OF RECENT HYPERTHERMOPHILIC ARCHAEA

From the walls of a black smoker at the Mid-Atlantic Ridge, we had isolated the archaeon, *Pyrolobus fumarii* (Blöchl *et al.* 1997). Cells are lobed cocci of about 0.7–2.5 µm in diameter (figure 9). *Pyrolobus fumarii* is optimally adapted to temperatures of superheated water, exhibiting an optimal growth temperature of 106°C and an upper temperature border of growth at 113°C. It is so dependent on high temperatures that it is unable to grow below 90°C. The cultures of *P. fumarii*, similar to *P. occultum*, are able to survive autoclaving for 1 h at 121°C. Finally, I want to introduce the phenotype and genotype of a member of a novel group of hyperthermophilic virus-sized archaea, which may have already existed at the dawn of life and had been completely overlooked so far. From a submarine hydrothermal system at the Kolbeinsey Ridge, North of Iceland, we were able to obtain our ultimate hyperthermophilic coccoid isolate *Nanoarchaeum*

Figure 10. Cells of *N. equitans* (small) attached to *Ignicoccus* strain Kin 4M (large). (a) Platinum-shadowing. (b) Freeze-etching. Transmission electron micrographs.

equitans, which represents a novel kingdom of Archaea (Huber *et al.* 2002). It is the smallest living organism known with a cell diameter of only 400 nm. Cells grow attached to the surface of a specific crenarchaeal host, a new member of the genus *Ignicoccus* (figure 10). Owing to their unusual ss rRNA sequence, members of *N. equitans* remained undetectable by commonly used 'universal' primers in the ecological studies based on the polymerase chain reaction. *N. equitans* represents the most deeply branching position within the Archaea, suggesting that the Nanoarchaeota diverged early within the Archaea (figure 1). The genome of *N. equitans* harbours some big surprises (Waters *et al.* 2003): with only 490 885 base pairs, it is the smallest microbial genome known to date and also the most compact, with 95% of the DNA predicted to encode proteins or stable RNAs. This genome encodes the complete machinery for information processing and repair, but lacks genes for lipid, cofactor, amino acid and nucleotide biosynthesis. The limited biosynthetic and catabolic capacity of *N. equitans* suggests that its symbiotic relationship to the *Ignicoccus* host may be parasitic, making it the only known archaeal parasite. However, unlike the small genomes of bacterial parasites that are undergoing reductive evolution, the small genome of *N. equitans* has very few pseudogenes and a well equipped DNA recombination system. In contrast to modern parasites, no free-living relatives of *Nanoarchaeum* could be detected so far. Therefore, it may be a very ancient parasite. Alternatively, the *Ignicoccus*–*Nanoarchaeum* system may even represent remains from pre-Archaea communities (Kandler 1994). At its molecular level, *Nanoarchaeum* harbours further unexpected, most probably primitive properties like separately encoded enzyme modules and—for the first time—tRNA gene fragments (Waters *et al.* 2003; Randau *et al.* 2005). At present, we are still far away from a deeper understanding of the *Nanoarchaeum*–*Ignicoccus* relationship and further investigations are

required. The Nanoarchaeota inhabit hot environments worldwide. Two ss rRNA sequences from Uzon Caldeira (Kamchatka, Russia) and Yellowstone National Park (USA) exhibit 83% sequence similarity to *N. equitans* and therefore represent a distinct family within the Nanoarchaeota (Hohn *et al.* 2002). These novel Nanoarchaeota are again tiny cocci about the size of *N. equitans*, however attached to a different (rod-shaped) *Pyrobaculum*-like host. The discovery of Nanoarchaeota suggests that further major groups of microbes may still be unrecognized, and are waiting for their isolation to tell us more about the origin and evolution of life.

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Discussion

M. K. Wallis (Cardiff Centre for Astrobiology, Cardiff University, UK). Your abstract mentions hyperthermophiles are tough survivors, including resistance to deep-freezing, making them potentially transported in impact ejecta and still viable when landing on planets and satellites. They also need the ability to survive space radiation. Do they have a general ability to stabilise their genome in spore states or via binding chemicals such as histones?

K. Stetter. The archaeal hyperthermophiles, *Pyrococcus furiosus* and *Thermococcus stetteri*, have been tested so far about their resistance against radiation. Although unable to form spores (like all archaea), these organisms indeed exhibit a remarkable resistance against γ -radiation (2500 Gy). After radiation, the chromosome of *Pyrococcus furiosus* is fully fragmented, but restored after incubation at 95°C (Kopylov et al. 1993; DiRuggiero et al. 1997).

W. R. Taylor (Mathematical Biology, The National Institute for Medical Research, London, UK). The nanoarchaea bacteria sitting on the outside of the larger bacterium remind me of mitochondria (waiting to be let in).

Is there any similarity between the nanobacteria and mitochondria, either in their energy relationship to their host or in the gene-set found in their genome?

K. Stetter. No similarity between *Nanoarchaeum equitans* and mitochondria is evident. Most likely, very much in contrast to mitochondria, *Nanoarchaeum* obtains its energy from its (*Ignicoccus*) host.

D. McL. Roberts (Zoology Department, The Natural History Museum, London, UK). As you know, there are many lineages of hyperthermophiles known only from environmental nucleic acid extraction. As uncultured organisms, nothing is known of their metabolism. Can you suggest any fundamental reason why hyperthermophiles should be hydrogenotrophs?

K. Stetter. Not all hyperthermophiles are hydrogenotrophs. For example, there are also sulphur and pyrite oxidizers. However, in hyperthermophiles, hydrogen is a very frequent energy source. This may be due to the presence of hydrogen within volcanic exhalations.

A. E. Walsby (School of Biological Sciences, University of Bristol, UK). Karl Stetter has produced evidence, from Woese's phylogenetic tree, that hyperthermophiles are closest to the root. But returning to my previous comment that the earliest cells would be the simplest, do not hyperthermophiles require histone-like proteins to prevent their DNA melting apart and other sophistications to withstand the high temperature? Might this therefore suggest that the hyperthermophiles were not the earliest forms but evolved from earlier forms that lacked these special characters?

K. Stetter. This question goes back to the origin of life ('earliest cells'). So far, there are no proofs about the kind of environments (and their temperatures!) inhabited by the earliest life forms. Nobody knows how the first information carrier may have looked like and how ancient histones are. However, in agreement with the geological settings of the early Earth, there should have been very early adaptations in order to colonize high temperature niches (like hot vents). In surviving the heavy meteorite bombardment, those high temperature organisms could have given rise to the three domains of life, as evidenced by Woese's phylogenetic tree. Therefore, by impact selection the last common ancestor of the present life forms most likely could have been a hyperthermophile. Its adaptation to high temperatures may have occurred much earlier, however.

J. J. McFadden (*Biological Sciences, University of Surrey, UK*). Is the nanoarchaeota a transport parasite? Is the nanoarchaeota infectious?

K. Stetter. These are crucial questions for a deeper understanding of the *Nanoarchaeum-Ignicoccus* system, which are still under investigation. Highly unexpected, there are only very few transporter genes in the *N. equitans* genome. *N. equitans* is infectious to its host, *Ignicoccus* sp. Although very rarely, we were able to reconstitute the system: single cells of two different components; the two single cells were combined and a coculture was obtained by cultivation. We were never able to detect the cells of *N. equitans* inside its *Ignicoccus* host. Therefore, the ways of supply of life-supporting cell components in *N. equitans* are still unknown.