A brief history of the discovery of hyperthermophilic life

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Abstract

Hyperthermophiles, growing optimally at 80°C and above were first discovered in 1981. They represent the upper temperature border of life and are found within water-containing terrestrial and submarine environments of active volcanism and geothermally heated subterranean rocks. The energy-yielding reactions represent mainly anaerobic and aerobic types of respiration rather than fermentation. Within the ss (single-stranded) rRNA phylogenetic tree, hyperthermophiles occupy all of the short deep branches closest to the root. Members of the deepest branch-offs are represented by the newly found Nanoarchaeota and Korarchaeota.

Introduction

In 1980, when Wolfram Zillig and I became interested in thermophilic Archaea, several thermophilic micro-organisms had already been known which grow optimally (fastest) at temperatures up to 75°C [1]. In following Pasteur’s principle, similar to mesophiles, they are all killed by incubation at 100°C. At that time, the organism with the highest growth temperature known was Sulfolobus acidocaldarius [2]. Itthrives aerobically at 75°C within acidic hot mud ponds in Yellowstone National Park. In addition, Tom Brock had already reported on non-culturable rod-shaped microbes (now known as Thermocrinis [3]) growing in boiling (92°C) hot springs of neutral pH in Yellowstone National Park [1]. Sulfolobus had been commonly seen as a highly derived species, a kind of curiosity (mis-classified) among the Pseudomonads. Its aerobic lifestyle with its suggested much higher yield of energy appeared essential to resist thermal destruction [4]. The much lower growth temperatures observed within the anaerobic thermophilic methanogens seemed to confirm this prejudice. In sea water, the high salt concentration was taken as additional stress preventing an extremely hot lifestyle [4]. Therefore the possibility of anaerobic extreme thermophiles within boiling terrestrial and marine environments had never been taken into consideration. At that time, Carl Woese had just published his discovery of the Archaea and his revolutionary concept of a three-domain living world [5]. As Woese had found out, Sulfolobus, in reality, belonged to the Archaea domain. Within this domain, it represented the most deeply branching-off lineage in his dendrogram. The RNA polymerase work with Wolfram Zillig confirmed Woese’s finding [6,7]. Therefore this novel view on universal phylogenetic relationships had convinced me rather early and became essential for my thinking.

Key words: Archaea, Bacteria, cultivation, evolution, phylogeny, thermophile
Abbreviation used: ss, single-stranded
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In the present paper, I give a short overview of the discovery and properties of hyperthermophiles [8], which we were able to isolate during the last 32 years. They grow fastest between 80 and 106°C and represent mainly strictly anaerobic archaea. Some hyperthermophiles grow at 113°C and possibly even higher, and survive autoclaving [9,10].

Discovery and cultivation of the first hyperthermophiles

On our first trip to Iceland in 1980, using field microscopy, Wolfram Zillig and I inspected boiling (98–100°C) springs and mud pools in several areas. Surprisingly, a great deal was teeming with micro-organisms with very unusual morphology like antler-shaped cells with true branchings (later to be named Thermoproteales [11]). When I poured the redox indicator resazurin into such boiling environments, the blue clouds became reduced immediately, indicating that they were anaerobic. I took several samples of boiling water and mud. In order to keep them anaerobic, after adding resazurin, sodium sulfide and sodium dithionite, I enclosed them in storage bottles with tightly fitting stoppers. From a sample taken from a strongly gassed little waterhole in the Kerlingarfjöll mountains, I was able to isolate Methanothermus fervidus, a novel rod-shaped methanogen [12]. For the first time, this organism grew at temperatures of up to 97°C and exhibited its fastest (optimal) growth at 82°C. Therefore, surprisingly, this strictly anaerobic archaean grew at much higher temperatures than the aerobic Sulfolobus acidocaldarius. It became the key organism of my thinking. In addition, from the anaerobic samples taken during this trip, Wolfram Zillig and I were able to isolate the first members of strictly anaerobic Thermoproteales [11]. Similar to Methanothermus, the Thermoproteales exhibited growth temperatures of up to 97°C and were unable to grow at 65°C or below. Therefore the novel isolates exhibited a previously unknown order of extremity of thermophily. Could there
be life even growing at 100°C and above? Of course, life in steam would be impossible owing to the lack of life-supporting components solubilized in liquid water. However, an increase of 1 bar (100 kPa) above atmospheric pressure raises the boiling point of water from 100 to 121°C and therefore keeps water liquid up to 121°C. This pressure corresponds to a water depth of only 10 m, which is easily accessible by scuba diving. In order to hunt for life above 100°C, during my holidays in 1981 at Vulcano Island (Italy), I took anaerobic samples from a submarine solfataric field close to the bay of Porto di Levante with temperatures up to 103°C. From these samples, after 3 months of cultivation experiments, members of the novel strictly anaerobic *Pyrodictium* grew up (Figure 1). For the first time, these organisms were able to grow above 100°C in superheated water with an optimal growth temperature of 105°C and an upper limit at 110°C [13]. On the basis of their volcanism-adapted primitive lifestyle, I raised the hypothesis that similar hyperthermophilic organisms could have existed already at the early Earth, 3.9 billion years ago. At those Hadean times, because of a still very brittle crust and very active volcanism, the sea had been much hotter than today.

**Continued hunting for novel hyperthermophiles**

Based on my new cultivation experience, in order to find more exciting hyperthermophiles, during the last 32 years, I visited high-temperature areas all over the world and isolated high-temperature organisms from there. At present, hot environments such as terrestrial and submarine heated soils, sediments and hot springs are mainly found in areas of active volcanism along tectonic fracture zones and hot spots. I had visited several of these sites including deep sea hot vents with their spectacular ‘black smokers’. In addition, I discovered communities of hyperthermophiles within deep subterranean (non-volcanic) geothermally heated oil-bearing sandstone and limestone with *in situ* temperatures of approximately 100°C some 3500 m below the bottom of the North sea and the surface of the Alaskan North Slope permafrost soil [14]. During that period of time, my laboratory isolated and described approximately 50 new species of hyperthermophiles, among those representatives of the novel bacterial genera *Thermotoga*, *Thermosipho*, *Aquifex*, *Thermocrinis* and the novel archaeal genera *Acidianus*, *Metallosphaera*, *Stygiolobus*, *Thermoproteus*, *Pyrococcus*, *Desulfurocococcus*, *Staphylothermus*, *Thermophila*, *Ignicoccus*, *Thermodiscus*, *Pyrodictium*, *Pyrolobus*, *Thermococcus*, *Pyrococcus*, *Archaeoglobus*, *Ferrolobus*, *Methanothermus*, *Methanopyrus*, *Nanoarchaeum* and ‘*Candidatus* Korarchaeum’.

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**Figure 1** | Disc-shaped cells of *Pyrodictium* within a network of ultrathin tubules
Scanning electron micrograph.
In Woese’s small subunit rRNA-based phylogenetic tree [16], hyperthermophiles exclusively represent all extremely short and deeply branching-off lineages within the Archaea and Bacteria, indicating a low rate of evolution of their ss (single-stranded) rRNAs.

Enrichment and cultivation
Enrichment cultures can be obtained by simulating the varying geochemical and geophysical composition of the environments. Various plausible electron donors and acceptors may be used under anaerobic, microaerophilic or (rarely) aerobic culture conditions. Depending on the (unknown) initial cell concentration and the doubling time of the organism, positive enrichment cultures of hyperthermophiles can be identified by microscopy within 1–7 days. For a deeper understanding of the organisms, the study of pure cultures is required. Owing to the high-incubation temperatures, the traditional way of cloning by plating does not work well. Therefore we developed a new procedure to clone single cells anaerobically under the laser microscope by employing optical tweezers [17,18]. Large cell masses are required for biochemical and biophysical investigations. For mass culturing of hyperthermophiles, in collaboration with an engineering company, a new type of high-temperature fermenter was developed, which became the basis of the University of Regensburg Archaea Center (Figure 2). The steel casing of these fermenters is enamel-protected to resist the highly corrosive culture conditions. Sharp-edged parts such as stirrers, gassing and sampling pipes and condensers are made of titanium. The cell yield of a 300-litre fermentation may vary from approximately 3 g to 2 kg (wet weight), depending on the hyperthermophilic isolate.

Energy sources and lifestyle
Usually, the energy sources of hyperthermophiles can be simple: most species exhibit a chemolithoautotrophic mode of nutrition. Anaerobic and aerobic types of respiration follow inorganic redox reactions (chemolithotrophic), and CO₂ is the only carbon source required to build up organic cell material (autotrophic). Molecular hydrogen serves as an important electron donor. Other electron donors may be sulfide, sulfur and ferrous iron. Whereas chemolithoautotrophic hyperthermophiles produce organic matter, there are some obligate heterotrophic hyperthermophiles which depend on organic material as energy and carbon sources. In addition, several chemolithoautotrophic hyperthermophiles are opportunistic heterotrophs.

Hyperthermophiles are adapted to distinct environmental factors including composition of minerals and gasses, pH, redox potential, salinity and temperature. Similarly to mesophiles, they grow within a temperature range of approximately 25–30 °C (in between the minimal and maximal growth temperature). Reproduction of cells at and even above 110 °C (maximal growth temperature) has been observed in members of Pyrodictium (110 °C), Methanopyrus (110 °C) and Pyrolobus (113 °C) [9,13,19]. A recent new Japanese isolate of Methanopyrus had been reported to grow, slowly, even at 122 °C [10]. Hyperthermophiles grow fastest (optimal) at temperatures between 80 and 106 °C, depending on the strains. At the low-temperature end, as a rule, hyperthermophiles do not propagate at 50 °C or below, some not even below 80 or 90 °C. Although unable to grow at ambient temperatures (and space temperatures of −140 °C, of course!), they are able to survive there for many years. On the basis of their simple growth requirements, hyperthermophiles could grow in any hot-water-containing site, even on other planets and moons.

My ultimate discoveries of hyperthermophiles
Finally, I want to introduce two members of novel groups of hyperthermophiles discovered in my laboratory recently. The first one is a hyperthermophilic virus-sized archaeon. In ecological studies based on PCR, it had been completely overlooked, so far. We isolated this novel organism from a
submarine hydrothermal system at the Kolbeinsey Ridge, north of Iceland. It is coccoid in shape. With a cell diameter of only 0.4 μm, it is among the smallest living organisms known. We named it Nanoarchaeum equitans. Most likely, it represents a novel kingdom of Archaea [20]. Cells of N. equitans grow only attached to the surface of a specific crenarchaeal host, Ignicoccus hospitalis (Figure 3). With 490885 bp, the genome of N. equitans is among the smallest microbial genomes known to date [21]. It 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 its Ignicoccus host is mandatory. The genome of the host organism, I. hospitalis, has also been analysed [22]. Although able to live free of its symbiont, I. hospitalis exhibits the

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*COG (Clusters of Orthologous Groups) functional categories: L, Replication, recombination and repair; D, Cell cycle control, cell division, chromosome partitioning, R, Translation, ribosomal structure and biogenesis, T, Transcription.

†Number of euryarchaeal (Eur) genomes containing that arCOG (of a total of 27).
‡Number of crenarchaeal (Cr) genomes containing that arCOG (of a total of 13).
smallest genome among all free-living Bacteria and Archaea. This organism exhibits a cellular organization unique to Archaea and all other living organisms [23]: two membranes; the outer membrane harbours the energy conservation (sulfur reductase and A1A2 ATP synthase), and the inner membrane comprises the DNA and the ribosomes (Figure 4). At present, we are still far away from a deeper understanding of the Nanoarchaeum–Ignicoccus relationship and further investigations are required.

On the basis of a unique environmental ss rRNA gene sequence within the hot Obsidian Pool in Yellowstone National Park [24], the second novel organism was obtained by a continuous enrichment culture from there. This hyperthermophile represents one of the deepest-branching lineages among the Archaea in the tree of life, tentatively named the Korarchaeota. We were able to physically enrich the Korarchaeota cells to approximately 99.4% purity. They consist of ultrathin rod-shaped cells, 10–100 μm long and only 0.16 μm in diameter, below the resolution of a regular light microscope. Owing to its difficult detection and unusual morphology, we named the first cultivated strain ‘Korarchaeum cryptofilum’ (‘*Candidatus Korarchaeum cryptofilum*’). Under the electron microscope, cells of ‘Ca. Korarchaeum cryptofilum’ can be easily recognized by their tiny cell diameter and their unique S-layer consisting of very tiny subunits (Figure 5). In collaboration with the JGI (Joint Genome Institute), the genome of ‘Ca. Korarchaeum cryptofilum’ was sequenced [25]. With a total of 1 590 757 bp, it is pretty small. Analyses of the genes revealed that the korarchaeal genome harboured an unprecedented combination of genes thought to be characteristic of either the Eurarchaeota or the Crenarchaeota (Table 1). The heterogeneous gene complement suggests that the Korarchaeota may have diverged very early from those two major lineages. Further comparisons may illuminate the early evolution of Archaea and the origins of life.

References


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