HYPERTHERMOPHILES - GEOCHEMICAL AND INDUSTRIAL IMPLICATIONS

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Abstract

Hyperthermophiles, growing optimally between 80 and 110 °C represent life at the upper temperature border. They have been isolated from terrestrial and marine volcanic areas. In the laboratory their temperature maximum of growth is so far between 110 and 113 °C (genera Pyrodictium and Methanopyrus). With the exception of the archaeal thermoacidophilic Sulfolobaceae and the bacterial neutrophilic Aquifex, hyperthermophiles are chemolithotrophic or heterotrophic anaerobes. They represent a reservoir for new (heat-stable) enzymes and biotechnological applications in the future.

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Introduction

Thermophilic microorganisms with upper temperature limits of growth between 50 and 70 °C are found within many natural and some anthropogenic high temperature habitats, like continental or marine geothermal fields, sun-heated environments or industrial cooling systems. These thermophilic organisms are related to mesophiles and can be found within the protozoa, fungi and many bacterial genera. During the last two decades, however, several procaryotes have been isolated from solfataric fields and submarine hydrothermal areas growing fastest between 80 and 110 °C[1,2]. These hyperthermophiles are usually unable to grow below 60 °C, some of them even below 80 °C[3-5]. They gain energy by oxidation of anorganic compounds (e.g. H_2, S°) or organic material. Autotrophic species use CO_2 as single carbon source and are therefore primary producers of organic matter in their biotope. Hyperthermophiles have been found within the Bacteria and the Archaea[6] but so far, no hyperthermophile is closely related to a mesophile. Therefore, they may be considered as still rather primitive descendants of early (thermophilic) live on earth, and they are consequently important for phylogenetic studies. In biotechnology the properties of enzymes or other cell components from hyperthermophiles may be utilized to develop new biocatalytic processes.

Biotopes

Natural habitats of hyperthermophiles are terrestrial and marine volcanic areas, which contain significant amounts of CO_2, H_2, CO, CH_4, H_2S and other sulfur compounds originating from magma chambers below[7]. In solfataric fields the pH of waters, mud holes or soils near the surface is usually acidic (pH 0.5 - 6) and sulfate and traces of ammonia are present[2,8]. In the deeper, oxygen-free areas of the soil (blackish-coloured due to high amounts of ferrous sulfide) a pH between 5 and 7 and temperatures around 100 °C are typical[9]. In some areas, also weakly alkaline springs rich in NaCl occur[2,8]. Terrestrial solfataric fields are found in Italy, Iceland, Indonesia, the United States, and many other countries in the world. Submarine hydrothermal systems exist in the deep sea and in shallow areas. They are slightly acidic to alkaline (pH 5 - 8.5) with high concentrations of NaCl (around 3 %) and sulfate (around 30 mmol/l). Special biotopes are the deep sea vents ("black smokers"), located at the East and South Pacific Rise or the Mid Atlantic Ridge. Temperatures of up to 400 °C have been measured in the emitted mineral rich water and a steep temperature gradient exists in the walls of the chimneys due to the cold surrounding seawater (around 2.8 °C). Furthermore hyperthermophiles have been isolated from anthropogenic habitats like hot outflows of geothermal power plants in Italy or Iceland or from burning coal refuse piles.

These biotopes of hyperthermophiles contain only low amounts of oxygen or are even anaerobic due to the presence of reducing gases and the low solubility of oxygen at higher temperatures. As mentioned above, volcanic gases contain molecular hydrogen, an important energy source for many hyperthermophiles. Recently a new hydrogen source (besides fermentation processes and water hydrolysis) has been discovered[10]. Pyrite and molecular hydrogen are formed from ferrous sulfide and H_2S at 100 °C according to the following equation[10]:

$$FeS + H_2S \dashrightarrow FeS_2 + H_2 \qquad (1)$$

By this reaction hydrogen-dependent hyperthermophiles could be grown due to continous generation of hydrogen in the culture media.

Morphology and Physiology

Aerobic Hyperthermophiles

So far, two groups of aerobic hyperthermophiles have been described (Table I): the extremely acidophilic representatives of the archaeal Sulfolobaceae and the bacterial Aquifex pyrophilus. The members of the Sulfolobaceae (genera Sulfolobus, Metallosphaera, Acidianus, Desulfurolobus, and Stygiolobus) are coccoid to lobed organisms with a cell envelope consisting of protein subunits (Figure 1, 2). With the exception of Acidianus they are adapted to low ionic strength. So far, they have been isolated only from terrestrial solfataric fields and recently from a smoldering refuse pile in a uranium mine in Germany (Isolate Ron 12, Segerer and Stetter, unpublished, Huber and Stetter, unpublished). Only a few strains of Sulfolobus are obligate chemolithoautotrophs, like Sulfolobus metallicus[11]. Most of them are facultative or strict heterotrophs[12]. With the exception of the strictly anaerobic Stygiolobus azoricus[13], elemental sulfur is oxidized to sulfuric acid by all members of the Sulfolobales. Several representatives of the Sulfolobaceae are able to reduce ferric iron or molybdate under anaerobic conditions[14, 15]. In addition some strains of Sulfolobus, Metallosphaera sedula[16], and less efficient strains of Acidianus are able to grow on sulfidic ores like pyrite, chalcopyrite, and sphalerite. Furthermore for the first time within the domain of the archaea, growth by aerobic oxidation of molecular hydrogen has been recently demonstrated for representatives of the genera Sulfolobus, Metallosphaera, and Acidianus[17]. Members of Acidianus and the closely related Desulfurolobus are further able to reduce elemental sulfur with molecular hydrogen under anaerobic conditions[18].

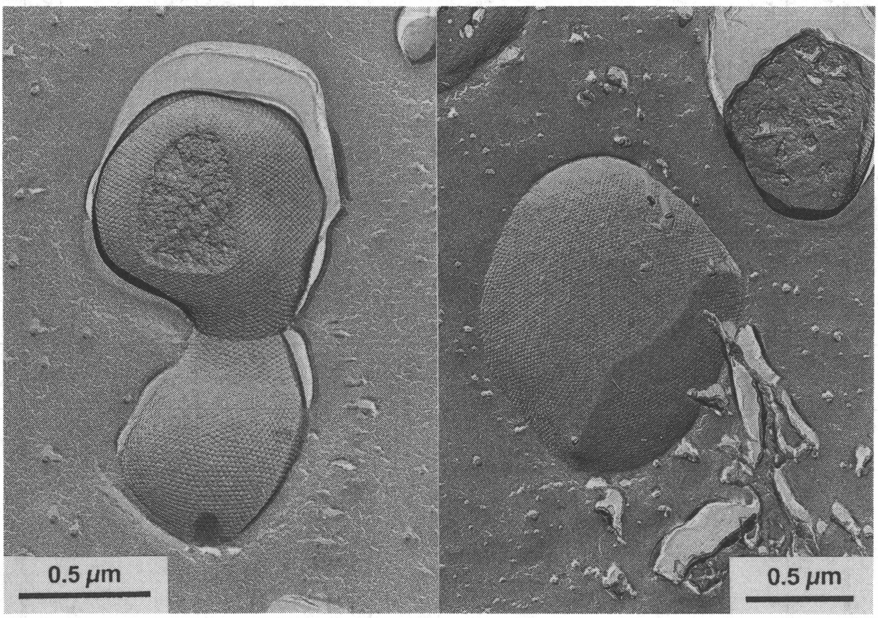


Figure 1, 2: Electron micrographs of freeze-etched[19] cells of Metallosphaera sedula (Fig. 1, left) and isolate Ron 12 (Fig. 2, right), exhibiting a surface layer with hexagonal symmetry.

Table 1: Growth conditions, morphological and biochemical characteristics of hyperthermophiles (modified from Ref.20)

Species	Growth conditions					Habitat	DNA	Morphology
	Temperature (°C)			pH	Aerobic (ae) anaerobic (an)	marine (m) terrestrial (t)	mol% G+C	
	Minimum	Optimum	Maximum					
Aquifex pyrophilus	67	85	95	5.4-7.5	ae/an	m	40	rods, refractile inclusions
Thermotoga maritima	55	80	90	5.5-9	an	m	46	rods with sheath
Thermotoga thermarum*	55	70	84	6-9	an	t	40	rods with sheath
Sulfolobus acidocaldarius	60	80	85	1-5	ae	t	37	lobed cocci
Sulfolobus metallicus*	50	70	75	1-4.5	ae	t	38	lobed cocci
Metallosphaera sedula*	50	75	80	1-4.5	ae	t	45	cocci
Acidianus infernus	60	88	95	1.5-5	ae/an	t	31	lobed cocci
Stygiolobus azoricus	57	80	89	1-5.5	an	t	38	lobed cocci
Thermoproteus tenax	70	88	97	2.5-6	an	t	56	regular rods
Pyrobaculum islandicum	74	100	103	5-7	an	t	46	regular rods
Pyrobaculum organotrophum	78	100	103	5-7	an	t	46	regular rods
Thermofilum pendens	70	88	95	4-6.5	an	t	57	slender regular rods
Desulfurococcus mobilis	70	85	95	4.5-7	an	t	51	cocci
Staphylothermus marinus	65	92	98	4.5-8.5	an	m	35	cocci in aggregates
Pyrodictium occultum	82	105	110	5-7	an	m	62	discs with fibres
Pyrodictium abyssi	80	105	110	4.7-7.5	an	m	60	discs with fibres
Thermodiscus maritimus	75	88	98	5-7	an	m	49	discs
Thermococcus celer	75	87	93	4-7	an	m	57	cocci
Thermococcus litoralis	55	85	98	4-8	an	m	38	cocci
Pyrococcus furiosus	70	100	103	5-9	an	m	38	cocci
Methanothermus sociabilis	65	88	97	5.5-7.5	an	t	33	rods in clusters
Methanopyrus kandleri	84	98	110	5.5-7	an	m	60	rods in chains
Methanococcus jannaschii	50	85	86	3-6.5	an	m	31	irregular cocci
Methanococcus igneus	45	88	91	5-7.5	an	m	31	irregular cocci
Archaeoglobus fulgidus	60	83	95	5.5-7.5	an	m	46	irregular cocci
Archaeoglobus profundus	65	82	90	4.5-7.5	an	m	41	irregular cocci

* = extremely thermophilic microorganism;

Aquifex pyrophilus (Figure 3) has recently been isolated from hot marine sediments at the Kolbeinsey Ridge near Iceland[19]. It represents a novel group of marine hydrogen oxidizers. The highly motile rods exhibit a complex cell wall including a surface layer with hexagonal symmetry (Figure 3). Aquifex is a strict chemolithoautotroph. Low concentrations of oxygen (below 0.5 %) and nitrate are used as electron acceptors to oxidize molecular hydrogen, thiosulfate, and elemental sulfur. Due to its temperature maximum of 95 °C Aquifex pyrophilus is the most extreme bacterial hyperthermophile known so far.

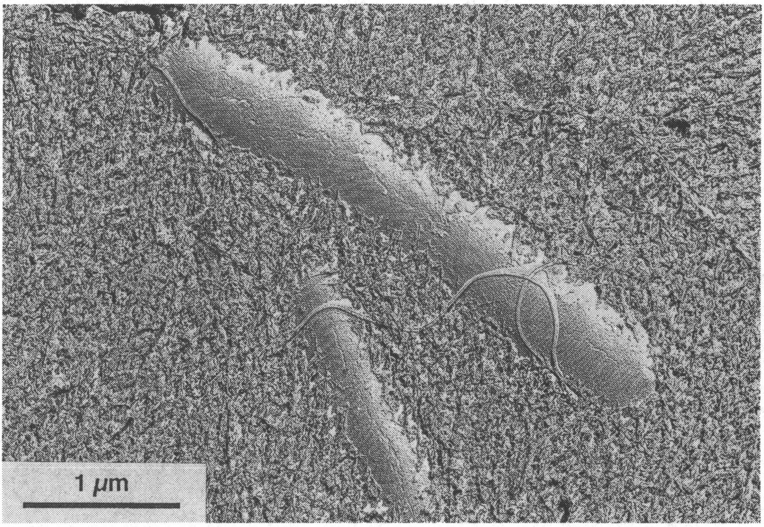


Figure 3: Electron micrograph of freeze-etched cells of Aquifex pyrophilus with flagella.

Anaerobic terrestrial hyperthermophiles

Moderate acidophilic and neutrophilic hyperthermophiles have been isolated from the deeper, oxygen-free layers in the soil of solfataric fields, representing the genera Desulfurococcus, Methanothermus, Pyrobaculum, Thermofilum, Thermoproteus, and Thermotoga[2,21,22,23] (Table 1). As expected, all representatives are strict anaerobes.

So far, the distribution of members of the genus Methanothermus seems to be restricted to some areas in the south west of Iceland. The rod-shaped organisms are obligate chemolithoautotrophs, gaining energy by reduction of CO_2 with molecular hydrogen. Cells of Thermofilum, Thermoproteus, and Pyrobaculum species are stiff rods, only 0.17-0.35 μm (Thermofilum) or 0.5 μm (Thermoproteus and Pyrobaculum) in width (Figure 4). Some species (e.g. Thermoproteus tenax) form spheres at one cell pole ("golf clubs"), possibly a kind of unusual budding. Thermoproteus tenax and Pyrobaculum islandicum are facultative heterotrophs growing either by sulfur respiration on organic matter or molecular hydrogen. Strains of Thermofilum, Pyrobaculum organotrophum, and the coccoid shaped Desulfurococcus are sulfur-respiring obligate heterotrophs. Thermotoga thermarum and T. neapolitana are characterized by a "toga" overballooning the ends of the rod-shaped cells. T. thermarum tolerates only low concentrations of salt and is therefore adapted to terrestrial hot springs, while T. neapolitana is mainly found in seawater.

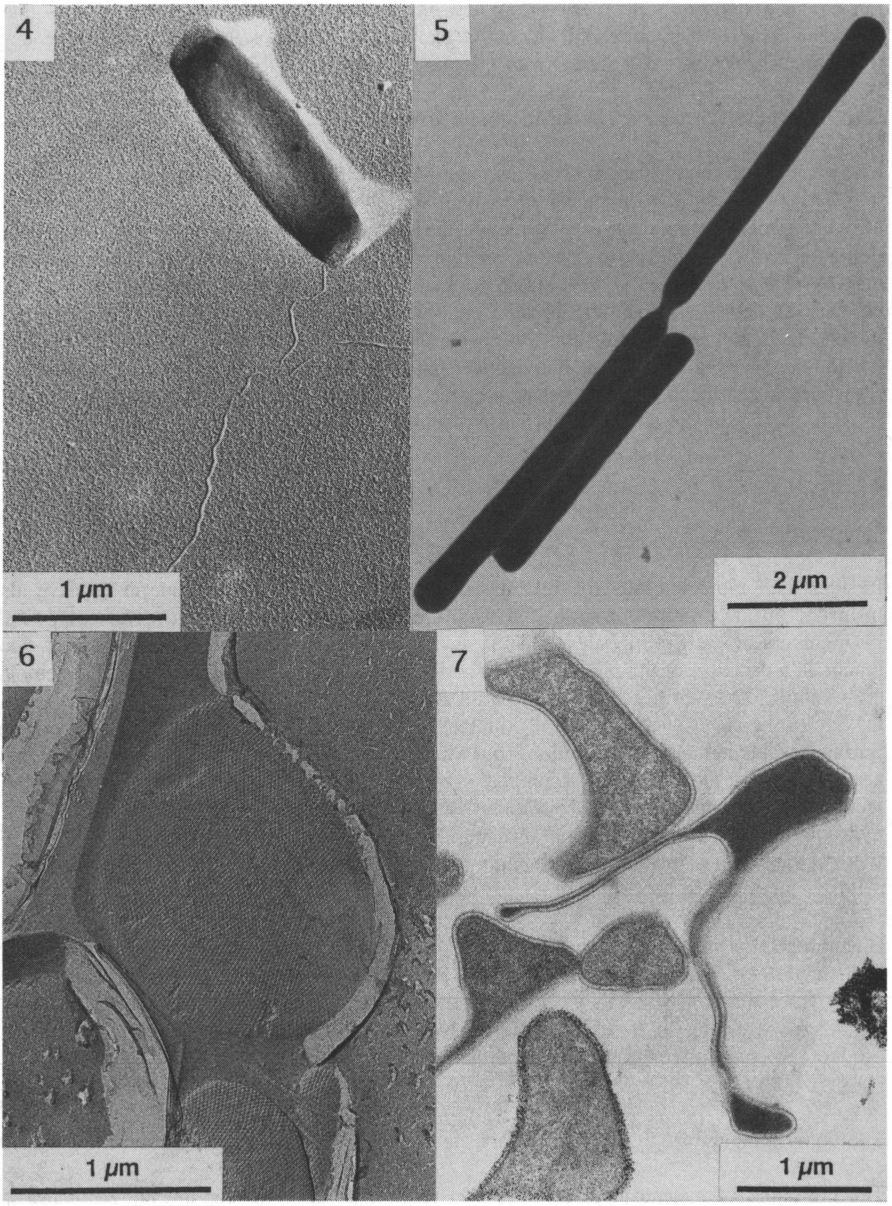
Anaerobic marine hyperthermophiles

Hyperthermophiles represented by the archaeal Archaeoglobus, Hyperthermus, Methanococcus, Methanopyrus, Pyrodictium, Staphylothermus, Thermococcus, Thermodiscus, and the bacterial Thermotogales (Table 1) thrive in hot marine environments. Organisms growing at the upper temperature border of life are found within these groups: the Pyrodictium species P. occultum, P. brockii, P. abyssi, and Methanopyrus kandleri[3, 4, 5]. The temperature maximum of these species is between 110 and 113 °C and no growth occurs at 80 °C or below. Methanopyrus cells occur as long (up to 14 µm) rods (Figure 5). Disk-shaped cells (Figure 6,7) with a cell envelope consisting of protein subunits (Figure 6) are typical for the Pyrodictium species. The cells are entrapped within a network of hollow fibres. P. occultum, P. brockii, and Methanopyrus are chemolithoautotrophs gaining energy by reduction of elemental sulfur (Pyrodictium) or CO_2 (Methanopyrus) with molecular hydrogen. In contrast Pyrodictium abyssi grows by fermentation of proteins, carbohydrates, cell extracts, and acetate. The two hyperthermophilic species Methanococcus jannaschii and Methanococcus igneus[24] are obligate chemolithoautotrophs. They have been isolated from deep and shallow submarine vents, respectively. For optimal growth of M. jannaschii selenium has to be present in the culture medium. Species of Archaeoglobus[25] are able to grow either chemolithotrophically or chemoorganotrophically by sulfate respiration. Similar to methanogens Archaeoglobus cells exhibit a green fluorescence during radiation with 436 nm under the microscope (due to the possession of factor 420) and traces of methane are formed during growth of A. fulgidus. Besides sulfate, thiosulfate or sulfite can be used as electron acceptors. Further marine archaeal hyperthermophiles are represented by members of Staphylothermus, Thermodiscus, Thermococcus, and Pyrococcus. They grow heterotrophically by fermentation of different organic substrates. Cells are coccoid or disc-shaped with diameters between 0.5 and 2 µm. Staphylothermus marinus[26] is able to form giant cells with diameters of up to 15 µm in minimal medium.

Although some strains have been obtained from continental solfataric fields, most representatives of the bacterial order Thermotogales have been isolated from volcanic marine habitats. They use various carbohydrates as energy source and produce L-lactate, acetate, CO_2, and molecular hydrogen[23]. Like Thermococcus, Pyrococcus, and Staphylothermus, the Thermotoga-strains are inhibited by molecular hydrogen. Therefore it has to be removed during cultivation (e.g. by addition of elemental sulfur and formation of H_2S).

Biotechnological implications

Due to their physiology and biochemistry hyperthermophiles are of increasing interest for biotechnological applications. In biohydrometallurgy members of the Sulfolobaceae are investigated for the application in leaching of low-grade ores, in the treatment of refractory gold ores, or in the removal of pyrite from coal. Reactor leaching with optimized strains may become an especially important application for ore concentrates (e.g. zinc, or gold ores), using continous cultures. In contrast to the mesophilic leaching organisms (e.g. Thiobacillus ferrooxidans), they are able to solubilize metals also within a heap or a refuse pile where temperatures of up to 80 °C occur[27] as the result of exothermic oxidation of mineral sulfides. Faster mobilization and higher amounts of extracted metals are the main advantages of the thermophilic leaching organisms[13], although considerable differences in metal extraction of various strains occur. These differences are possibly based on the individual capability of cells to attach to the mineral surface and to grow along the vein in the mineral particles (Huber and Stetter, unpublished).



Electron micrographs of anaerobic archaeal hyperthermophiles.

Figure 4: Pyrobaculum islandicum **Figure 5:** Methanopyrus kandleri

Figure 6: Pyrodictium abyssi (Freeze-edched)

Figure 7: Pyrodictium abyssi (Ultrathin section)

For biochemistry and molecular biology new enzymes for laboratory use have been isolated and some are already commercially available like the DNA polymerase "Pfu" from Pyrococcus furiosus or the "Vent" polymerase from Thermococcus litoralis for Polymerase chain reaction (PCR) experiments. Both enzymes are more heat stable than the "Taq" polymerase and the "Pfu" enzyme includes even proof-reading activity.

Many hyperthermophiles are able to grow heterotrophically on polymers like proteins or starch (e.g. Thermococcus, Pyrococcus, Thermotoga, Sulfolobus). Enzymes with activities at 130 °C have been isolated from these organisms, like the amylase from Pyrococcus woesei[28]. The isolation of these (heat stable) decomposition enzymes is also of industrial interest, and the enzymes may be used in tanneries. A heat stable pullulanase, isolated from Pyrococcus furiosus may be used in bakeries preventing bread from staling. Furthermore, and perhaps even more important for a possible application, it appears, that the thermostability of these enzymes is not their only advantage: there is evidence that these enzymes are characterized by a high resistence to chemicals (e.g. detergents, organic solvents, oxidizing agents) and extreme pH[29]. In addition thermostability prevents the molecules to some extent from enzymic cleavage[29].

Geochemical aspects

Besides their biotechnological implications hyperthermophilic thermoacidophiles have also influence on their natural environment. Due to the oxidation of different sulfur compounds by members of the Sulfolobales, sulfuric acid is produced in aerobic volcanic soils[30]. This results in a decrease of the pH in this area and as a consequence, minerals and trace elements are solubilized into springs and draining waters. A great variety of neutrophilic hyperthermophiles live in the anaerobic areas of the solfataric soils beyond the aerobic acidic surface layer. Sulfate and elemental sulfur are mobilized by reduction to H_2S gas. Therefore soluble metals can precipitate resulting in local accumulation of metal sulfides[3]. Autotrophic hyperthermophilic methanogens produce methane in solfataric areas at temperatures near 100°C and in marine hydrothermal systems methanogenesis occurs up to 110 °C. So far biological methanogenesis seemed to be impossible at these temperatures with consequences for the determination of the origin of methane in deposits.

Chemolithoautotrophic hyperthermophiles are the important producers of primary organic matter in their biotope[5]. At temperatures of up to 110 °C anaerobic representatives thrive independently from sunlight, since they do not require oxygen. Life by all mixo- and heterotrophic organisms, occuring in these volcanic ecosystems depends on growth and the metabolic products of chemolithoautotrophic hyperthermophiles.

The upper temperature border of life

The principles of thermostabilization of hyperthermophilic organisms are under intense investigation. Some strategies are identified like an increased salt concentration of the cytoplasma or proteins induced under thermal stress[31]. Nevertheless many thermostabilizing principles are so far unknown. Since the stability of biomolecules at temperatures above 100 °C decreases rapidly[32, 33], the upper temperature border of life may possibly not exceed 150 °C, and high rates of resynthesis of heat-sensitive molecules are necessary at these temperatures.

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