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Life above the boiling point of water?

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Summary. Various extremely thermophilic archaebacteria exhibit optimum growth at above 80 °C. *Pyrodictium* is the most thermophilic of these organisms, growing at temperatures of up to 110 °C and exhibiting optimum growth at about 105 °C. All of these organisms grow by diverse types of anaerobic and aerobic metabolism.
Key words. Archaebacteria; thermophilic bacteria; *Pyrodictium.*

1. Introduction

For a long time, thermophilic bacteria with temperature optima above 45 °C have been recognized to be widely distributed in soils, self-heated hay, and geothermally heated areas. Most of them show an upper temperature limit of growth between 60 and 80 °C and are members of genera also containing mesophiles, such as *Bacillus* and *Clostridium.* About 15 years ago, bacteria living in the hot springs of Yellowstone National Park were observed[3] and the first extremely thermophilic organism with a temperature maximum at 85 °C was isolated[4]. Since that time, various extremely thermophilic bacteria with temperature optima well above 80 °C were obtained which, as a rule, do not grow at 60 °C or below. *Pyrodictium,* the most extreme thermophilic organism existing in pure culture does not even grow at 82 °C or below[17]. Almost all of these organisms (one exception[10]) belong to the methanogenic and S°-metabolizing archaebacteria[23], the properties of which are reviewed here.

2. Habitats

All the extremely thermophilic, methanogenic and S°-dependent archaebacteria isolated have been found in geothermal areas. Sulfur is formed there by the oxidation of H_2S and by the reaction of H_2S with SO_2. Both of these gases are often present in volcanic exhalations[22]. Liquid water is one important requirement for life[3]. The maximum temperatures for liquid water are pressure-dependent, and in deep-sea hydrothermal areas 2500 m below the surface water temperatures may exceed 300 °C[5]. Terrestrial solfataric springs and mud holes exhibit temperatures of up to 100 °C. They include neutral to weakly alkaline (pH 7–9) springs rich in Cl^- as well as acidic sulfate-rich water- or mudholes[3,19]. The examination of soil profiles within solfatara fields in Iceland, Italy and the Azores showed that these water-containing soils typically consist of two layers which have quite different properties; there is an oxidized, strongly acidic ochre-colored upper layer of about 15–30 cm in thickness over-

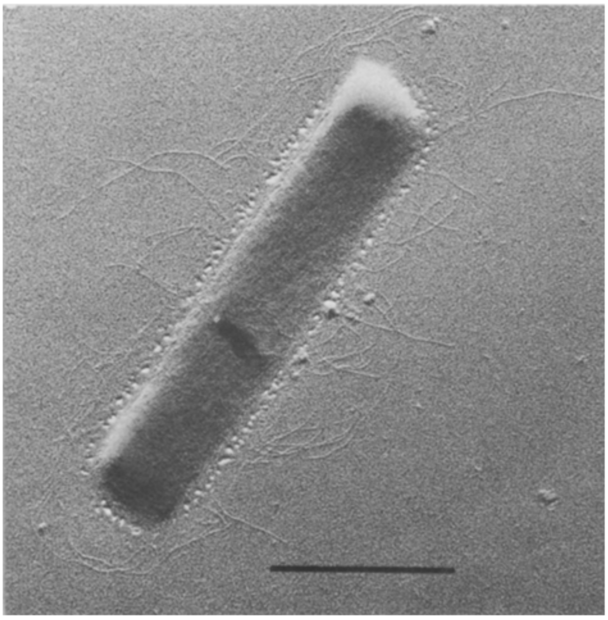

Figure 1. Isolate H 10 grown at 100°C. EM micrograph, Pt-shadowing. Bar 1 µm.

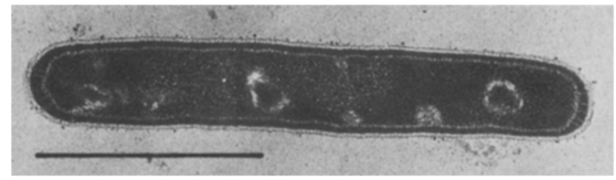

Figure 3. *Methanothermus fervidus*. EM micrograph, thin section. Bar 1 µm.

Table 1. Growth temperatures of extremely thermophilic archaebacteria

Species	Growth temperature Minimal	Optimal	Maximal
Methanothermus sociabilis	60	88	97
Acidianus infernus	60	88	95
Staphylothermus marinus	65	92	98
Sulfolobus acidocaldarius	60	80	90
Pyrococcus furiosus Vc-1	70	100	103
Isolate H 10	75	100	102
Thermodiscus maritimus	75	88	98
Thermofilum librum	70	80	95
Thermoproteus neutrophilus	70	85	97
Thermococcus celer	75	88	97
Pyrodictium occultum	82	105	110

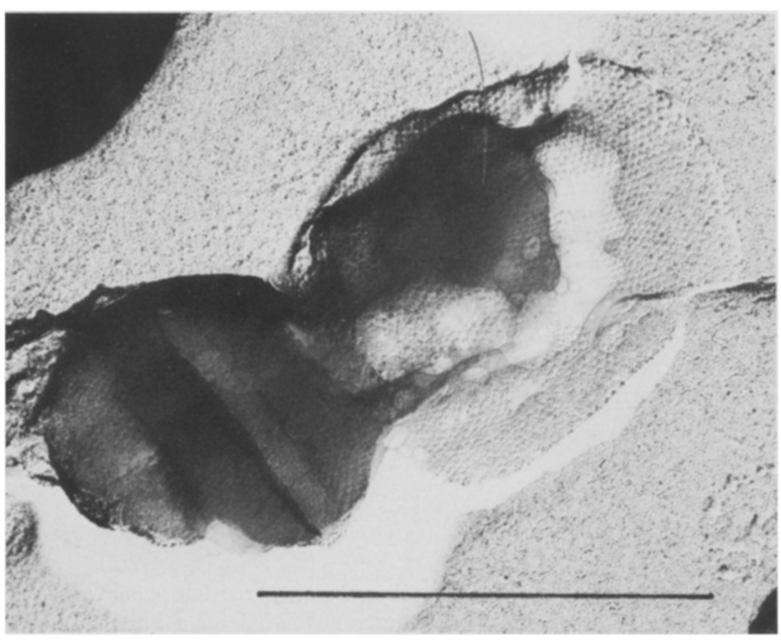

Figure 2. Extremely thermophilic ore-leaching isolate TH2. EM micrograph, Pt-shadowing. Bar 1 µm.

laying a reduced, bluish-black lower zone exhibiting a slightly acidic pH of between 4 and 6.5. In addition to their presence in natural habitats, extremely thermophilic archaebacteria also thrive within boiling outflows of geothermal powerplants in Larderello, Italy, and Krafla, Iceland.

With respect to their growth requirements, e.g. pH, salts, possible substrates and high temperatures, extremely thermophilic archaebacteria appear to be well adapted to their natural environment. They are usually found to proliferate at temperatures between 60 and 98°C (table 1). We obtained isolate 'Geo 3' from the Krafla geothermal power plant. This organism resembles *Thermoproteus* in shape (fig. 1) and metabolism but differs from the latter by its much lower GC-content and its upper growth temperature limit of 102°C. The marine archaebacterium *Pyrococcus furiosus* shows a temperature optimum of growth at 100°C (38 min doubling time[6]). *Pyrodictium* grows at the highest temperatures found for any organism in the laboratory, exhibiting an optimum at 105°C and a maximum of approximately 110°C. Due to its adaptation to the extremely high temperatures of its biotope, this organism is unable to grow at temperatures below 82°C[17].

3. Metabolism

The extremely thermophilic methanogens grow exclusively by formation of methane from H_2 and CO_2, both

Table 2. Energy-yielding reactions of extremely thermophilic archaebacteria

Mode of nutrition	Metabolism	Energy-yielding reaction	Example
Lithoautotrophic	Methanogenesis	$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$	*Methanothermus sociabilis* *Methanothermus fervidus* *Methanococcus jannaschii*
	S/H Autotrophy	$H_2 + S \rightarrow H_2S$	*Pyrodictium occultum* *Thermoproteus neutrophilus* *Thermoproteus tenax** *Acidianus infernus***
	S-oxidation	$2S + 3O_2 + 2H_2O \rightarrow 2H_2SO_4$	*Sulfolobus acidocaldarius** *Acidianus infernus***
	Pyrite oxidation	$4FeS_2 + 15O_2 + 2H_2O \rightarrow 2Fe_2(SO_4)_3 + 2H_2SO_4$	Isolates TH2*; Kra23; VE2
Heterotrophic	S-respiration	Organic [H] + $S \rightarrow H_2S$	*Thermoproteus tenax** *Desulfurococcus mobilis* *Thermofilum pendens*
	Unknown anaerobic	Yeast extract $\rightarrow CO_2$ + ?	*Thermodiscus maritimus*
	Fermentation	Yeast extr. $\rightarrow$ acetate, isovalerate, CO_2 + ?	*Staphylothermus marinus*
	O-respiration	Organic [H] + $O_2 \rightarrow 2H_2O$	*Sulfolobus acidocaldarius**

* facultatively autotrophic. ** facultatively aerobic.

gases present in volcanic exhalations[18]. The sulfur-dependent archaebacteria are able to obtain metabolic energy either by the oxidation or by the anaerobic reduction of elemental sulfur, or require S° for anabolic reactions (table 2). Anaerobic conditions in the volcanic environment are maintained by the escaping gases (e.g. CO_2, SO_2, H_2O, H_2, CO).

The aerobic and facultatively aerobic acidophilic representatives of the genera *Sulfolobus* and *Acidianus* thrive by formation of sulfuric acid either autotrophically or mixotrophically, depending on the isolate[4,14,25]. Some *Sulfolobus* strains can also grow organotrophically[3]. Some recent extremely thermophilic isolates[9] (table 2) are able to grow autotrophically on sulfidic ores, solubilizing heavy metals at temperatures of up to 95°C (fig. 2). *Acidianus infernus* is able to grow anaerobically via the formation of H_2S from H_2 and S° (table 2)[8,14].

The strictly anaerobic *Thermoproteus tenax* can grow autotrophically on H_2 and S° or heterotrophically on yeast extract, carbohydrates and simple organic compounds by means of sulfur respiration[24]. *Pyrodictium occultum* is an obligate S/H autotroph (table 2)[15].

The heterotrophic anaerobic S°-metabolizing archaebacteria consume organic material in the solfataric and hydrothermal areas[19]. Some fermentative organisms are also present in such biotopes, e.g. *Staphylothermus marinus*[7]. Methanogenic bacteria are also very efficient S°-reducers, some of them (e.g. *Methanothermus*) sharing the habitats of S°-metabolizing archaebacteria[13,16].

4. Morphology

The sulfur-metabolizing archaebacteria are variously coccoid, rod- or plate-shaped (table 3). Coccoid and plate-shaped cells are often highly variable in size even within the same culture. The rod-shaped *Thermoproteus* and *Thermofilum* form 'normal' cells of about 1–5 µm in length or filaments more than 100 µm long depending upon growth conditions[19]. Cell division usually takes place by constriction (e.g. *Thermococcus*) or budding (e.g. *Thermoproteus*), but never by septa formation. *Methanothermus* species are gram-positive and show a rigid cell wall composed of pseudomurein (fig. 3). All S°-metabolizing archaebacteria are gram-negative with enve-

Table 3. Morphology of extremely thermophilic archaebacteria

Shape	Genus	Size (µm)	Comments
Rods	*Methanothermus*	0.3–0.5 ∅; 1–3 µm	Gram-positive; pseudomurein covered by S-layer
	Thermoproteus	0.4–0.5 ∅; 1–100 µm	Spheres protruding terminally; true branchings
	Thermofilum	0.15–0.2 ∅; 1–100 µm	Spheres protruding terminally; rarely true branchings
Coccoid	*Sulfolobus*	0.8–2 ∅; irregular	–
	Acidianus	Aerobic: 1–1.5 ∅; anaerobic: 0.5–1 ∅; irregular	–
	Desulfurococcus	0.5–1 ∅	*D. mobilis* is flagellated
	Thermococcus	1 ∅	Tuft of flagella
	Staphylothermus	0.5–1 ∅	Grows in aggregates. Growth of giant cells (10 µm ∅) in the presence of 0.2% yeast extract
	Pyrodictium	Plates: 0.2 thick; 0.3–2.5 ∅ Filaments: 0.04–0.08 ∅; up to 40 long	Plate- to dish-shaped cells; network formed; grows like a mold
	Thermodiscus	0.2 thick; 0.3–3 ∅	Plate- to dish-shaped

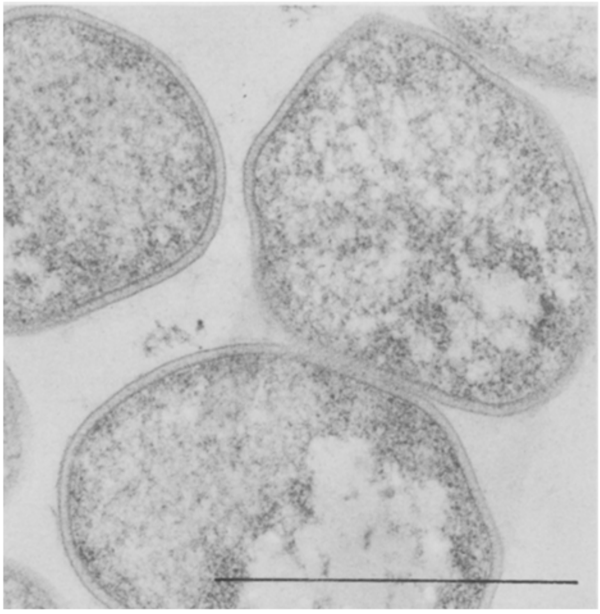

Figure 4. *Acidianus infernus*. EM micrograph, thin section. Bar 1 μm.

lopes composed of protein subunits which cover their cytoplasmic membranes (fig. 4)[11, 12]. Some coccoid (fig. 5) and rod-shaped isolates (fig. 1) are motile by means of flagella. *Pyrodictium* forms pellicles consisting of networks of fibers 0.04 to 0.08 μm in diameter[17], which entrap the cells during exponential growth (fig. 6).

5. Prerequisites and limits of extremely thermophilic life

Since some extremely thermophilic bacteria grow even in super-heated water, the question whether there is a general upper temperature limit for life arises. It depends primarily on the thermostability of cell components. The S°-metabolizing archaebacteria, which are the most thermophilic organisms known, are able to grow within a range of temperatures spanning approximately 30 °C (table 1). This relatively narrow range may be due to the intrinsic properties of the cell material, e.g. the fluidity of the membranes and the optimal conformation of enzymes and nucleic acids. Possibly on account of this phenomenon, extremely thermophilic S°-metabolizers do not grow at temperatures below 60–82 °C, depending on the isolate (table 1). On the other hand, they are able to survive for years at low temperatures[17]. The molecular stabilization mechanisms enabling growth at very high temperatures of up to 110 °C are still unknown.

At temperatures of the order of 100 °C even some low molecular weight compounds such as ATP and NAD hydrolize quite rapidly (half life below 30 min in vitro; Stetter, unpublished) and some thermolabile amino acids, e.g. cystein and, less markedly, glutamic acid, are decomposed[2]. The survival of organisms growing at these temperatures may be ensured by successful re-synthesis of sensitive compounds. This suggestion is in line with the observations that (a) maximal and optimal growth temperatures of *Staphylothermus marinus* are about 7 °C lower in minimal medium than in full medium[7] and (b) that *Pyrodictium* is rapidly killed at 110 °C in the absence of substrate (Stetter, unpublished).

Under 'black smoker' conditions (e.g. 250 °C; 26 MPa) existing within hydrothermal deep-sea vents[5], macromolecules and simple organic molecules, e.g. amino acids, are highly unstable (e.g. DNA: half life 20 μs in vitro)[2, 21]. Even the 'heat-stable' proteins of *Pyrodictium* are rapidly decomposed under such extreme conditions[2]. Despite an early report of bacterial growth at 250 °C[1] life

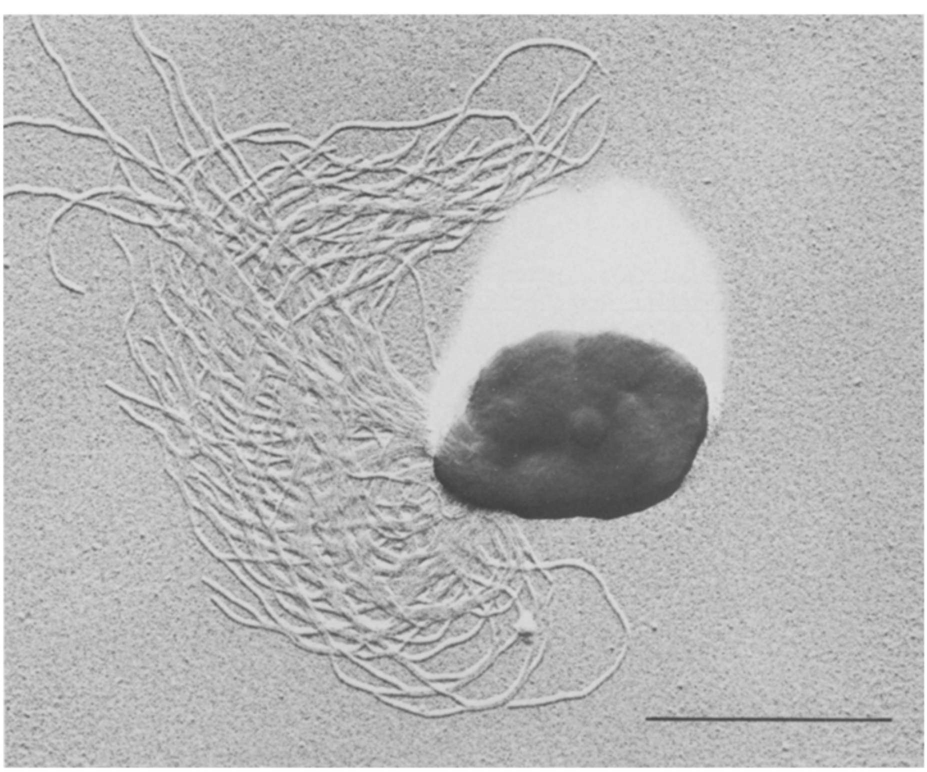

Figure 5. *Pyrococcus furiosus*. EM micrograph, Pt-shadowing. Bar 1 μm.

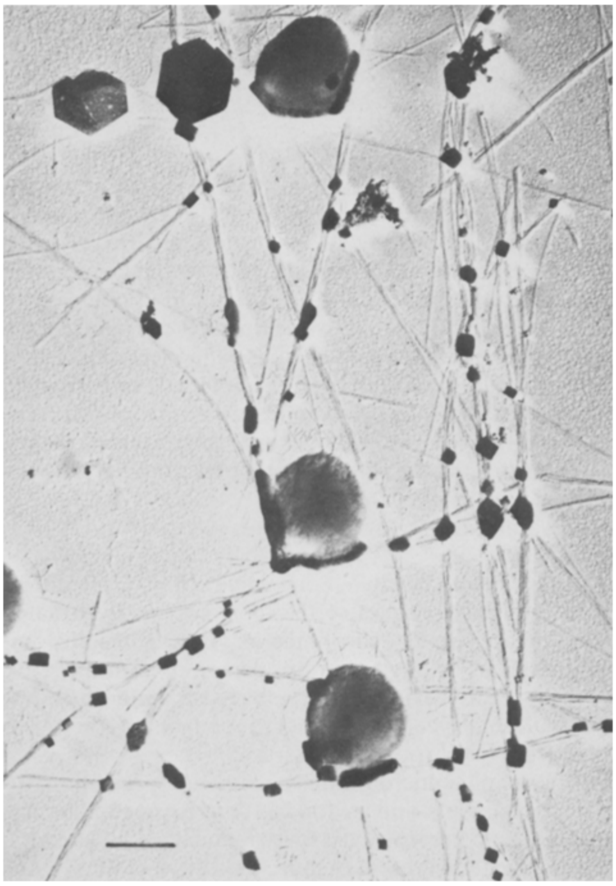

Figure 6. *Pyrodictium occultum*. EM micrograph, Pt-shadowing. Bar 1 μm.

under these conditions does not seem possible[20,21]. Although the upper temperature limit for life is still unclear, it is probably much lower than 250 °C, possibly in the range between 110 and 150 °C, at which heat-sensitive molecules could be successfully resynthesized.

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