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Hyperthermophilic microorganisms

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1. INTRODUCTION

The most extremely thermophilic living beings known to date are bacteria growing at temperatures between 80 and 110 °C [1-3]. Some of them are so well adapted to the high temperatures that they do not even grow below 80 °C [4]. As a rule, non of these hyperthermophilic bacteria are able to grow at 60 °C or below. Hyperthermophiles are occurring mainly within the archaebacterial kingdom [5]; some of them are also present within the eubacteria [6,7]. Due to their existence within phylogenetically highly divergent groups, the lack of closely related mesophiles, and their biotopes existing already since the Archean age, hyperthermophiles may have adapted to the hot environment already billions of years ago.

2. BIOTOPES

The hyperthermophilic bacteria known at present have been isolated from submarine hydrothermal areas and from continental solfataras. The surface of the solfataras is usually rich in sulfate and exhibits an acidic pH (0.5 to 6; [1,2]). In the depth, solfataras are less acidic or even neutral (pH 5 to 7; [8]). Sometimes, solfataric fields may also contain some weakly alkaline hot springs (pH 7 to 9) rich in NaCl. Also man-made hot environments such as the boiling outflows of geothermal power plants are suitable environments for hyperthermophiles [9]. Submarine hydrothermal systems are slightly acidic to alkaline (pH 5 to 8.5) and normally contain high amounts of NaCl and SO_4^{2-} due to the presence of sea water (Table 1). Due to the low solubility of oxygen at high temperatures and the presence of reducing gases, most biotopes of hyperthermophiles are anaerobic. Within continental solfataric fields, oxygen is only present within the upper acidic layer which appears ochre-coloured due to the existence of ferric iron [8].

Although not growing below 60 °C, hyperthermophiles are able to survive at low temperatures at least for years. Some of the anaerobic hyperthermophiles are able to tolerate oxygen much better at low (non-growth) temperatures than at high (growth) temperatures. This property may be essential for dispersal of these organisms through oxygen-rich low-temperature areas.

3. TAXONOMY OF HYPERTHERMOPHILIC BACTERIA

Up to now, about 35 different species of hyperthermophilic bacteria are known. They belong to different taxa within the eu- and archaebacteria (Table 2). Within the eubacteria, hyperthermophiles are within the Thermotogales order, which is the deepest phylogenetic branch-off based on 16S rRNA sequences within this kingdom [7]. The phylogenetic tree within the archaebacterial king-

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dom exhibits two main branches: the branch containing methanogens and halophiles and the branch of the sulfur metabolizing archaebacteria [7]. The latter consists almost exclusively of hyperthermophiles [3,7] while there are only a few groups of hyperthermophiles within the other branch (Archaeoglobales, Thermococcales, Methanothermaceae, *Mc. jannaschii*). Within the sulfur metabolizers, acidophilic (e.g. *Sulfolobus, Metallosphaera, Acidianus*) and neutrophilic (e.g. *Pyrodictium, Desulfurococcus, Staphylothermus, Thermococcus*) genera were discovered during the last years. Therefore, the old designation "Thermoacidophiles" which was primarily used to characterize the genera *Sulfolobus* and *Thermoplasma* [1] is not suitable anymore for the designation of this branch.

3.1. Extremely acidophilic hyperthermophiles

Extremely acidophilic hyperthermophiles were found up to now exclusively within continental solfataric fields. The organisms are coccoid-shaped strict and facultative aerobes. They are extreme acidophiles due to their requirement of acidic pH (opt. approx. pH 3). Phylogenetically, they belong to the archaebacterial genera *Sulfolobus, Metallosphaera, Acidianus*, and *Desulfurolobus*. The less extremely thermophilic archaebacterium *Thermoplasma* is an extreme acidophile, too. It is a facultative anaerobe occurring both in smoldering coal refuse piles and continental solfataric fields [1,10].

Members of the genus *Sulfolobus* are strict aerobes growing autotrophically by oxidation of S^0 and S^{2-}, forming sulfuric acid (Table 3, 4). Many *Sulfolobus* isolates are facultative heterotrophs and a few, still unnamed, are even strict heterotrophs (Stetter, unpublished). The type strain of *Sulfolobus acidocaldarius* (DSM 639) shows excellent growth on organic matter [11]. Autotrophically cultured on S^{2-} and S^0, however, this strain shows only extremely weak growth and sulfuric acid formation [12–14]. Some members of the Sulfolobales like *Metallosphaera sedula* are able to oxidize sulfidic ores like pyrite, chalcopyrite and sphalerite, forming sulfuric acid and solubilizing the heavy metal ions (Table 3,4; [15]). Under microaerophilic conditions, *Sulfolobus* is able to reduce ferric iron and molybdate, which therefore act as electron acceptors under these conditions [16,17]. Members of the genus *Sulfolobus* grow only at very low ionic strength and were therefore not found within the marine environment ([11]; Segerer and Stetter, unpublished). Members of the genus *Acidianus* similar to *Sulfolobus* are able to grow by oxidation of S^0, forming sulfuric acid [18,19]. Some strains grow also on sulfidic ores, but much less efficient than *Metallosphaera* (Table 4; [14]). In addition, members of *Acidianus* are able to grow by anaerobic oxidation of H_2, using S^0 as electron donor (Table 4; [18]). A thermoacidophilic isolate, which had been tentatively named "*Sulfolobus ambivalens*" [20] was later described as *Desulfurolobus ambivalens*, representing a new genus [21]. By DNA : DNA hybridization, however, *Desulfurolobus ambivalens* shows a close relationship to the

Table 1

Biotopes of hyperthermophilic bacteria

Characteristics	Type of thermal area	
	Solfataric fields	Submarine hydrothermal systems
Sites	Steam-heated mud holes, soils and surface waters; deep hot springs; geothermal power plants	Hot sediments and vents
Temperatures	Up to 100 °C	Up to about 374 °C
pH	0.5 to 9	5 to 8.5
Presence of O_2	Surface oxic; depth anoxic	Anoxic
Major gases and sulfur compounds	CO_2, CO, CH_4, SO_4^{2-}, NH_4^+	H_2, H_2S, S^0, SO_3^{2-}

Table 2

Taxonomy of hyperthermophilic archaebacteria

Order	Genus	Species
I. Eubacterial kingdom		
Thermotogales	*Thermotoga*	*T. maritima*
		T. neapolitana
		T. thermarum
	Thermosipho [a]	*T. africanus*
	Fervidobacterium [a]	*F. nodosum*
		F. islandicum
II. Archaebacterial kingdom		
Sulfolobales	*Sulfolobus*	*S. acidocaldarius*
		S. solfataricus
	Metallosphaera	*M. sedula*
	Acidianus	*A. infernus*
		A. brierleyi [a]
	Desulfurolobus	*D. ambivalens*
Thermoproteales	*Thermoproteus*	*T. tenax*
		T. neutrophilus
	Pyrobaculum	*P. islandicum*
		P. organotrophum
	Thermofilum	*T. pendens*
		T. librum
	Desulfurococcus	*D. mobilis*
		D. mucosus
		D. saccharovorans
	Staphylothermus	*S. marinus*
Pyrodictiales	*Pyrodictium*	*P. occultum*
		P. brockii
		P. abyssum
	Thermodiscus	*T. maritimus*
Thermococcales	*Thermococcus*	*T. celer*
		T. stetteri
	Pyrococcus	*P. furiosus*
		P. woesii
Archaeoglobales	*Archaeoglobus*	*A. fulgidus*
		A. profundus
Thermoplasmales [a]	*Thermoplasma*	*T. acidophilum*
		T. volcanium
Methanobacteriales [b]	*Methanothermus*	*M. fervidus*
		M. sociabilis
Methanococcales	*Methanococcus* [b]	*M. thermolithotrophicus* [a]
		M. jannaschii

[a] Growth only up to about 70 °C, mentioned due to their close relationship to hyperthermophiles

[b] Contain also mesophilic species.

type species of *Acidianus*, *Acidianus infernus* [22]. Members of the genus *Acidianus* are able to grow in the presence of up to 4% salt. In agreement with this result, they also have been isolated (rarely) from a marine hydrothermal system [19].

3.2. Slightly acidophilic and neutrophilic hyperthermophiles

Slightly acidophilic and neutrophilic hyperthermophiles were found both in continental solfataric fields and in submarine hydrothermal systems and they show specific adaptations to their environments. All of them are strict anaerobes. Solfataric fields contain members of the genera *Thermoproteus, Pyrobaculum, Thermofilum, Desulfurococcus*, and *Methanothermus* (Table 3; [9,23–26]). Cells of members of the genera *Thermoproteus, Pyrobaculum*, and *Thermofilum* are characteristically stiff rods with protruding spheres ("golf clubs") mainly at their ends, possibly due to a mode of budding. Cells of *Thermofilum* are only about 0.17 to 0.35 μm in width and are therefore different from *Thermoproteus* and *Pyrobaculum. Thermoproteus tenax, Thermoproteus neutrophilus* and *Pyrobaculum islandicum* are able to grow autotrophically by anaerobic reduction of S^0 with H_2 as electron donor [9,27]. Strains of *Thermofilum* and *Pyrobaculum organotrophum* are obligate heterotrophs. They are growing by sulfur respiration, using different organic substrates (Table 4). *Thermofilum pendens* shows an obligate requirement for a lipid fraction of *Thermoproteus tenax. Thermoproteus tenax* and *Pyrobaculum islandicum* are also able to grow heterotrophically by sulfur respiration (Table 4). Also in solfataric fields coccoid heterotrophic organisms occur which gain energy by respiring organic material using sulfur as electron acceptor. They belong to the genus *Desulfurococcus* [25]. From solfataras in the southwest of Iceland, rod-shaped methanogens growing at temperatures up to 97 °C (Table 3) were isolated [26,28]. Two species are known: *Methanothermus fervidus* and *Methanothermus sociabilis*, cells of the latter growing in clusters up to 3 mm. Both species are strict autotrophs, gaining energy by reduction of CO_2 by H_2 (Table 4). Within neutral continental hot springs in Djibouti, Africa, the extremely thermophilic eubacterial species *Thermotoga thermarum* was found, which grows only at low ionic strength (Table 3,4; [29]). Members of this group are strictly anaerobic heterotrophs, growing by fermentation of carbohydrates. Most of them are found within the marine biotopes, however.

Many hyperthermophiles are adapted to the

Table 3

Growth conditions and morphological and biochemical features of hyperthermophiles

Species	Growth conditions						DNA (mol% G+C)	Morphology
	Temperature (°C)			pH	Aerobic (ae) anaerobic (an)	Habitat (marine (m)/ solfataric (s))		
	Minimum	Optimum	Maximum					
Sulfolobus acidocaldarius	60	80	85	1 –5	ae	s	37	Lobed cocci
Metallosphaera sedula	50	75	80	1 –4.5	ae	s	45	Cocci
Acidianus infernus	60	88	95	1.5–5	ae/an	s	31	Lobed cocci
Thermoplasma volcanium	33	60	67	1 –4	ae/an	s	38	Irregular cocci
Thermoproteus tenax	70	88	97	2.5–6	an	s	56	Rods with terminally protruding spheres
Pyrobaculum islandicum	74	100	103	5 –7	an	s	46	Rods with terminally protruding spheres
Pyrobaculum organotrophum	78	100	102	5 –7	an	s	46	Rods with terminally protruding spheres
Thermofilum pendens	70	88	95	4 –6.5	an	s	57	Slender rods with terminal spheres
Desulfurococcus mobilis	70	85	95	4.5–7	an	s	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 abyssum	80	100	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	97	4 –7	an	m	57	Cocci
Pyrococcus furiosus	70	100	103	5 –9	an	m	38	Cocci
Archaeoglobus fulgidus	60	83	95	5.5–7.5	an	m	46	Irregular cocci
Methanothermus sociabilis	65	88	97	5.5–7.5	an	s	33	Rods in clusters
Methanococcus jannaschii	50	85	86	5.5–6.5	an	m	31	Irregular cocci
Thermotoga maritima	55	80	90	5.5–9	an	m	46	Rods with sheath
Thermotoga thermarum	55	70	84	6 –9	an	s	40	Rods with sheath

marine thermal environments (Table 3). They are represented by the genera *Staphylothermus, Pyrodictium, Thermodiscus, Thermococcus, Pyrococcus, Archaeoglobus*, and by members of the genera *Methanococcus* and *Thermotoga* (Table 3; [3,4,6,30–34]). The organisms with the highest growth temperatures known are members of the genus *Pyrodictium*, growing up to 110°C [4,35]. Cells of *Pyrodictium* are so well-adapted to high temperatures that they do not even grow below 80°C (Table 3). Cultures of *Pyrodictium* grow in flocs. Cells are disc-shaped and are connected by a network of very thin hollow fibres. *Pyrodictium occultum* and *Pyrodictium brockii* are able to grow autotrophically, gaining energy by reduction of S^0 by H_2. Alternatively, these species are able to grow mixotrophically on H_2 and thiosulfate in the presence of cell extracts (Table 4). In contrast, *Pyrodictium abyssum* is an obligate heterotroph, gaining its energy from an up to now unknown type of fermentation. Elemental sulfur stimulates growth, but is not essential (Table 4). A group of coccoid hyperthermophilic sulfate-reducing archaebacteria is represented by *Archaeglobus fulgidus* [33,36]. It is a facultative autotroph. During autotrophic growth, *Archaeoglobus fulgidus* gains its energy by reduction of thiosulfate by H_2 (Table 4). Only very little growth is obtained when thiosulfate is replaced by sulfate under autotrophic conditions. During heterotrophic growth,

Table 4

Metabolism of hyperthermophiles

Species	Autotrophic growth [a]	Substrates	Electron acceptors	End products
Sulfolobus acidocaldarius	f	S^0; H_2S; cell extracts; sugars	O_2	H_2SO_4; ?
Metallosphaera sedula	f	S^0; sulfidic ores; cell extracts	O_2	H_2SO_4; ?
Acidianus infernus	o	S^0; sulfidic ores; H_2	O_2; S^0	H_2SO_4; H_2S
Thermoplasma volcanium	–	Yeast extract; cell extracts	O_2; S^0	Acetate, CO_2; H_2S; ?
Thermoproteus tenax	f	H_2; yeast extract; cell extracts	S^0; $S_2O_3^{2-}$; SO_3^{2-}	H_2S; ?
Pyrobaculum islandicum	f	H_2; yeast extract; cell extracts	S^0; $S_2O_3^{2-}$; SO_3^{2-}; cystine	H_2S; ?
Pyrobaculum organotrophum	–	Yeast extract; cell extracts	S^0; $S_2O_3^{2-}$; SO_3^{2-}; cystine	H_2S; ?
Thermofilum pendens	–	Yeast extract; tryptone; +lipid fraction	S^0	H_2S; ?
Desulfurococcus mobilis	–	Yeast extract; tryptone; casein	S^0	H_2S; ?
Staphylothermus marinus	–	Yeast extract; peptone; meat extract	S^0	H_2S; acetate; isovalerate
Pyrodictium occultum	f [b]	H_2 (H_2 + cell extract)	S^0; ($S_2O_3^{2-}$)	H_2S
Pyrodictium abyssum	–	Yeast extract; gelatine; starch; formate	Fermentative? (S^0)	?
Thermodiscus maritimus	–	Yeast extract; (cell extracts + H_2)	S^0; unknown respiration?	H_2S; ?
Thermococcus celer	–	Tryptone; yeast extract; casein	S^0; fermentation?	H_2S; ?
Pyrococcus furiosus	–	Yeast extract; casein; starch; maltose	S^0; fermentation?	H_2S; H_2; CO_2; ?
Archaeoglobus fulgidus	f [c]	H_2; formate; lactate; sugars; proteins	SO_4^{2-}; $S_2O_3^{2-}$; SO_3^{2-}	H_2S; traces of CH_4
Methanothermus sociabilis	o	H_2	CO_2	CH_4
Methanococcus jannaschii	o	H_2	CO_2	CH_4
Thermotoga maritima	–	Yeast extract; sugars; starch; cellulose	Fermentative	L-Lactate; acetate; H_2; CO_2
Thermotoga thermarum	–	Yeast extract; (yeast extract + carbohydrates)	Fermentative	n.d.

[a] o = obligately autotrophic; f = facultatively autotrophic; – = heterotrophic.
[b] Autotrophic growth only with S^0 as electron acceptor.
[c] Autotrophic growth only with $S_2O_3^{2-}$ as electron acceptor.
n.d. = not determined.

various substrates like lactate, formate, glucose, starch and proteins can be used. As electron acceptors, sulfate, thiosulfate, and sulfite can be used heterotrophically. S^0 is inhibitory during autotrophic and heterotrophic growth. In addition, traces of methane (up to 0.1 μmol/ml) are formed via an unknown pathway [36]. Similar to the methanogens, cells of *Archaeoglobus* show a blue-green fluorescence at 420 nm due to the possession of factor 420 [36]. Due to its unique type of RNA polymerase and its 16S rRNA sequence, *Archaeoglobus* forms a separate phylogenetic branch situated between the thermophilic sulfur metabolizers and the methanogens [33,36,37]. A further autotrophic marine hyperthermophile is represented by *Methanococcus jannaschii*, which had been isolated up to now only from submarine deep sea vents (34). *Methanococcus jannaschii* is a strictly autotrophic methanogen growing at temperatures up to 86 ° C (Table 3, 4).

The marine thermal environment contains also a variety of strictly heterotrophic hyperthermophiles. Members of the genus *Staphylothermus* are obligate sulfur respirers. Cells of *Staphylothermus marinus* are coccoid and grow in grape-like aggregates and form giant cells under special nutritional conditions [30]. Cells of isolates of the genus *Thermodiscus* are disc-shaped. In contrast to *Pyrodictium*, they do not form fibres, grow only up to 98 ° C and show a much lower GC-content of their DNA (Table 3, 4). Growth occurs by sulfur respiration on yeast- and cell extracts and is stimulated by H_2. Good growth is also obtained without sulfur possibly due to an unknown respiration of fermentation. The genera *Thermococcus* and *Pyrococcus* are belonging to the Thermococcales, which represent up to now the deepest branch-off within the phylogenetic archaebacterial tree [7,31,32,38]. The cells are coccoid shaped (Table 3). *Thermococcus celer* utilizes tryptone, yeast extract and protein as carbon sources. Growth is stimulated by sucrose. In closed culture vessels, *Thermococcus celer* grows optimally in the presence of sulfur and about 1.5 mol of H_2S are formed per mole of CO_2 [31]. *Thermococcus* can also grow without sulfur. Under optimal conditions, the generation time of *Thermococcus celer* is close to 50 min. *Pyrococcus furiosus* grows at temperatures up to 103° C and shows a much lower GC content than *Thermococcus celer* (Table 3). At 100 ° C, the doubling time is only 37 min [32]. Cells of *Pyrococcus furiosus* are highly motile due to monopolar polytrichous flagellation, They grow on yeast extract, peptone, casein, starch and maltose and possess a powerful protease and amylase. As metabolic products in the absence of sulfur CO_2 and H_2 were found, the latter being inhibitory to growth. Hydrogen inhibition can be prevented by the addition of S^0, whereupon H_2S is formed in addition. The mode of fermentation of *Pyrococcus* and *Thermococcus* is still unclear. Many submarine hydrothermal fields contain also members of the eubacterial genus *Thermotoga* which are thriving together with hyperthermophilic archaebacteria in the same environment. *Thermotoga maritima* and *Thermotoga neapolitana* are fermentative hyperthermophiles growing at temperatures up to 90 ° C (Table 3; [6,39]). They are using various carbohydrates as energy source. As end products, L-lactate, acetate, H_2 and CO_2 are formed (Table 4). Hydrogen is inhibitory to growth and has to be removed during cultivation. In the presence of S^0, H_2S is formed and H_2 inhibition is overcome [6].

4. CONCLUSIONS

The isolation of various groups of hyperthermophilic bacteria from geothermally and hydrothermally heated environments demonstrates an unexpected complexity of these up to now almost unexplored ecosystems. Within these, primary production and consumption of organic matter is going on at temperatures around 100 ° C. The energy-yielding reactions of primary production is based on reduction or oxidation of inorganic sulfur compounds by H_2 or O_2, or in the case of methanogens on reduction of CO_2 by H_2. Since H_2, CO_2 and S^0 are formed within the environment, anaerobic autotrophs using these compounds are completely independent of sunlight. The consumers of organic matter are most likely using cell components of the decaying primary producers. Most of them are growing by sulfur respiration or by unknown types of respiration and fermentation. A great deal of the autotrophs are 'opportunistic' heterotrophs, too. This property may be important for effective competition within the extreme environment.

The upper temperature border of life is still unknown. The existence of *Pyrodictium* and other hyperthermophiles demonstrates that still unrecognized thermostabilizing principles must exist. Since stability of biomolecules at temperatures above 100 ° C decreases rapidly [40–42], the maximal growth temperature at which microbial life can exist may be possibly found between 110 and 150 ° C. Within this temperature range, heat-sensitive biomolecules could possibly still be resynthesized at biologically feasible rates. Beyond the interesting questions in basic research, hyperthermophiles may be well suited for the development of novel biotechnological processes due to their novel metabolic properties and the outstanding heat resistance of their cell components.

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