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Extremely Thermophilic Sulfur-Metabolizing Archaebacteria[*]

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Summary

Various extremely thermophilic coccoid, rod- or plate-shaped S_0-metabolizing 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 proliferate on the basis of diverse types of anaerobic and aerobic lithoautotrophy and heterotrophy.

Key words: Thermophilic – Archaebacteria – Volcanism – Sulfur Metabolism

1. Introduction

Two main subdivisions of the archaebacterial kingdom (*Fox* et al., 1980) are evident based on 16S rRNA: one comprising the strictly anaerobic methanogens and the aerobic extreme halophiles and the other the thermophilic, aerobic and anaerobic, sulfur metabolizers (*Woese* et al., 1984). The moderately thermophilic *Thermoplasma acidophilum* is a further minor distinct branch of the archaebacteria, and may occupy an intermediate position between the two major groups (*Fox* et al., 1980; *Stetter* and *Zillig,* 1985). The only extremely thermophilic representatives of the methanogenic-halophilic branch are the genus *Methanothermus* (*Stetter* et al., 1981) and the species *Methanococcus jannaschii* (*Jones* et al., 1983). The branch of S_0-metabolizing archaebacteria consists of thermophiles and almost exclusively of extreme thermophiles. They characteristically use elemental sulfur in their metabolism, but some are able to grow alternatively without S_0 and are therefore not strictly S_0-dependent. Their pH requirements range from acidophilic to neutrophilic. This paper reviews the properties of the previously described extremely thermophilic S_0-metabolizing archaebacteria, and presents information on new isolates.

2. Habitats

All S_0-dependent archaebacteria known at present have been isolated from geothermal areas abundant in elemental sulfur. This substance is formed 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 (*Williams* and *McBirney,* 1979). Liquid water is one important requirement for life (*Brock,* 1978). 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 (*Corliss* et al., 1979). Terrestrial solfataric springs and mud holes exhibit temperatures of up to 100 °C, depending upon their height above sea level. These solfataric fields sometimes contain neutral to weakly alkaline (pH 7–9) springs rich in Cl^-. But usually solfataric springs, water- or mudholes are mildly (pH 6 to 4) or strongly (pH 3–0.5) acidic and rich in sulfate (*Brock,* 1978; *Stetter* and *Zillig,* 1985). 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 having quite different properties: an oxidized, strongly acidic ochre-colored upper layer of about 15 to 30 cm in thickness is overlaying a reduced, bluish-black lower zone exhibiting a slightly acidic pH of between 4 and 6.5. In addition to their presence in these natural habitats, extremely thermophilic archaebacteria also thrive within manmade habitats, such as the 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 S_0-metabolizers appear to be well adapted to their natural environment. They are usually found to proliferate within a temperature range of between 60 and

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98 °C and exhibit a growth optimum at 80 to 90 °C, depending on the isolate. We obtained isolate "Geo 3" from the Krafla geothermal power plant, an organism which resembles Thermoproteus in shape and metabolism but differs from the latter by its much lower GC-content and its upper growth temperature limit of 102 °C. *Pyrodictium* grows at the highest tempertures of 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 (*Stetter* et al., 1983).

3. Metabolism

The sulfur-dependent archaebacteria are either able to obtain metabolic energy from the oxidation or reduction of elemental sulfur, or require S_o for anabolic reactions (Table 1). Most of the anaerobic representatives of this type of archaebacteria require extremely low redox potentials (e. g. –300 mV) for growth. These potentials are maintained in the volcanic environments by the escaping gases (e. g. CO_2, SO_2, H_2O, H_2, CO).

Table 2. Autotrophic and heterotrophic growth of *Sulfolobus* spec. and some new isolates

Substrate Strains	ore mixture[a] G1 (3.3%)	S_o (0.3%)	yeast extract (0.1%)	sucrose (0.1%)
S. brierleyi DSM 1651[b]	–	–	+	+
S. acidocaldarius DSM 639	–	–	+	n. d.
S. solfataricus DSM 1616	–	–	+	n. d.
Isolate TH2	+	+	+	–
Isolate Kra 23	+	+	–	–
Isolate VE2	+	–	–	–

[a] = pyrite, chalcopyrite, sphalerite and pitchblende
[b] = moderately thermophilic
n. d. = not determined

The aerobic and facultatively aerobic acidophilic representatives of the genera *Sulfolobus* and *"Acidothermus"* thrive in the upper oxidized layer of solfatara fields and within solfataric mudholes, where they form sulfuric acid either autotrophically or mixotrophically, depending on the isolate (*Brock* et al., 1972; *Zillig* et al., 1980; *Segerer* et al., 1985). A moderately thermophilic strain of *Sulfolobus* (*Brierley* and *Brierley*, 1973; *Brierley*, 1978) and some recent *Sulfolobus*-shaped, extremely thermophilic isolates (Table 1, 2) are able to grow autotrophically on sulfidic ores, solubilizing heavy metals. Some strains of *Sulfolobus* and the new isolates cited above also grow heterotrophically on yeast extract and sugars (*Brock* et al., 1972; *Brock*, 1978; Table 1, 2). *"Acidothermus infernus"* and similar isolates are able to grow anaerobically via the formation of H_2S from H_2 and S_o (Table 1; *Segerer* et al., 1985; *Zillig* et al., 1985).

The strictly anaerobic lithoautotrophic *Thermoproteus* species are found in the reduced soils as well as within the springs and mudholes of the solfatara fields (*Fischer* et al., 1983).

Thermoproteus tenax can also grow heterotrophically on yeast extract, carbohydrates and simple organic compounds by means of sulfur respiration (*Zillig* et al., 1981). *Pyrodictium occultum* is an obligate S/H autotroph found

Table 1. Energy-yielding reactions of sulfur-metabolizing archaebacteria

Mode of nutrition	Metabolism	Energy-yielding reaction	Example
lithoautotrophic	S/H autotrophy	$H_2+S \rightarrow H_2S$	*Pyrodictium occultum* *Thermoproteus neutrophilus* *Thermoproteus tenax*[a] *"Acidothermus infernus"*
	S-oxidation	$2S+3O_2+2H_2O \rightarrow 2H_2SO_4$	*Sulfolobus acidocaldarius*[a] *"Acidothermus infernus"*[b]
	Pyrite oxidation	$4FeS_2+15O_2+2H_2O \rightarrow 2Fe_2(SO_4)_3+2H_2SO_4$	*"Sulfolobus"-isolates* TH2[a]; Kra23; VE2
heterotrophic	S-respiration	"organic" [H]$+S \rightarrow H_2S$	*Thermoproteus tenax*[a] *Desulfurococcus mobilis* *Thermofilum pendens* *Thermococcus celer*
	unknown anaerobic respiration	yeast extract $\rightarrow CO_2+?$	*"Thermodiscus maritimus"*[c]
	Fermentation	yeast extract$\rightarrow$?	*"Staphylothermus marinus"*[c]
	O-respiration	"organic" [H]$+O_2 \rightarrow 2H_2O$	*Sulfolobus acidocaldarius*[a] *"Sulfolobus"-isolate TH2*[a]

[a] = facultatively autotrophic
[b] = facultatively aerobic
[c] = S_o strictly required in addition

in the shallow, geothermally heated sea floor area close to Vulcano, Italy (Table 1; *Stetter,*1982).

The heterotrophic anaerobic S_o-metabolizing archaebacteria consume organic material in the solfataric and hydrothermal areas (*Stetter* and *Zillig,* 1985). Most of these organisms respire anaerobically, using S_o or undefined organic components as hydrogen acceptors and forming CO_2 as a metabolic by-product. Some fermentative organisms are also present in such biotopes, e.g. *Staphylothermus marinus,* which in the laboratory converts undefined components of yeast extract into CO_2, acetate and isovalerate (*Fiala* and *Stetter,* unpublished).

Methanogenic bacteria are also very efficient S_o-reducers, some of them (e.g. *Methanothermus*) sharing the habitats of S-metabolizing archaebacteria (*Stetter* and *Gaag,* 1983). However, it is still not clear whether methanogens are able to obtain metabolic energy from the S_o-reduction.

4. Morphology

The sulfur-metabolizing archaebacteria are variously rod-, coccoid- or plate-shaped (Table 3). Coccoid- and plate-shaped cells are often highly variable in size even within the same culture. *Staphylothermus* grows in aggregates of up to 100 individuals. In the presence of 0.2% yeast extract, the cultures consist exclusively of "giant" cells about 7 to 15 µm in diameter. The rod-shaped *Thermoproteus* and *Thermofilum* form "normal" cells of about 1 to 5µm in length or filaments more than 100 µm long depending upon growth conditions (*Stetter* and *Zillig,* 1985). Cell division usually takes place by constriction (e.g. *Thermococcus; Zillig* et al., 1983) or budding (e.g. *Thermoproteus; Zillig* et al., 1981), but never by septa formation. All S-metabolizing archaebacteria are Gram-negative with envelopes composed of protein subunits which cover their cytoplasmic membranes (*König* and *Stetter,* 1986; *Kandler* and *König,* 1985). *Desulfurococcus mobilis* (*Zillig* et al., 1982 a), *Thermococcus celer* (*Zillig* et al., 1983) and some *Thermoproteus*-like isolates are motile due to flagella. *Pyrodictium* forms pellicles consisting of networks of fibres 0.04 to 0.08 µm in diameter, which are composed of subunits in helical array (*Stetter* et al., 1983) and which are entrapping the cells during exponential growth.

5. Prerequisites and Limits of Extremely Thermophilic Life

The upper temperature limit at which growth can take place depends primarily on the thermostability of cell components. The S_o-metabolizing archaebacteria, which are the most thermophilic organisms known, are able to grow within a range of temperature spanning approximately 30 °C (Table 4), a range similar to that within which mesophilic bacteria grow (e.g. *Lactobacillus bavaricus:* 2–39 °C). This relatively narrow growth temperature 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_o-metabolizers do not grow at temperatures below 60 to 82 °C, depending on the isolate (Table 4). On the other hand, they are able to survive for years at low temperatures (e.g. *Stetter* et al., 1983). The molecular stabilization mechanisms enabling growth at very high temperatures of up to 110 °C are still unknown. Few enzymes from extremely thermophilic S_o-metabolizers have been studied; however β-Galactosidase from *Sulfolobus* (*Buonocore* et al., 1980) and the RNA polymerases from *Sulfolobus, Thermoproteus, Desulfurococcus* and *Thermococcus* (*Zillig* et al., 1982 b) are thermostable *in vitro* at temperatures comparable to those allowing growth. Another unexplored area is the stability of the nucleic acids, e.g. the maintenance of the double helical structure, at the very high growth temperatures. Some stabilization could be obtained by basic DNA binding proteins (*Thomm* et al., 1982; *Green* et al., 1983) or by introduction of positive superhelical strains into DNA by a reverse gyrase (*Kikuchi* and *Asai,* 1984). The thermostability of DNA could also be improved by an increased GC-content (*Marmur* and *Doty,* 1962), but no correlation has been

Table 3. Morphology of S_o-metabolizing archaebacteria

Shape	Genus	Size (µm)	Comments
rods	*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
	Sulfolobus	0.8–2 ∅; irregular	–
	"Acidothermus"	aerobic: 1–1.5 ∅; anaerobic: 0.5–1 ∅; irregular	–
	Desulfurococcus	0.–1 ∅	D. mobilis is flagellated
coccoid	*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

Table 4. GC-contents and growth temperatures of extremely thermophilic S_0-metabolizing archaebacteria

Species	GC-content (mol%) of DNA	Growth temperature		
		minimal	optimal	maximal
"Acidothermus infernus"	31	60	88	95
"Staphylothermus marinus"	35	65	92	98
Sulfolobus acidocaldarius	37	60	80	90
"Pyrococcus furiosus" Vc-1	38	70	100	103
"Thermoproteus" sp. Geo 3	46	75	100	102
"Thermodiscus maritimus"	49	75	88	98
Desulfurococcus mobilis	51	70	85	95
Thermofilum "librum"	56	70	80	95
Thermoproteus neutrophilus	56	70	85	97
Thermococcus celer	57	75	88	97
Pyrodictium occultum	62	82	105	110

found between the GC-content of DNA and the growth temperature of extremely thermophilic S_0-metabolizers (Table 4). Clearly, other still unknown thermostabilizing principles must exist.

At temperatures in the order of 100 °C even some low molecular weight compounds such as ATP and NAD hydrolyze 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 (*Bernhardt* et al., 1984). The survival of organisms growing at these temperatures may be ensured by rapid resynthesis of these 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 (*Fiala* and *Stetter,* unpublished) 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 (*Corliss* et al., 1979), macromolecules and simple organic molecules, e. g. amino acids, are highly unstable (e. g. DNA: half life 20 µsec *in vitro: White,* 1984; *Berhardt* et al., 1984). Even the "heat-stable" proteins of *Pyrodictium* are rapidly decomposed under such extreme conditions (*Bernhardt* et al., 1984). Despite an early report of bacterial growth at 250 °C (*Baross* and *Deming,* 1983) life under these conditions does not seem possible (*Trent* et al., 1984; *White,* 1984). Although the upper limit of temperature at which life can exist is still unclear, it should be much lower than 250 °C and will possibly be found to be between 110 and 150 °C, at which heat-sensitive molecules could be successfully resynthesized at biologically feasible rates. Whether or not organisms exist above the present upper temperature limit for life has yet to be determined.

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