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AEROBIC AND ANAEROBIC EXTREMELY THERMOPHILIC AUTOTROPHS

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1. INTRODUCTION

During the last years, various extremely thermophilic bacteria with optical growth temperatures above 80°C have been isolated from geo- and hydrothermally heated areas [14, 15, 17]. All of these organisms belong to the archaebacteria, the third kingdom of life [19]. The mode of nutrition is lithoautotrophic or organotrophic, depending on the isolates. In this paper, lithoautotrophic extreme thermophiles including some very recent isolates are described which are the primary producers of organic matter at high temperatures.

2.BIOTOPES

Up till now, all extremely thermophilic autotrophic bacteria have been isolated from terrestrial solfataric fields and submarine hydrothermal systems. Both biotopes are situated usually above magma chambers about 400 to 2000 m within the floor [10]. From there, steam consisting of water and volcanic gasses, mainly CO_2, H_2, SO_2 and H_2S, escape and are heat the soil and surface water. The examination of soil profiles within solfataric fields showed that these soils consist of two layers with different properties: On the top, there is an oxygen-containing strongly acidic layer of about 15 to 30 cm in thickness, which shows an ochre colour due to the presence of ferric iron. Below, there is an oxygen-free, slightly acidic to neutral bluish-black zone, which contains ferrous sulfide. Submarine hydrothermal systems contain seawater of neutral to slightly acidic pH which remains liquid even above 100°C due to the prevention of boiling by the hydrostatic pressure. Geothermal as well as hydrothermal areas are abundant in elemental sulfur which is formed by the oxidation of H_2S and by the reaction of H_2S with SO_2. Seawater contains about 30 mmoles of sulfate per litre.

3. ACIDOPHILIC AEROBIC AND FACULTATIVELY ANAEROBIC AUTOTROPHS

The upper oxidized layer within solfataric fields including solfataric mudholes contains coccoid lobe-shaped strictly aerobic organisms which belong to the genus *Sulfolobus* which was described by T.D. Brock [4]. The type species *Sulfolobus acidocaldarius* and most other isolates can gain their energy

Table 1. Properties and energy sources of aerobic and facultatively aerobic S-metabolizing archaebacteria

Species	DNA mol% GC	T_{max} (^0C)	Ore mixture G1	Ore mixture G1 + YE	S^0	S^0 + YE	YE	Sucrose	Anaerobic growth $H_2 + S^0$
Sulfolobus acidocaldarius DSM 639	37	85	-	-	-	+	+	+	-
Sulfolobus solfataricus DSM 1616	36	87	-	+ (no acidification !)	-	+	+	+	-
TH 2	45	80	+	n.d.	+	+	+	-	-
Kra 23	38	70	+	n.d.	+	+	-	-	-
VE 2	37	85	+	n.d.	-	-	-	-	-
VE 6	31	95	+	n.d.	-	+	+	-	-
HV 5	30	95	+	n.d.	+	-	-	n.d.	-
Acidianus brierleyi DSM 1651	31	75	-	+	-	+	+	-	+
Acidianus infernus DSM 3191	31	95	-	-	-	+	-	-	+

n.d. = not determined

chemolithotrophically by oxidation of S^o, forming H_2SO_4. Some isolates (Tab. 1) are able to grow lithoautotrophically in S^o-containing mineral medium in the presence of CO_2 and oxygen, others require the addition of traces of yeast extract (e.g. 0.01%) , the function of which is not yet clear (source of minerals or of organic carbon). Alternatively both *Sulfolobus*-species and many *Sulfolobus*-shaped isolates (Tab. 1) grow without S^o organotrophically on yeast extract, sugars, peptone and amino acids [3, 4, 15]. Many *Sulfolobus*-isolates are able to oxidize ferrous iron [2, 5]. However, due to extensive precipitates of jarosite, even at pH 2 and in uninoculated controls, no significant growth could be observed [3, Huber and Stetter, unpublished]. *Sulfolobus brierleyi*, which has now been renamed *Acidianus brierleyi* [12] and some still unnamed *Sulfolobus*-shaped isolates [8] are moderate thermophiles, growing at temperatures up to 70°C. They are able to grow on sulfidic ores, forming sulfuric acid and solubilizing heavy metals. New ore-leaching bacteria could be isolated aerobically in mineral medium in the presence of ore mixture G1, consisting of pyrite, chalcopyrite, sphalerite and pitchblende [Tab. 1; 6]. Isolates HV5 and VE2 from samples of Hveragerthi and Hveravellir in Iceland are strictly dependent on ores and can not grow on S^o. Strains HV5 and VE6 are the most extremely ore-leaching bacteria known, growing at temperatures up to 95°C (Tab. 1). In contrast, *Sulfolobus acidocaldarius* and *Sulfolobus solfataricus* are unable to grow by the oxidation of sulfidic ores.

Recently, the new genus *Acidianus* was recognized (tentatively named *'Acidothermus"*, [14, 17]. Members have been isolated from acidic solfatara fields in Italy, Iceland, the Azores and the United States, and from a submarine hydrothermal vent in Italy [11, 12]. Although the cells of these thermoacidophiles have a coccoid, lobed shape similar to *Sulfolobus* (Fig. 1), they are different in DNA composition (G+C-content; DNA homology) and in metabolic properties.

Figure 1. Thin section of *Acidianus infernus* contrasted with lead citrate and uranyl acetate. Bar 1 µm.

Table. 2: Anaerobic growth of autotrophic S-dependent archaebacteria on H_2, yeast extract and different sulfur compounds

Mode of growth	Electron donor	Electron acceptor (?)	H_2S *) (μmoles/10^8 cells)	Species
			54	*Acidianus (S.) brierleyi*
			68	*Acidianus infernus*
			122	*Thermoproteus neutrophilus*
lithoautotrophic	H_2	S^0	54	*Thermoproteus tenax*
			63	*"Pyrobaculum islandicum"*
			158	*Pyrodictium occultum*
			237	*Pyrodictium brockii*
		S^0	2	
		$S_2O_3^{2-}$	0	*"Pyrobaculum islandicum*
		SO_3^{2-}	2	
organotrophic	YE			
		S^0	2	
		$S_2O_3^{2-}$	5	*Thermoproteus tenax*
		SO_3^{2-}	2	

*) corrected by the uninoculated control

Acidianus infernus, the first species to be recognized [12], is able to grow aerobically by S^o-oxidation, forming sulfuric acid. Surprisingly, this organism is also able to grow under extremely anaerobic conditions by S^o-reduction: H_2S is formed from H_2 and S^o (Tab, 1, 2). *Sulfolobus brierleyi* also processes this previously unrecognized metabolic versatility, and was therefore considered to also belong to the genus *Acidianus* [11, 12]. A further isolate, which contains a prophage (W. Zillig, pers. comm.) and had been tentatively named "*Sulfolobus ambivalens*" [21] exhibits 60% DNA homology with the type species, *Acidianus infernus* , indicating that it is another species of the genus *Acidianus*.

4. NEUTROPHILIC AND SLIGHTLY ACIDOPHILIC ANAEROBIC AUTOTROPHS

From the lower reduced zone of solfataric fields, extremely thermophilic methanogens and S^o-dependent autotrophs could be isolated. Previously, the methanogens could be only obtained from Icelandic solfataric fields. They are rod-shaped, Gram-positive lithoautotrophs, growing by formation of methane from H_2 and CO_2 at temperatures up to 97°C. Until now, the two species *Methanothermus fervidus* and *Methanothermus sociabilis* (Fig. 2) are the only known representatives of *Methanoathermaceae* [7,8].

Figure 2. Electron micrograph of *Methanothermus sociabilis*, platinum-shadowed. Bar 1 µm.

The S^o-dependent autotrophs are rod-shaped organisms which divide by budding [20]. Until now, members of the two new genera *Thermoproteus* and "*Pyrobaculum*" (Fig. 3) could be isolated growing lithoautotrophically on S^o, H_2 and CO_2, gaining energy by H_2S formation (Tab. 2). "*Pyrobaculum*" has a G+C-content of only 46 mol% which is 10% lower than that of *Thermoproteus* [20]. In addition, isolates belonging to "*Pyrobaculum*" show an optimal growth temperature of 100°C which is 12°C higher than that of the *Thermoproteus*-isolates [17, 20]. Alternatively, "*Pyrobaculum islandicum*" and *Thermoproteus tenax* are able to grow without H_2 organotrophically on yeast extract in the presence of S^o, $S_2O_3^{2-}$ and SO_3^{2-} (Tab. 2).

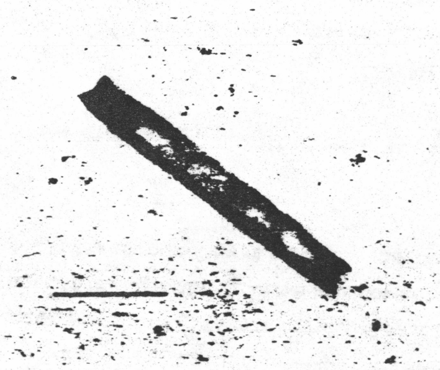

Figure 3. Electron micrograph of "*Pyrobaculum islandicum*" Geo 3, platinum-shadowed. Bar 1 µm.

During organotrophic growth, only low amounts of H_2S are formed compared with lithotrophic growth of these organisms. In the case of "*Pyrobaculum Islandicum*", organotrophic growth is also obtained when S^o is replaced by cysteine. It is most likely, these bacteria are growing organotrophically by an

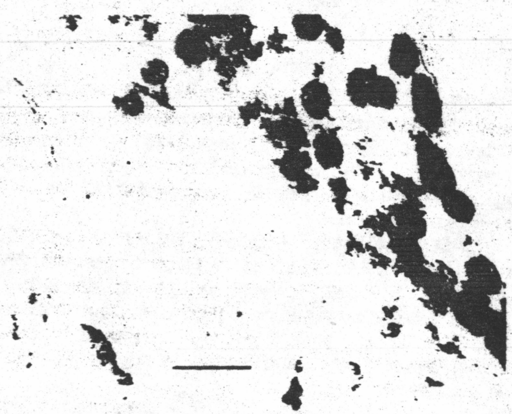

Figure 4. Electron micrograph of *Pyrodictium occultum*, platinum-shadowed. Bar 5 µm.

unknown fermentation rather than by sulfur respiration.

From the submarine hydrothermal vents of Vulcano, Italy, the most extremely thermophilic organisms known until now have been isolated. They have an optimal growth temperature of 105°C, and a maximum at around 110°C [under slight overpressure, 13]. These disc- to dish-shaped cells form unusual networks of fibers connecting the cells (Fig.4).

The new genus "*Pyrodictium*" currently contains the species *Pyrodictium occultum* and *Pyrodictium brockii*, which are strict H_2/S-autotrophs (Tab. 2). During growth, the fiber networks cover the sulfur like cobwebs [13, 16].

5. ISOLATION OF EXTREMELY THERMOPHILIC SULFATE-REDUCING ARCHAEBACTERIA

Until now, no archaebacteria thriving by dissimilatory sulfate reduction were known [9]. During our recent in situ enrichments within the hydrothermal systems close to Vulcano, Italy, coccoid to disc-shaped irregular cells could be isolated. These grew anaerobically without S^0 at temperatures between 65 and 95°C with an optimum at around 83°C. They use formate, L(+) and D(-) lactate and, less efficiently, glycogen, starch, yeast extract, meat extract and bacterial homogenates as substrates. Sulfate, thiosulfate and sulfite are used as electron acceptors, while S^0 is inhibitory for growth. As end products, H_2S, CO_2, and traces of methane (about 4 mmoles/l) could be detected. Growth is inhibited by 0.1 mmoles/l of Na-molybdate. Under the UV microscope, the cells show a blue-green fluorescence due to the presence of F_{420} [1]. In addition, methanopterin, but not F_{430} [A. Pfaltz, pers. comm.]or coenzyme M could be detected. The RNA polymerase of isolate VC-16 shows homology to subunits B' and B" of the methanogens, but is unique in serological cross-reaction of the heaviest subunit with components A and C of methanogens and S-dependent archaebacteria, indicating an outstanding position for the new isolates within the phylogenetic tree of the archaebacteria.

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