Extremophiles (1997) 1:67–73

ORIGINAL PAPER

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Stetteria hydrogenophila, gen. nov. and sp. nov., a novel mixotrophic sulfur-dependent *crenarchaeote* isolated from Milos, Greece

Received: September 4, 1996 / Accepted: November 12, 1996

Abstract A new hyperthermophilic, strictly anaerobic *crenarchaeote*, *Stetteria hydrogenophila* DSM11227 representing a new genus within the family of *Desulfurococcaceae*, was isolated from the sediment of a marine hydrothermal system at Paleohori Bay in Milos, Greece. Cells are gram-negative irregular and disc-shaped cocci, 0.5–1.5 μm in diameter, which are flagellate and can form cytoplasmatic protrusions up to 2 μm in length. The strain grew optimally at 95°C at pH 6.0 and at a NaCl concentration of 3%. The organism grew mixotrophically on peptide substrates. It required elemental sulfur as an external electron acceptor, and in addition, its growth was completely dependent on the presence of molecular hydrogen. Sulfur could be replaced by thiosulfate. H_2S, CO_2, acetate, and ethanol were identified as products of metabolism. The G + C content of DNA was 65 mol%. Analysis of its phylogenetic position by sequence analysis of 16S rRNA placed this organism in the family of *Desulfurococcaceae*. The dependence of this organism on both hydrogen and sulfur during growth on peptide substrates distinguishes *Stetteria* from all previously described species of *Crenarchaeota*.

Key words Hyperthermophilic · Crenarchaeota · Hydrogen and sulfur dependence · Hydrothermal · *Stetteria hydrogenophila*

Communicated by: G. Antranikian

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Marine hyperthermophilic archaea have been isolated from shallow submarine hydrothermal systems and abyssal "black smokers" (Stetter 1992, 1996). The biodiversity of hyperthermophiles in shallow marine hydrothermal systems has been extensively studied at the beaches of Vulcano and Ischia (Italy), off Kodakara-Jima (Japan), and to a lesser extent in Sangeang (Indonesia), Sao Miguel (Azores), and off Iceland (Zillig 1990; Huber et al. 1991; Hokai et al. 1995; Stetter 1996). The communities of hyperthermophiles in hydrothermal systems of the South Aegean volcanic arc have not previously been described. The island of Milos is located in this volcanic zone. Paleohori Bay at Milos is a geothermally active area characterized by extensive venting of both gas and hot water (Dando et al. 1995a,b). The venting liquids originating from a reservoir located underneath the seafloor are enriched in Na, K, Ca, and CO_2 compared with seawater, and depleted in Mg and sulfate. This unusual composition of the brine, the high temperature, and the sulfur content of the sediment, together with the efflux of gas, which is mainly CO_2 but contains up to 3% hydrogen, makes this bay a good habitat for the isolation of hyperthermophilic archaea. We report here the isolation and characterization of a novel, heterotrophic hyperthermophile whose growth is strictly dependent upon the presence of hydrogen and elemental sulfur.

Materials and methods

Collection of samples. Samples from marine sediments at a depth of 10 m were taken in 20-ml syringes by scuba divers at different locations of the white mats overlying hot brine seeps (Dando et al. 1995a) at Paleohori Bay, Milos. These samples were combined and stored in 28-ml serum tubes

(Fiala et al. 1986). The samples were reduced by the addition of sulfide and dithionite as described by Stetter (1982). They were then transported and stored at 4°C until enrichment.

Organisms. *Desulfurococcus mobilis* (DSM 2161), *Hyperthermus butylicus* (DSM 5456), *Pyrodictium abyssi* (DSM 6158), *Pyrodictium occultum* (DSM 2709), and *Staphylothermus marinus* (DSM 3639) were obtained from the Deutsche Sammlung von Mikroorganismen (Braunschweig, Germany) and were grown in either the medium of Allen (modified as described by Huber and Stetter 1992; *D. mobilis*) or synthetic marine (SME) medium (Stetter et al. 1983; all other strains). M5 originated from a hot vent site (temperature of the sediment 107°C) at Paleohori Bay, Milos and was enriched and isolated at 95°C in SME medium.

Culture conditions. Enrichment of the sample of interest (4ABC) was done in 100-ml type-III serum bottles (Pharmapack Stute, Königswinter, Germany) filled with 20 ml of medium comprising half-strength SME medium (Stetter et al. 1983), supplemented with 2% sulfur and containing in addition 0.5 M NaCl, 25 μM $MnSO_4$, 500 μM Na_2SiO_4, and 400 μM NH_4Cl under a H_2/CO_2 (80:20) atmosphere (300 kPa). Mass cultures were grown in a 10-l titanium fermentor (Braun Biotech, Melsungen Germany). For the analysis of optimal pH, different buffers (20 mM) were used: glycyl/glycine for the pH range 4–4.5; 2-morpholinoethane sulfonic acid (MES), pH 5.0–6.5; and 1,4-piperazine-diethane sulfonic acid (PIPES), pH 7–7.5.

Table 1. Analysis of nutritional requirements of *Stetteria hydrogenophila*

Substrate	Gas atmosphere	
	H_2/CO_2 (300 kPa)	N_2/CO_2 (300 kPa)
Ribose	−	−
Xylose	−	−
Glucose	−	−
Saccharose	−	−
Lactose	−	−
Xylane	−	−
Starch	−	−
Yeast extract	+++	−
Peptone	+++	−
Tryptone	+++	−
Caseine	++	−
Casamino acids	+++	−
Pyruvate	−	−
Na-acetate	−	−
Tri-sodium citrate	−	−
Extract of *Thermococcus celer*	+++	−
Extract of *Pyrococcus furiosus*	+++	−
Yeast extract and Peptone (SME medium)	+++	−

− indicates that no growth occurred, ++ indicates a final cell-density of 1×10^7–5×10^7, +++ 5×10^7–1×10^8 cells/ml. Note that hydrogen is required for growth on all carbon sources tested.

Growth measurements. Bacterial growth was determined by direct cell counting in a Thoma chamber (depth 0.02 mm) under a phase contrast microscope (Zeiss Standard 16). The growth of parallel cultures was followed for the determination of optimal temperature, pH, and salt concentration, respectively.

Analyses of carbon sources. The various compounds listed in Table 1 were added at a final concentration of 0.2% to SME medium not containing organic substances.

Analysis of metabolic products. Lactate (not detected as a metabolic product), acetate, and ethanol were determined enzymatically (Bergmeyer 1974; Dorn et al. 1978). For analysis of the formation of CO_2, the carbonate contained in SME medium was replaced by K_2HPO_4 (3.7 mM) and cells were grown under a hydrogen atmosphere. CO_2 was analyzed in a gas chromatograph model CP 9002 (Chrompack, Frankfurt, Germany) equipped with a thermal conductivity detector and a 10-m Carboplot capillary column with the following conditions: oven temperature 80°C; detector temperature 180°C; injector temperature 80°C; flow rate of the carrier gas nitrogen, 10 ml/min. CO_2 dissolved in the medium was calculated according to Henry–Dalton's law as described by Umbreit et al. (1972).

DNA techniques and sequencing analyses. Genomic DNA extractions, polymerase chain reaction (PCR)-mediated amplification of the 16S rRNA, and purification of PCR products were carried out as described by Barns et al. (1994), Rainey et al. (1992), and Rainey and Stackebrandt (1993). Purified PCR products were sequenced using the *Taq* Dye-Deoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Weiterstadt Germany) as directed in the manufacturer's protocol. Sequencing reactions were electrophoresed using the Applied Biosystems 373A DNA Sequencer. The sequence is available from the European Molecular Biology Laboratory (EMBL) under the accession number Y07784. The 16S rRNA sequence was manually aligned against sequences of members of the domain *Archaea*. Pairwise evolutionary distances were computed using the correction of Jukes and Cantor (1969). The least-squares distance method of De Soete (1983) was used in the construction of the phylogenetic dendrogram from distance matrices.

DNA for DNA–DNA hybridization experiments and for analysis of the G + C content was isolated by the procedure described by Jarrel et al. (1992), slightly modified. Analysis of the G + C content of DNA by HPLC was performed according to Mesbah et al. (1989). Dot-blot DNA–DNA hybridization was performed using the digoxigenin (DIG) random primed DNA labeling protocol (Boehringer-Mannheim, Germany) according to the recommendation of the supplier. As a modification, the filter membranes (hybond-N, Amersham, Braunschweig, Germany) were washed in the final washing step twice for 15 min in 1.25 × standard saline citrate (SSC) containing 0.1% (w/v) sodium dodecylsulfate at 68°C. The conditions used for DNA–

DNA hybridization were appropriate to detect homology of DNA molecules showing a similarity of ≥70%.

Light microscopy. Cells were mounted on agar-coated slides (Pfennig and Wagener 1986) and photographs were taken on Kodak plus-x-pan films with a Zeiss photomicroscope fitted with phase-contrast optics.

Electron microscopy. Cells were prepared for shadow casting (35°) with Pt/C and sections were cut with a Reichert Ultratome S after fixing cells with glutaraldehyde (3%) and OsO_4 (1%), dehydrating in a graded series of ethanol, and embedding in epoxy resin "Spurr" (ERL). Sections were stained with uranyl acetate and lead citrate and visualized in a Philips EM 300. Photographs were taken on Kodak electron microscope films.

Results

Enrichment and isolation

Interstitial water was collected from geothermally heated sediment in the brine seep area of Paleohori Bay (Dando et al. 1995a) by scuba diving at a depth of 10 m (for isolate 4ABC). The water originated from a sediment depth of approximately 10 cm. Sample (0.5 ml) was used to inoculate 20 ml of half-concentrated SME medium (Stetter et al. 1983) adjusted to the salt conditions encountered in the upper layer of the brine seep (Materials and methods). The elemental sulfur content of bulk sediment in this area was up to 1.1 g l^{-1} with temperatures up to 113°C. Serum bottles were incubated at 95°C without agitation under a H_2/CO_2 (80:20; 300 kPa) atmosphere. After 2 weeks of incubation irregular coccoid organisms became visible. Pure cultures were obtained by repeated serial dilution. Isolated strain 4ABC also grew well in the SME medium as described by Stetter et al. (1983). SME medium contains 2% elemental sulfur. The isolate grew with identical doubling time in this medium and in SME medium containing 0.5% sulfur. Therefore, SME medium supplemented with 0.5% sulfur was used for further cultivation experiments.

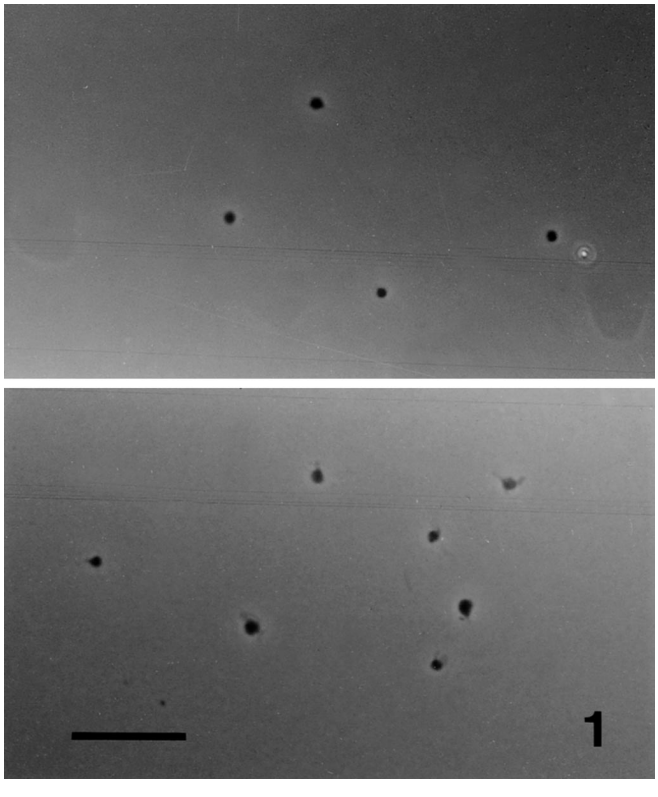

Fig. 1. Phase-contrast micrographs of the isolate 4ABC showing irregular shaped cocci, often forming one or more protrusions. *Bar*, 10 μm

Morphology

Cells of isolate 4ABC were irregular cocci (Figs. 1, 2) ranging in diameter from 0.5 to 1.5 μm. Many cells formed cytoplasmatic protrusions, in particular during the stationary growth phase (Fig. 1; Fig. 2b,d), when the concentration of S° in the medium was lower than 0.5% or when sulfur was replaced by thiosulfate (data not shown; see later). Cells were flagellate, with one flagellum (Fig. 2a). Ultrathin sections revealed the presence of a unilayer cell envelope ("S"-layer) above the membrane (Fig. 2b). This hexagonal array, probably consisting of protein subunits, was also detected by analysis of platinum shaded cells (Fig. 2c).

Optimal growth conditions

Growth was obtained between 70°C and 102°C with an optimum at 95°C. The doubling time at 95°C was 3.7 h (Fig. 3). No growth was observed at 68°C or 103°C. The isolate grew in a pH range between 4.5 and 7. The optimal pH for growth was 6.0 (Fig. 4). No growth could be detected at pH 4 or pH 7.5. Strain 4ABC grew at [NaCl] between 0.5% and 6% (Fig. 5). The optimal NaCl concentration in SME medium was between 2% and 3.5%. No growth was possible at 0.075% or 7% NaCl.

Metabolism

Growth of the isolate was strictly dependent upon the presence of elemental sulfur in the culture medium and of hydrogen in the gas phase of the culture. Large amounts of H_2S were formed during growth. When the H_2/CO_2 (80:20) atmosphere was replaced by a 100% hydrogen atmosphere, growth was still possible, but the doubling time of strain 4ABC was increased by a factor of six. These findings indicate that the isolate grows by reduction of elemental sulfur to H_2S using hydrogen as the major electron donor, and that the growth of this organism is strongly stimulated by CO_2.

However, in addition to H_2, the 0.1% yeast extract contained in the SME medium was also required for growth. When SME medium containing 0.1% yeast extract as the single organic component was used for cultivation of the

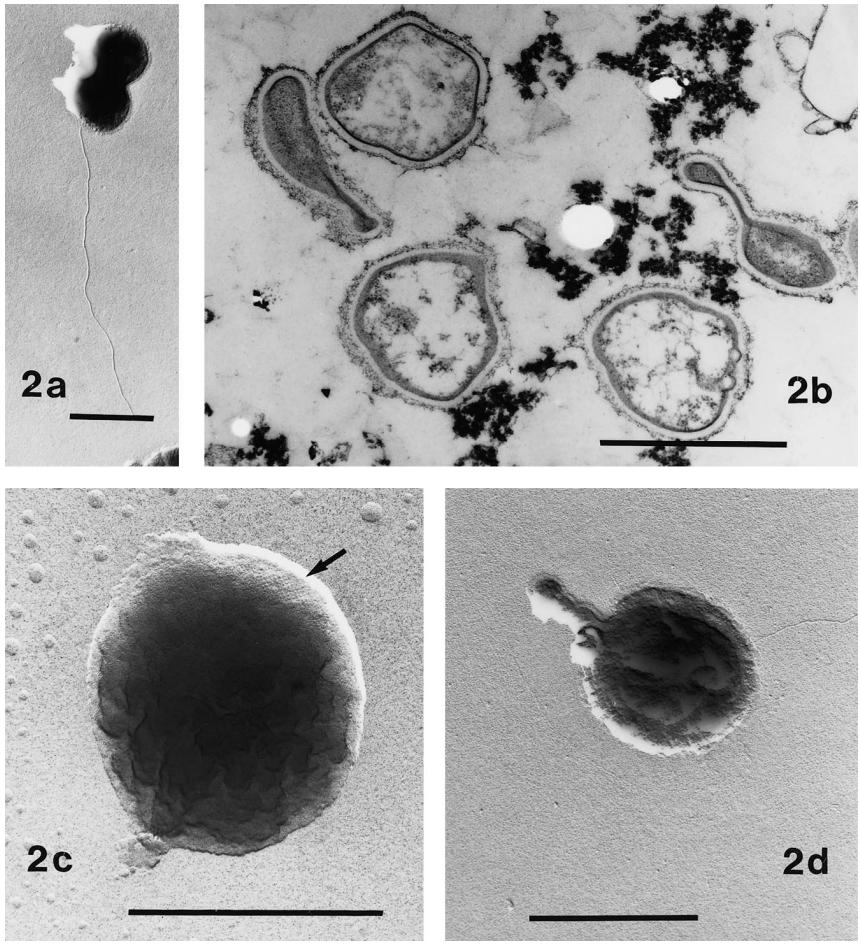

Fig. 2a–d. Electron micrographs of isolate 4ABC. *Bars*, 1 μm. **a** Pt/C shaded dividing cells of 4ABC with flagellum. **b** Thin section of strain 4ABC showing the irregular shape of cells and the protrusions; note the S-layer surrounding the cells. **c** Pt/C shaded cell showing a hexagonal array of cell surface proteins (S-layer, indicated by an *arrow*). **d** Pt/C shaded cell from stationary growth phase showing a protrusion

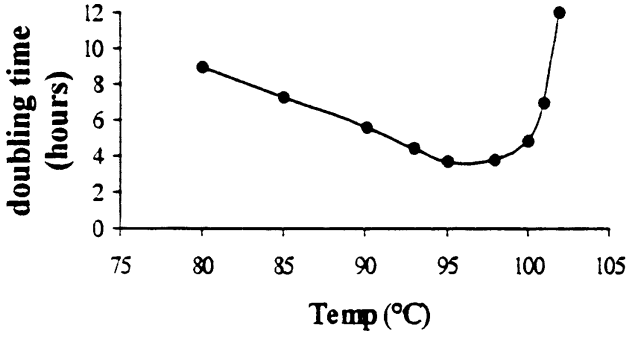

Fig. 3. Optimal growth temperature of *Stetteria hydrogenophila*. The doubling times were calculated from the slopes of the growth curves (5 parallel cultures were analyzed; not shown)

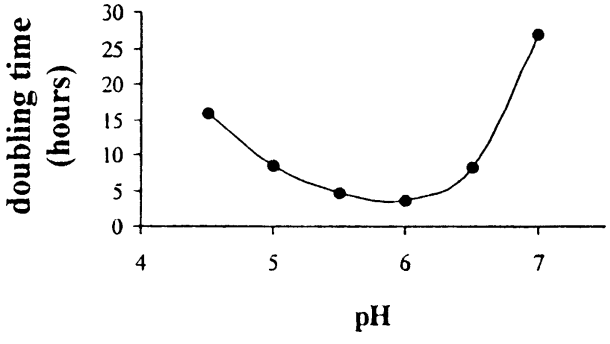

Fig. 4. Influence of pH on growth of *Stetteria hydrogenophila*. The doubling times were calculated from growth curves (5 parallel cultures were analyzed; not shown)

organism, the doubling time was 3.6 h. When the concentration of yeast extract was lowered to 0.05% and to 0.02%, the doubling times were 6 and 14 h, respectively. No growth was observed in the absence of yeast extract, although it could be replaced by peptone, tryptone, casein, casamino acids, or cell-free extracts of *Thermococcus celer* or *Pyrococcus furiosus* (Table 1). These findings demonstrate that 4ABC grows mixotrophically on peptide substrates in the presence of H_2. No growth was observed in the presence of ribose, xylose, glucose, saccharose, lactose, xylane, starch, pyruvate, acetate, or citrate as the single carbon source excepting CO_2 (Table 1). Although strain 4ABC grew in the presence of casamino acids, no growth could be detected when single amino acids or pairwise combinations of L-amino acids (final concentration 0.2% each) were used as the carbon source. The amino acids tested were: alanine, arginine, aspartate, cysteine, glycine, methionine, histidine, hydroxy-proline, lysine, proline, and valine (final concentration each 0.2%); the pairs of amino acids tested were: alanine + arginine; alanine + glycine; methionine + glycine;

and methionine + arginine. Strain 4ABC also did not grow in the presence of the four amino acids alanine, arginine, glycine, and methionine (final concentration 0.2% each).

To identify possible additional electron-donors, formate (0.5% final concentration) was added to the SME medium and the strain cultivated under a N_2/CO_2 (80:20; 300 kPa) atmosphere. No growth was observed, indicating that formate could not replace hydrogen as electron donor.

To detect additional electron acceptors, sulfur was replaced by nitrate, malate, or fumarate (final concentration 10 mM each), Fe^{III}-chloride (final concentration 1.5 mM), sulfite, cysteine, or thiosulfate (final concentration 15 mM each) and the cultures were incubated under a $H_2/C0_2$ atmosphere. Growth was observed only in the presence of

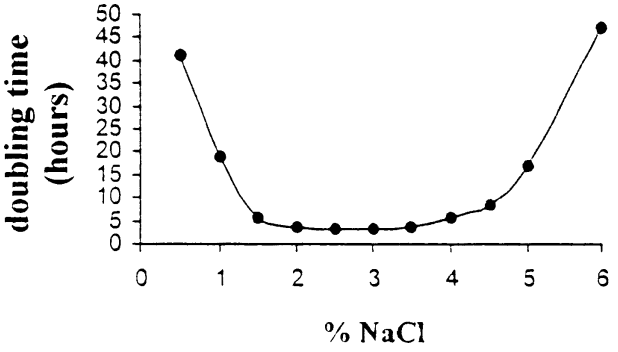

Fig. 5. Growth of *Stetteria hydrogenophila* at different concentrations of NaCl. The doubling times were calculated from growth curves (3 parallel cultures were analyzed; not shown)

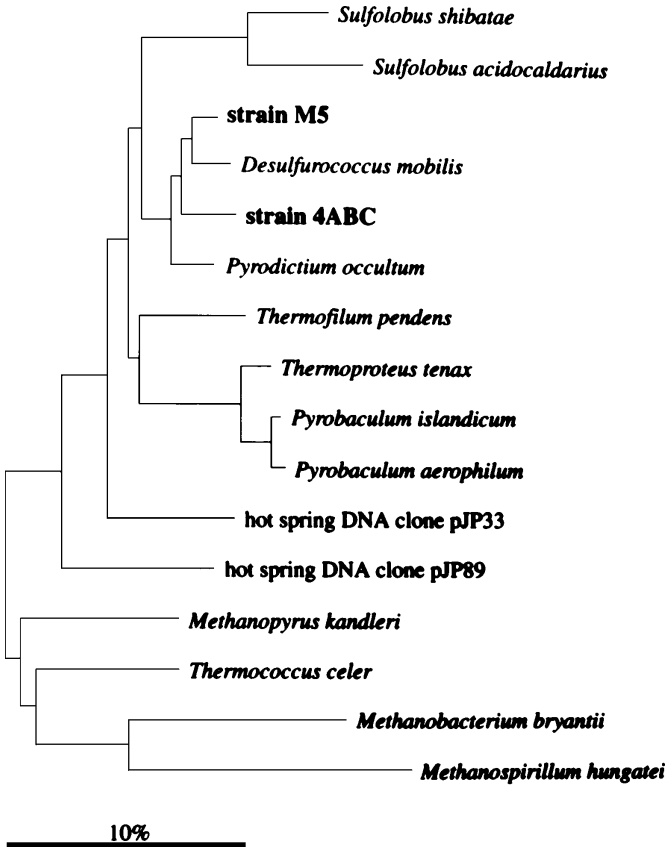

Fig. 6. Unrooted phylogenetic dendrogram indicating the position of strain 4ABC (*Stetteria hydrogenophila*) and isolate M5 from Paleohori Bay which is closely related to *Staphylothermus marinus*. *Scale bar* indicates 10 inferred nucleotide substitutions per 100 nucleotides

Table 2. Distance matrix indicating the similarity of the sequence of the 16S rRNA of *Stetteria hydrogenophila* (4ABC) and strain M5 with that of described species of *Archaea*. DNA–DNA hybridization experiments revealed that isolate M5 is closely related to *Staphylothermus marinus*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	–															
2	95.9	–														
3	95.1	95.8	–													
4	95.0	96.9	94.5	–												
5	90.0	91.0	90.5	89.7	–											
6	89.6	90.0	90.6	89.5	89.8	–										
7	89.7	89.7	89.8	89.0	89.3	95.6	–									
8	89.2	89.1	89.2	88.0	88.8	95.8	98.8	–								
9	88.8	90.3	90.8	89.0	90.5	88.6	88.4	87.7	–							
10	87.9	88.8	89.5	89.4	85.9	85.9	85.9	85.4	85.4	–						
11	86.8	87.9	88.3	87.9	85.9	84.9	84.7	84.0	84.4	91.4	–					
12	86.8	88.5	87.7	87.5	87.4	84.5	84.5	84.1	89.2	85.4	82.9	–				
13	84.9	85.7	86.0	84.9	85.3	83.0	82.6	82.3	84.8	79.7	79.3	84.3	–			
14	84.7	84.7	84.7	84.4	84.4	83.7	83.5	83	84.3	80.2	78.8	84.4	86.7	–		
15	78.7	79.8	78.8	79.1	79.1	77.3	77.5	76.8	79.3	76.8	76.6	81.1	81.2	82.5	–	
16	75.4	76.0	75.5	75.6	74.9	74.7	73.8	73.7	76.0	73.3	73.3	76.8	78.7	79.4	79	–

1. 4ABC
2. M5 (*Staphylothermus marinus*)
3. *Pyrodictium occultum*
4. *Desulfurococcus mobilis*
5. *Thermofilum pendens*
6. *Thermoproteus tenax*
7. *Pyrobaculum islandicum*
8. *Pyrobaculum aerophilum*
9. DNA clone jJP33
10. *Sulfolobus acidocaldarius*
11. *Sulfolobus shibatae*
12. DNA clone pJP89
13. *Methanopyrus kandleri*
14. *Thermococcus celer*
15. *Methanobacterium bryantii*
16. *Methanospirillum hungatei*

thiosulfate, indicating that this compound can act as an alternative electron acceptor.

To identify some products of metabolism of strain 4ABC, the isolate was grown in SME medium for 3 days at 95°C. Acetate (0.12 μmol/ml) and ethanol (0.11 μmol/ml) were detected as products of metabolism. To investigate the possibility that 4ABC also forms CO_2, the strain was grown in SME medium not containing carbonate under a hydrogen atmosphere (300 kPa). After 3 days of incubation at 95°C, 3.7 μmol/ml CO_2 was formed.

Phylogenetic position

The G + C content of the DNA of strain 4ABC was 65 mol%. PCR reactions using kingdom-specific primers indicated that the isolate is a *crenarchaeote* (data not shown). In dot-blot DNA–DNA hybridization experiments, the DNA of strain 4ABC did not show homology with DNA of *Staphylothermus marinus*, *Pyrodictium occultum*, *P. abyssi*, or *Desulfurococcus mobilis* (data not shown). To identify the phylogenetic position of the isolate, the complete sequence of 16S rRNA was determined. A distance matrix analysis revealed that strain 4ABC is related to the *Desulfurococcaceae* (Table 2). The closest relatives of strain 4ABC are *Staphylothermus marinus* (similarity of 16S rRNA 95.9%), *Pyrodictium occultum* (95.1%), and *Desulfurococcus mobilis* (95%). The phylogenetic dendrogram (Fig. 6) showed that 4ABC represents a new lineage within the *Desulfurococcaceae*.

Discussion

We report here the isolation of a marine hyperthermophilic *crenarchaeote* from the Aegean. DNA–DNA hybridization experiments revealed that this isolate was not closely related to representatives of the genera *Staphylothermus*, *Desulfurococcus*, *Pyrodictium*, or *Hyperthermus*. Analysis of 16S rRNA sequences and phenotypic properties clearly indicate that this isolate represents a new genus within the family of *Desulfurococcaceae* (Huber and Stetter 1992). According to the analysis of 16S rRNA, the closest relative of strain 4ABC is isolate M5, which is almost identical to *Staphylothermus marinus* (unpublished data). Strain 4ABC can be clearly distinguished from this organism by its G + C content which is 65 mol% rather than 35 mol%. In addition, *Staphylothermus* cells are different in their morphology and physiology. *Staphylothermus* cells occur characteristically as large aggregates consisting of up to 100 cells and form giant cells during growth on yeast extract. This has not been observed with cells of 4ABC. Although *Staphylothermus* is a heterotroph like 4ABC, its growth, unlike that of 4ABC, is not dependent on the presence of hydrogen.

The 16S rRNA of strain 4ABC shows 95% similarity with that of *Pyrodictium* and also the G + C values of the DNA of *Pyrodictium* isolates are high (60–62 mol%; Huber and Stetter 1992). However, *Pyrodictium* is clearly different from 4ABC in morphology and metabolism. All *Pyrodictium* isolates form ultrathin fibers which build cobweb-like networks connecting the cells. Furthermore, *Pyrodictium* forms macroscopically visible flakes when grown without shaking. Neither fibers nor these flakes were observed during cultivation of 4ABC. *P. abyssi* is a heterotroph like 4ABC, but unlike 4ABC this strain shows growth in the absence of hydrogen (Pley et al. 1991). In contrast to *P. abyssi*, strain 4ABC is unable to grow on carbohydrates, acetate (Table 1), or formate. *Hyperthermus butylicus*, whose 16S rRNA sequence has not yet been completely established, shows a lower G + C content of DNA (56 mol%) and it can grow fermentatively in the absence of hydrogen (Zillig et al. 1990). "*Thermodiscus*" and *Desulfurococcus* show a considerably lower G + C content of DNA (ca. 50 mol%). Both strains are obligate heterotrophs but also are able to grow without hydrogen. In addition, and in contrast to 4ABC which is adapted to marine salt concentrations, *Desulfurococcus* was isolated from continental solfataras and grows only at low salinity (Huber and Stetter 1992). *Stetteria hydrogenophila* represents the first novel hyperthermophile isolated from an Aegean hydrothermal system.

Description of *Stetteria*, Jochimsen, Peinemann-Simon, and Thomm gen. nov.

Stetteria, Jochimsen, Peinemann-Simon, and Thomm (Stet.te´ria, M.L. fem. of Stetter, named for Karl Otto Stetter, who pioneered the discovery of microorganisms growing at the boiling point of water). Cells are gram-negative irregular cocci, about 0.5–1.5 μm in diameter. Cells are flagellate, with a single flagellum. Cells form cytoplasmatic protrusions, in particular during the stationary growth phase. They grow heterotrophically on yeast extract, peptone, tryptone, casamino acids, or cell-free extracts of *Pyrococcus furiosus* or *Thermococcus celer*, but only in the presence of both molecular hydrogen and elemental sulfur. Hydrogen is obligately required as an electron donor. H_2S is formed from sulfur during growth. Sulfur or thiosulfate act as electron acceptors. The presence of CO_2 stimulates growth. Cells are hyperthermophiles growing between 68°C and 102°C with an optimum at 95°C, between pH 4.5 and 7.0 with the optimum at 6, and between 0.5% and 6% NaCl. The doubling time at 95°C under optimal growth conditions is 3.1 h. The G + C content of DNA is 65 mol%. Isolated from a shallow submarine hydrothermal system at Paleohori Bay in Milos, Greece.
Type species *Stetteria hydrogenophila* DSM11227.

Description of *Stetteria hydrogenophila* Jochimsen, Peinemann-Simon and Thomm Sp. nov.

Stetteria hydrogenophila, Jochimsen, Peinemann-Simon, and Thomm (hy.dro.ge.no.phi´la, M.L. fem. adj.; Gr. n. *hydrogenium* hydrogen; Gr. n. *philos* friend; *hydrogenophila*, liking hydrogen, since growth depends upon the presence of hydrogen). The description is the same as that of the genus.

Acknowledgments We would like to thank Horst-Dieter Pokojski for the large-scale cultivation of cells and Angelika Brandenburger and Marita Beese for excellent technical assistance. This work was supported by the EC MAST contracts 95-0021 and 93-0058, and the Fonds der Chemischen Industrie. The work of the scuba diving team of the Institute for Geology and Paleontology of the University of Kiel and of Peter Haushahn during sampling is highly appreciated. We would like to thank Alan Hughes and Ioanna Akoumianaki for logistical support in Milos.

References

Barns SM, Fundyga RE, Jeffrics MW, Pace NR (1994) Remarkable archaeal diversity in a Yellowstone National Park hot spring environment. Proc Natl Acad Sci USA 91:1609–1613

Bergmeyer HH (1974) Methoden der enzymatischen Analyse. Verlag Chemie, Weinheim

De Soete G (1983) A least-square algorithm for fitting additive trees to proximity data. Psychometrika 48:621–626

Dando PR, Hughes JA, Thiermann F (1995a) Preliminary observations on biological communities at shallow hydrothermal vents in the Aegean Sea. In: Parson LM, Walker CL, Dixon DR (eds) Hydrothermal vents and processes 87. Geological Society Special Publication, London, pp 303–317

Dando PR, Hughes JA, Leahy Y, Niven SJ, Taylor LJ, Smith C (1995b) Gas venting rates from submarine hydrothermal areas around the island of Milos, Hellenic Volcanic Arc. Cont Shelf Res 15:913–929

Dorn M, Andreesen JR, Gottschalk G (1978) Fermentation of fumarate and L-malate by *Clostridium formicoaceticum*. J Bacteriol 133:26–32

Fiala G, Stetter KO, Jannasch HW, Langworthy T, Madon J (1986) *Staphylothermus marinus* sp. nov. represents a novel genus of extremely thermophilic submarine heterotrophic archaebacteria growing up to 98°C. Syst Appl Microbiol 8:106–113

Hokai T, Nishijima M, Miyashita H, Maruyama T (1995) Dense community of hyperthermophilic sulfur-dependent heterotrophs in a geothermally heated shallow submarine biotop near Kodakara-Jima Island, Kagoshima, Japan. Appl Environ Microbiol 61:1931–1937

Huber R, Stetter KO (1992) The order *Thermoproteales*. In: Balows A, Trüper HG, Dworkin M, Harder W, Schleifer KH (eds) The prokaryotes. Springer Berlin Meidelberg New York, pp 677–683

Huber G, Huber R, Jones BE, Lauerer G, Neuner A, Segerer A, Stetter KO, Degens ET (1991) Hyperthermophilic archaea and bacteria occurring within Indonesian hydrothermal areas. Syst Appl Microbiol 14:397–404

Jarrel KF, Faguy D, Hebert AM, Kalmakoff ML (1992) A general method of isolating high molecular weight DNA from methanogenic archaea (archaebacteria). Can J Microbiol 38:65–68

Jukes TH, Cantor CR (1969) Evolution of protein molecules. In: Munro HN (ed) Mammalian protein metabolism. Academic, New York

Meshbah M, Permachandran U, Whitman WB (1989) Precise measurement of the G + C content of deoxyribonucleic acid by high performance liquid chromatography. Int J Syst Bacteriol 39:159–167

Pfennig N, Wagener S (1986) An improved method of preparing wet mounts for photomicrographs of microorganisms. J Microbiol Methods 4:303–306

Pley U, Schipka J, Gambacorta A, Jannasch HW, Fricke H, Rachel R, Stetter KO (1991) *Pyrodictium abyssi* sp. nov. represents a novel heterotrophic marine archaeal hyperthermophile growing at 110°C. Syst Appl Microbiol 14:245–253

Rainey FAM, Stackebrandt E (1993) 16S rDNA analysis reveals phylogenetic diversity among the polysaccharolytic clostridia. FEMS Microbiol Lett 113:125–128

Rainey FAM, Dorsch HW, Morgan HW, Stackebrandt E (1992) 16S rDNA analysis of *Spirochaeta thermophila*: its phylogenetic position and implications for the systematics of the order *Spirochaetales*. Syst Appl Microbiol 16:224–226

Stetter KO (1982) Ultrathin mycelia-forming organisms from submarine volcanic areas having an optimal growth temperature of 105°C. Nature 300:258–260

Stetter KO (1992) Life at the upper temperature border. In: Tran Than Van J, Tran Than Van K, Mounolou JC, Schneider J, McKay C (eds) Frontiers of life. Editions Frontiers, Gif-sur-Yvette, pp 195–219

Stetter KO (1996) Hyperthermophilic procaryotes. FEMS Microbiol Rev 18:149–158

Stetter KO, König H, Stackebrandt E (1983) *Pyrodictium* gen. nov., a new genus of submarine disc-shaped sulfur reducing archaebacteria growing optimally at 105°C. Syst Appl Microbiol 4:535–551

Umbreit WW, Burris RH, Stauffer JF (1972) Manometric and biochemical techniques. Burgess, Minneapolis

Zillig W, Holz I, Janekovic D, Klenk HP, Jmsel E, Trent J, Wunderl S, Fortjatz VH, Coutinho R, Ferreira T (1990) *Hyperthermus butylicus*, a hyperthermophilic sulfur-reducing archaebacterium that ferments peptides. J Bacteriol 172: 3959–3965