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Thermococcus aegaeicus sp. nov. and *Staphylothermus hellenicus* sp. nov., two novel hyperthermophilic archaea isolated from geothermally heated vents off Palaeochori Bay, Milos, Greece

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Two novel, hyperthermophilic, anaerobic, heterotrophic archaea were isolated from shallow hydrothermal vents off Palaeochori Bay, Milos, Greece. Strain P5^T (BK17S6-3-b2^T) is an irregular coccus, with a single polar flagellum, growing optimally at 90 °C, pH 6 and 2% NaCl. The DNA G+C content was 45 mol%. Due to its morphology, phylogenetic analyses based on 16S rRNA gene sequencing, DNA–DNA hybridization experiments, physiological properties and nutritional features, this strain represents a new species within the genus *Thermococcus* for which the name *Thermococcus aegaeicus* is proposed. The type strain is P5^T (= DSM 12767^T = JCM 10828^T). Strain P8^T (BK20S6-10-b1^T) is a coccus that forms aggregates. It grew optimally at 85 °C, pH 6 and 3% NaCl. The DNA G+C content was 38 mol%. Physiological properties and sequence analysis of the 16S rRNA gene, as well as DNA–DNA hybridization experiments, indicate that this strain is a new species belonging to the genus *Staphylothermus* for which the name *Staphylothermus hellenicus* is proposed. The type strain is P8^T (= DSM 12710^T = JCM 10830^T).

Keywords: hyperthermophiles, Aegean Sea, *Thermococcus*, *Staphylothermus*, hydrothermal vents

INTRODUCTION

More than 20 different genera of hyperthermophilic archaea have been isolated from geothermal and hydrothermal environments (Stetter, 1996). These micro-organisms are a valuable source for the exploitation of novel biotechnological processes and provide unique models for the investigation of principles of thermoadaptation.

The hyperthermophilic archaea appear to occupy the deepest branches of various phylogenetic trees (Stetter, 1996). Hyperthermophiles are normally isolated from areas undergoing vigorous hydrothermal activity and they are subjected to various geochemical challenges (Gonzalez *et al.*, 1998). Consequently, marine hydrothermal vents are normally colonized by hyperthermophilic strains representing diverse physiological types (Jannasch *et al.*, 1992).

In the Aegean Sea, venting of hot water has been described in the Caldera of Santorini (Holm, 1987; Varnavas *et al.*, 1990) and off the coast of Kos and Yali (Varnavas & Cronan, 1991). Furthermore, hydrothermal activity has been detected at several areas around the island of Milos as reported by Dando *et al.* (1995). The genus *Stetteria* has been isolated from marine sediments at Palaeochori Bay in Milos (Jochimsen *et al.*, 1997), but otherwise the diversity and ecological role of archaea in Aegean hot vent systems are unexplored. In this study we report the isolation and characterization of two novel hyperthermophilic archaea from hydrothermal vents off Palaeochori Bay near the island of Milos, Greece.

METHODS

Sample collection. Samples of marine sediment were collected from a shallow water area (K1 site at 4–5 m depth and

The GenBank accession numbers for the 16S rRNA sequences of *Thermococcus aegaeicus* strain P5^T and *Staphylothermus hellenicus* strain P8^T are AJ012643 and AJ012645, respectively.

W1 site at 9·5 m depth) during a fieldtrip to Palaeochori Bay (Milos, Greece; 14–29 September 1996). The sediment temperature at this area was between 90 and 103 °C. The samples were placed into 100 ml screw cap bottles, reduced with sodium sulfide at a final concentration of 0·05% and then stored at 4 °C for further analyses.

Reference strains. *Staphylothermus marinus* (DSM 3639), *Pyrococcus furiosus* (DSM 3638), *Pyrococcus woesei* (DSM 3773), *Thermococcus celer* (DSM 2476) and *Pyrodictium occultum* (DSM 2709) were obtained from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany).

Enrichment, isolation and cultivation. For the enrichment, 20 ml half-strength SME medium (Stetter *et al.*, 1983) supplemented with 2% elemental sulfur in 120 ml serum bottles (type III glass; Bormioli) was inoculated with 1 ml marine sediment and incubated without shaking at 90 °C in a H_2/CO_2 atmosphere (80:20, v/v; 300 kPa). For the isolation of pure cultures, the enrichment cultures were plated on the same medium solidified with 1% (w/v) Phytagel (Sigma) and 2–3% (w/v) sodium alginate (Roth) according to the method of Kovacs & Rakhely (1996). The Petri dishes were incubated in pressurized stainless steel cylinders at 90 °C (Balch *et al.*, 1979). Positive colonies were then transferred into liquid medium and incubated at 90 °C. Thereafter, the cultivated strains were stored at 4 °C for further investigations.

Large-scale cultivation was performed in a 100 l enamel-coated fermenter (Braun Biotech International) in half-strength SME medium containing 0·25 g sulfur l^{-1}. The stirring rate was 150 r.p.m. for the cultivation of P5^T and 100 r.p.m. for P8^T. The gassing rate at the beginning of the fermentation was 0·8 l H_2/0·2 l CO_2 min^{-1} for P5^T and 0·75 l H_2/0·3 l CO_2 min^{-1} for P8^T. When the cells entered the exponential growth phase, the gassing rate was increased for both strains to 4·0 l H_2/1·0 l CO_2 min^{-1}. Simultaneously, the stirring rate was increased to 300 r.p.m. (P5^T) or 350 r.p.m. (P8^T).

Light microscopy. Cells were mounted on agar-coated slides and photographs were taken on Kodak plus-X-pan film with a Zeiss photomicroscope fitted with phase-contrast optics.

Electron microscopy. Shadow-casting of air-dried cells with Pt/C was done with Balzers BAF 300 at an angle of 35°. Electron micrographs were taken with a Philips EM 300 on Kodak electron microscope film no. 4489.

Optimal growth. The optimal growth conditions of the strains were determined by varying the NaCl concentration and pH of the medium and by incubating the cultures at different temperatures. The growth response to pH values from 4 to 9 was tested. Unless otherwise stated, the buffers Glycyl/glycine (20 mM) for the pH range 4–5, MES (20 mM) for pH 5·5–6·5 and PIPES (20 mM) for pH 7–9 were used. The pH buffer was adjusted at 20 °C. The $\Delta pK_a/\Delta T$ (change of pK_a with temperature) values for Glycyl/glycine, MES and PIPES are −0·025, −0·011 and −0·0085, respectively (Stoll & Blanchard, 1990).

Growth of the strains was determined by counting cells in a Thoma chamber (depth 0·02 mm) under a phase-contrast microscope (Zeiss standard 16).

Determination of growth requirements. Unless otherwise indicated, individual carbon sources were added at a final concentration of 0·2% to the mineral medium (half-strength SME medium without organic compounds) supplemented with 2% elemental sulfur and 0·2% NH_4Cl. The atmosphere was H_2/CO_2 (80:20, 300 kPa) or N_2/CO_2 (80:20, 300 kPa).

The ability of strains to utilize alternate electron acceptors was tested using mineral medium without elemental sulfur and sulfate, but with yeast extract and peptone at a final concentration of 0·01%. The concentration of sodium thiosulfate, sodium sulfite or magnesium sulphate was 20 mM. Growth was inspected after an incubation period of 2 d, 4 d and 1 week.

Analysis of DNA G+C content. DNA was isolated using the method described by Hensiek (1992). The DNA was denatured, digested with Nuclease P1 (Zillig *et al.*, 1980) and then analysed for G+C content according to the method of Mesbah *et al.* (1989) using DNA of *Escherichia coli* strain K12 (50 mol% G+C; Sigma) and DNA of phage λ (48·9 mol% G+C; Roche Diagnostics) as standards.

Dot-blot DNA–DNA hybridization. Hybridization analyses were carried out according to the DIG System User's Guide for Filter Hybridization (Boehringer Mannheim). If two DNA's give a hybridization signal under the conditions suggested in the supplier's manual, the corresponding organisms are considered to belong to the same species (Jahnke, 1994)

16S rRNA analyses. PCR was used for the amplification of the 16S rDNA gene as described by Saiki *et al.* (1985, 1988) using the following archaeal primer pairs (M = A/C, R = A/G, Y = C/T): Arch 21F, 5′-TTCCGGTTGATCCYGCCGGA-3′/Arch 958R, 5′-YCCGGCGTTGAMTCCAATT-3′; Universal 1100F, 5′-AACGAGCGMRACCC-3′/Universal 1400R, 5′-GACGGGCGGTGTGTRC-3′ (DeLong, 1992); CAF545, 5′-TTGAGCTCAAGCTTCCGCGGTAATACCAGCYCCGC-3′/CAR952, 5′-TTTTGGATCCCCGGCGTTGACTCCAATTRARCCG-3′ (Hellwig, 1994); EAF545, 5′-TTGAGCTCAAGCTTCCGCGGTAAYACCGRCRGYYC-3′/EAR952, 5′-TTTTGGATCCCCGGCGTTGARTCCAATTRAACCG-3′. The amplified PCR products were sequenced with the ABI Prism Dye Termination Kit (Applied Biosystems) and then electrophoresed using a 373A automated DNA sequencer (Applied Biosystems). The sequence alignments and database searching were done using the HUSAR-Heidelberg Server. The PHYLIP 3.5c package was used to establish the evolutionary distance matrix and the phylogenetic trees (Felsenstein, 1985).

Quantitative DNA hybridization. DNA was isolated by column chromatography using hydroxyapatite, according to the procedure of Cashion *et al.* (1977). DNA–DNA hybridization was performed according to the procedure of De Ley *et al.* (1970) using a Gilford System 2600 spectrophotometer equipped with a Gilford 2527-R thermoprogrammer and plotter. The hybridization procedure was modified as described by Huss *et al.* (1983) and Escara & Hutton (1980). The renaturation rate was established using the TRANSFER.BAS program described by Jahnke (1992).

RESULTS

Isolation and morphology

Half-strength SME medium (Stetter *et al.*, 1983) containing yeast extract, peptone and elemental sulfur in a H_2/CO_2 atmosphere (80:20, 300 kPa) was

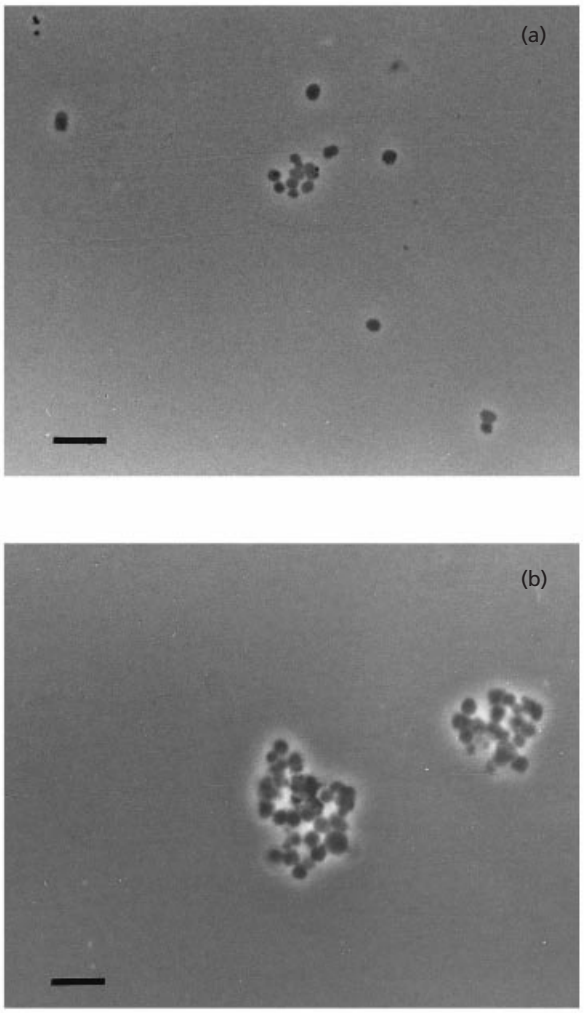

Fig. 1. Light micrographs of *Thermococcus aegaeicus* (a) and *Staphylothermus hellenicus* (b). Bars, 5 μm.

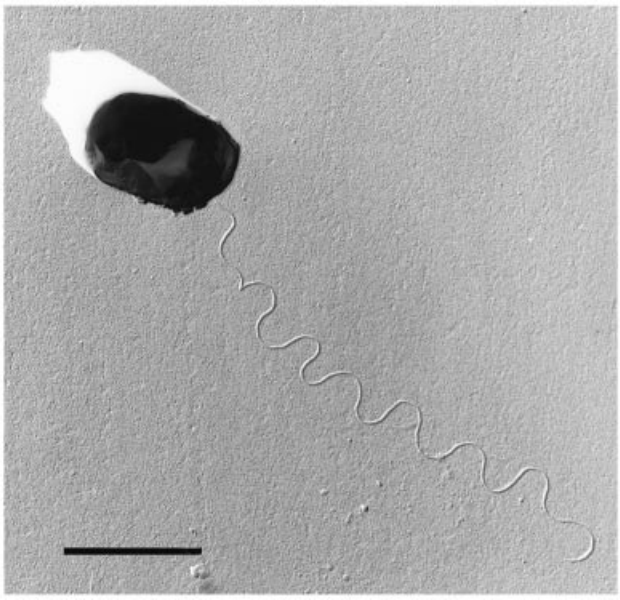

Fig. 2. Electron micrograph of an air-dried and platinum-shaded cell of P5^T showing a single polar flagellum. Bar, 1 μm.

inoculated with 1 ml samples collected from various locations around Palaeochori Bay, Milos, Greece, as described in Methods and incubated at 90 °C. After 1–2 weeks, regular and irregular cocci were observed in the various enrichment cultures. These cultures were transferred successfully and pure cultures were obtained by plating the cultures on half-strength SME medium solidified with Phytagel and alginate. From 36 isolates obtained, 20 were arbitrarily selected for further investigations. Dot-blot DNA–DNA hybridization and partial sequence analysis of 16S rRNA suggested that ten isolates were very similar or almost identical to *Staphylothermus marinus*, three to *Pyrococcus woesei*, one to *Pyrococcus horikoshii* and two to *Thermococcus celer*. Dot-blot DNA–DNA hybridization identified strains P3, P5^T, P6 and P8^T as not being closely related to the described archaeal species *Pyrococcus furiosus*, *Pyrococcus woesei*, *Staphylothermus marinus*, *Pyrodictium occultum* and *Thermococcus celer*. Quantitative DNA–DNA hybridization revealed that strain P3 exhibited a DNA hybridization value of 95·2% with *Pyrococcus woesei*. Therefore, this strain most likely belongs to this species and was not analysed in more detail. However, two of the isolates, P5^T and P8^T, were chosen for further analysis and are described in this study as novel species. Strain P6 has also been subjected to further analysis (data not shown). The cells of strain P5^T were irregular cocci about 0·8–1·5 μm in diameter (Fig. la) with a single flagellum of an extraordinarily short wavelength (Fig. 2). The length of the flagellum was 4–6 μm. Cells of P5^T could occasionally form aggregates of up to ten cells (Fig. la). Cells of strain P8^T were regular cocci, 0·8–1·3 μm in diameter, often forming large aggregates of up to 50 cells (Fig. 1b). The cells were not flagellated. At room temperature motility of both strains could not be detected by microscopic inspection.

Growth conditions and nutritional requirements for P5^T

Strain P5^T grew at pH 6 and at an NaCl concentration of 2·7% between 50 and 90 °C, with an optimum between 88 and 90 °C. The doubling time at 90 °C was 1·1 h and the growth yield was up to 10^9 cells ml^{-1}. In large-scale fermentations usually 0·3 g wet wt l^{-1} was obtained. No growth occurred at 45 or 100 °C. It grew at a temperature of 90 °C and at an NaCl concentration of 2·7% in a pH range between 4·5 and 7·5 with an optimum around pH 6. The strain grew at between 0·5 and 6·5% NaCl with an optimum of 2%. The conditions for the determination of the optimal NaCl concentration were 90 °C, pH 6. The strain is an

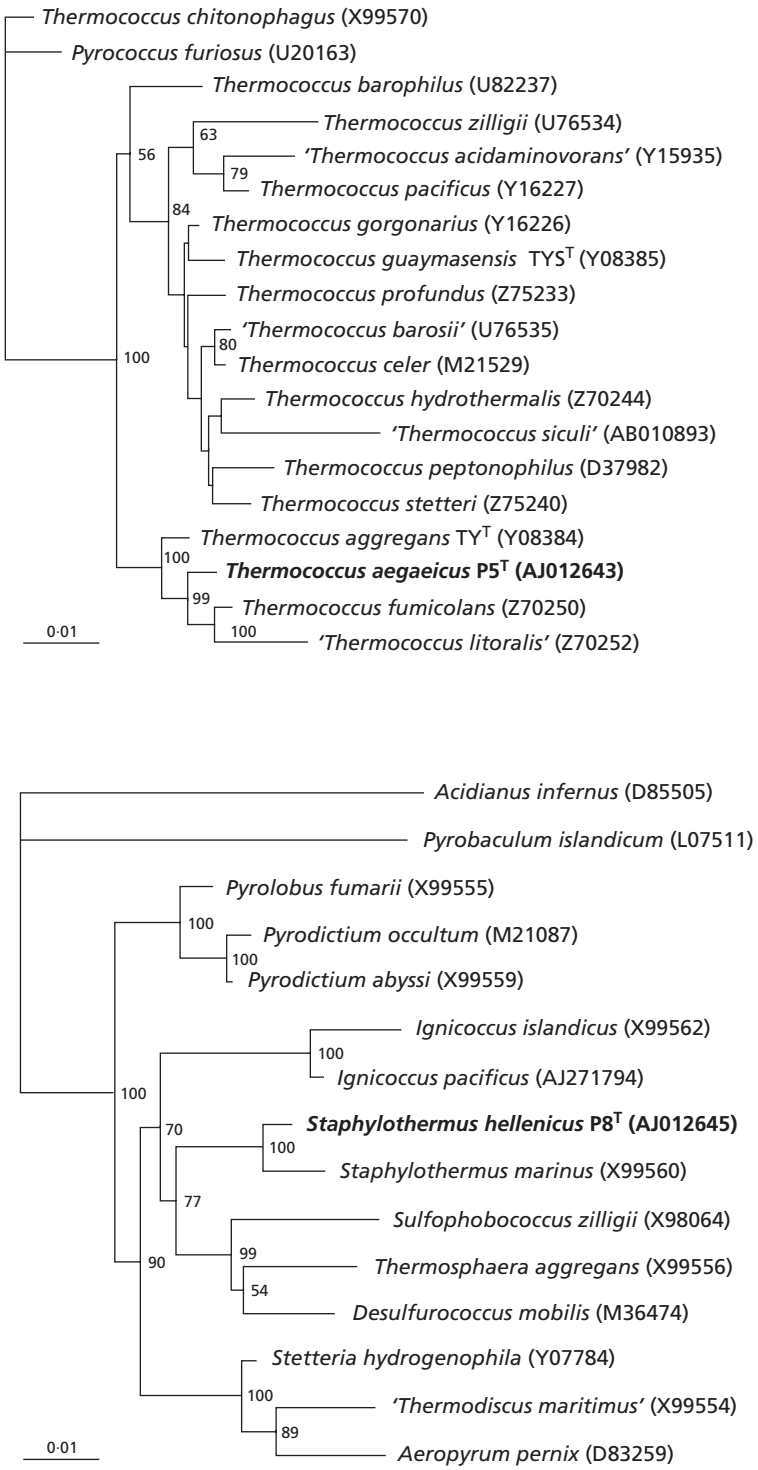

Fig. 3. Unrooted phylogenetic trees based on 16S rRNA sequences analysis showing the relationship of P5^T (*Thermococcus aegaeicus*) to members of the kingdom *Euryarchaeota* and representatives of the genus *Thermococcus*, and of P8^T (*Staphylothermus hellenicus*) with representatives of the kingdom *Crenarchaeota*. The distance matrix was calculated using the Kimura method. The trees were constructed using the neighbour-joining method. The scale bar indicates five estimated substitutions per 100 nt. The internal numbers indicate absolute bootstrap values per 100 bootstraps performed.

obligate heterotroph. Under H_2/CO_2 (80:20, 300 kPa) strain P5^T grew on yeast extract, peptone or tryptone as carbon sources under H_2/CO_2 and N_2/CO_2. It was able to utilize starch, but only under N_2/CO_2. It was not able to grow in the presence of casein, chitin, pyruvate, cellulose, sodium formate, maltose, glucose, cellobiose, trehalose, ribose, sorbose, sucrose or methanol as sole carbon sources under H_2/CO_2 or N_2/CO_2. The same carbon sources were utilized in the absence of sulfur. In a culture medium containing low amounts of yeast extract (0·01%), elemental sulfur as electron acceptor could not be replaced with sodium thiosulfate, sodium sulfite and magnesium sulphate. Strain P5^T grew in a sulfur-free medium containing 0·2% yeast extract, but its growth was stimulated in the presence of elemental sulfur. The addition of 40 ml air

to cultures inhibited growth, indicating that the strain is a strict anaerobe.

Growth conditions and nutritional requirements for P8^T

Strain P8^T grew at pH 6 and at an NaCl concentration of 2·7% between 70 and 90 °C, with an optimum at 85 °C. The doubling time at 85 °C was 2·9 h and the growth yield was 2×10^8 cells ml^{-1}. In large-scale fermentations usually about 0·25 g wet wt l^{-1} was obtained. At 85 °C and at pH 6 growth of the strain was observed between 2 and 8% NaCl with an optimum of 4%. No growth was observed at NaCl concentrations below 2% or at 8·5%. The pH range for growth was between 4·5 and 7·5 with an optimum at pH 6. The conditions for the determination of the pH optimum were 85 °C, 2·7% NaCl. Strain P8^T grew on yeast extract under H_2/CO_2 and N_2/CO_2. Poor growth was observed on peptone. No growth occurred in the presence of casein, pyruvate, cellulose, sodium formate, maltose, glucose, cellobiose, trehalose, ribose, sorbose, sucrose or methanol as sole carbon sources under H_2/CO_2 or N_2/CO_2. The growth of P8^T was absolutely dependent upon the presence of elemental sulfur. The addition of 20 ml air to the head space of 120 ml serum bottles used for cultivation inhibited growth, indicating that the strain is a strict anaerobe.

G+C content

Strain P5^T exhibited a G+C content of 45·5 mol%, as determined by analysis of mononucleotides after digestion of DNA with Nuclease P1 (Zillig *et al*., 1980), whereas strain P8^T had a G+C content of 38·8 mol%.

16S rRNA sequencing and phylogenetic position of the new isolates

To identify the phylogenetic positions of the new isolates, the 16S rRNA genes of the new strains were amplified and sequenced. The DNA sequences (P5^T, 1311 nt; P8^T, 1363 nt) of the 16S rRNA gene were aligned with those of archaea and then compared in a phylogenetic tree (Fig. 3) based on distance matrix methods (Olsen, 1987). The resulting tree was tested using bootstrap analysis (Felsenstein, 1985).

Strain P5^T was related to the genus *Thermococcus*. *Thermococcus aggregans* (Canganella *et al*., 1998) (99·2% similarity) and *Thermococcus fumicolans* (Godfroy *et al*., 1997) (99% similarity) were the closest relatives. Strain P8^T was closely related to *Staphylothermus marinus* (99·3% similarity). The bootstrap analysis confirmed the relationships between the strains isolated and reference species of the domain *Archaea*. Strain P5^T was associated with *Thermococcus aggregans* in all of the 100 bootstrap trees tested and *Thermococcus fumicolans* in 99 of 100 (Fig. 2). Strain P8^T was related to *Staphylothermus marinus* in all of the 100 bootstrap trees tested (Fig. 3). This strain has 98% similarity to *Staphylothermus marinus*, 95% similarity to *Desulfurococcus mobilis* (Huber & Stetter, 1982) and 81% similarity to strain P5^T.

DNA–DNA hybridization

In dot-blot DNA–DNA hybridization experiments at 70% homology, strain P3^T showed a weak signal with *Pyrococcus woesei* (Zillig *et al*., 1987) and therefore it seemed to be related to the genus *Pyrococcus*. Strain P5^T showed a hybridization signal only with two other isolates from Milos (P2 and P4; data not shown) and strain P8^T showed a weak hybridization signal with *Staphylothermus marinus* (data not shown).

To investigate the relationship of strains P5^T and P8^T to their closest relatives in more detail, a quantitative DNA–DNA hybridization of strain P5^T with *Thermococcus aggregans* and *Thermococcus fumicolans*, and of strain P8^T with *Staphylothermus marinus* was performed. P5^T exhibited hybridization values of 47% with *Thermococcus aggregans* and 38.5% with *Thermococcus fumicolans*. Strain P8^T showed a hybridization value of 37·5% with *Staphylothermus marinus*.

DISCUSSION

We report here the isolation and characterization of two hyperthermophiles, P5^T and P8^T, belonging to the phylogenetic domain *Archaea* (Woese *et al*., 1990).

Strain P5^T is an irregular coccus. Sequence analyses of the 16S rRNA gene place P5^T in the kingdom *Euryarchaeota* and the order *Thermococcales* (Fig. 3) that contains the genera *Thermococcus* and *Pyrococcus*. Dot-blot and quantitative DNA hybridization show that members of the genus *Thermococcus* are the closest relatives of P5^T. This is consistent with physiological characteristics, e.g. an optimal growth temperature below 90 °C typical for members of the genus *Thermococcus*, whereas *Pyrococcus* species grow optimally at temperatures higher than 90 °C and are able to grow at temperatures higher than 100 °C (Fiala & Stetter, 1986; Zillig, 1989; Godfroy *et al*., 1997; Canganella *et al*., 1998). The members of the genus *Thermococcus* can be divided into two groups based on their DNA G+C content. One group contains species with high G+C values (50–60 mol%) and the other group contains six described species listed in Table 1. DNA–DNA hybridization showed that *Thermococcus aggregans* and *Thermococcus fumicolans* were the closest relatives of P5^T. DNA–DNA hybridization with these species revealed that the hybridization values were lower than 70%, indicating that P5^T is a new and distinct species in accordance with the recommendations of the committee on reconciliation of approaches to bacterial systematics (Wayne *et al*., 1987). Some physiological characteristics discriminate P5^T from *Thermococcus aggregans* and *Thermococcus fumicolans* and from the *Thermococcus* species with low G+C content (Table 1). The ability of this organism to utilize starch

Table 1. Phenotypic features distinguishing *Thermococcus aegaeicus* from its closest relatives, *Thermococcus aggregans* and *Thermococcus fumicolans*, and from described *Thermococcus* species with a low G+C content

Data were taken from Marteinsson *et al.* (1999), Dirmeier *et al.* (1998), Canganella *et al.* (1998), Godfroy *et al.* (1996), Huber *et al.* (1995), Keller *et al.* (1995) and Neuner *et al.* (1990). ND, Not determined.

Strain	G+C content (mol%)	Utilization of:				pH optimum	NaCl concentration range for growth (%)
		Maltose	Starch	Casein	Malt extract		
Thermococcus aggregans	42	+	+	+	ND	7	1–3
Thermococcus fumicolans	55	+	−	−	+	8	1.3–2.6
Thermococcus aegaeicus	45	−	+	−	−	6	0.5–6.5
Thermococcus barophilus	37	−	−	+	ND	7	1–4
Thermococcus chitonophagus	46	−	ND	ND	ND	6.7	0.8–8
Thermococcus alcaliphilus	42	−	−	+	ND	9	1–6
'*Thermococcus litoralis*'	38	−	−	+	ND	7.2	1.8–6.5
'*Thermococcus acidaminovorans*'	49	−	−	+	ND	9	1–6

distinguishes P5^T from all other strains listed in Table 1 except *Thermococcus aggregans*. It can be distinguished from *Thermococcus aggregans* and most *Thermococcus* species with low G+C content by its inability to utilize casein. It also shows the lowest pH optimum of growth in this group. It shares some phenotypic properties with *Thermococcus chitonophagus* (Table 1) but, in contrast, strain P5^T is unable to utilize chitin as sole carbon source. It can be distinguished from all *Thermococcus* species listed in Table 1 by its monopolar flagellum (Fig. 2). This property and its G+C content of 45 mol% discriminate P5^T also from *Thermococcus* species with a high G+C content, which, as well as the *Thermococcus* species listed in Table 1, show polytrichous flagellation or are not flagellated. For strain P5^T the name *Thermococcus aegaeicus* sp. nov. is proposed.

Sequence analysis of 16S rRNA indicated that P8^T belonged to the kingdom *Crenarchaeota*. It showed the highest sequence similarity to *Staphylothermus marinus* (Fig. 3). The genera *Desulfurococcus*, *Stetteria* and *Staphylothermus* have been proposed to belong to the family *Desulfurococcaceae* (Jochimsen *et al.*, 1997). *Staphylothermus marinus* has been described to form large aggregates, consisting of up to 100 cells (Fiala *et al.*, 1986). Strain P8^T showed a similar morphology (Fig. 1b). The temperature optimum for growth of P8^T was lower than for *Staphylothermus marinus* (85 versus 92 °C), but P8^T was able to grow at higher NaCl concentrations (2–8% compared to 1–3·5% NaCl). This property seems to be an adaptation to the high salt concentration encountered in this ecosystem (Dando *et al.*, 2000). Like *Staphylothermus marinus*, P8^T was able to grow on complex protein substrates like yeast extract and could not utilize carbohydrates as carbon source. However, in contrast to *Staphylothermus marinus* (Fiala *et al.*, 1986) only poor growth of P8^T on peptone as sole carbon source was observed. The DNA G+C content of 38·8 mol% was similar to that of *Staphylothermus marinus* (35 mol%), but quantitative DNA–DNA hybridization revealed less than 70% similarity with *Staphylothermus marinus*. In view of this result and various physiological properties we propose strain P8^T is classified as a new *Staphylothermus* species, *Staphylothermus hellenicus* sp. nov.

Description of *Thermococcus aegaeicus* sp. nov.

Thermococcus aegaeicus (ae.gae′i.cus. M.L. adj. *aegaeicus* of the Aegean Sea, from where the organism was isolated).

Cells are irregular cocci with a diameter of 0·8–1·5 μm. Growth occurs between 50 and 95 °C with an optimum between 88 and 90 °C. Doubling time at 90 °C is 1.1 h. pH range of growth is between 4·5 and 7·5 with an optimum around pH 6. Optimal NaCl concentration is 2%, but growth is observed up to 6·5%. Obligate anaerobe, utilizing complex organic compounds like yeast extract, peptone and tryptone as carbon source. Starch can be utilized under an N_2/CO_2 atmosphere but not under an H_2/CO_2 atmosphere. No growth on pyruvate, malt extract, maltose and casein. Elemental sulfur stimulates growth. DNA G+C content is 45 mol%. Sequence comparisons place P5^T in the order *Thermococcales*; DNA–DNA hybridization indicates that it belongs in the genus *Thermococcus*. The type strain was isolated from sediments of Palaeochori Bay, Milos, Greece, located at a depth of 4 m. The type strain is P5^T (= DSM 12767^T = JCM 10828^T).

Description of *Staphylothermus hellenicus* sp. nov.

Staphylothermus hellenicus (hel.le′ni.cus. Gr. adj. *hellenikos*, M.L. adj. *hellenicus* Greek, pertaining to Greece, from where the organism was isolated).

Cells are irregular cocci with a diameter of 0·8–1·3 μm, often growing in large aggregates. Growth occurs

between 70 and 90 °C with an optimum at 85 °C. Doubling time at 85 °C is about 2·9 h. pH range of growth is between 4·5 and 7·0 with an optimum around pH 6. Growth occurs between 2 and 8 % NaCl with an optimum at 4 %. Obligate anaerobe. Grows with yeast extract as carbon source, but growth on peptone as carbon source is poor. Growth is absolutely dependent on elemental sulfur. DNA G+C content is 38 mol %. Sequence analyses place P8^T in the family *Desulfurococcaceae*; DNA–DNA hybridization indicates that it belongs in the genus *Staphylothermus*. The type strain was isolated from a geothermally heated sediment at Palaeochori Bay, Milos, Greece located at a depth of 9·4 m. The type strain is P8^T (= DSM 12710^T = JCM 10830^T).

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