***Thermococcus barophilus* sp. nov., a new barophilic and hyperthermophilic archaeon isolated under high hydrostatic pressure from a deep-sea hydrothermal vent**

Viggó Thór Marteinsson,^{1†} Jean-Louis Birrien,¹
Anna-Louise Reysenbach,² Marc Vernet,¹ Dominique Marie,¹
Agata Gambacorta,³ Paul Messner,⁴ Uwe B. Sleytr⁴ and Daniel Prieur^{1,5}

Author for correspondence: Viggó Thór Marteinsson. Tel: +354 5707213. Fax: +354 5707111.
e-mail: vigg@iti.is

¹ Station Biologique, CNRS, UPR 9042, BP 74, 29681 Roscoff cedex, France

² Rutgers University Institute for Marine and Coastal Sciences, Cook College, New Brunswick, NJ 08903, USA

³ Istituto per la Chimica di Molecole di Interesse Biologico, CNR, via Toiano 6, 80072 Arco Felice, Napoli, Italy

⁴ Zentrum für Ultrastrukturforschung und Ludwig Boltzmann-Institut für Molekulare Nanotechnologie, Universität für Bodenkultur, A-1180 Wien, Austria

⁵ Université de Bretagne Occidentale, Brest, France

A novel barophilic, hyperthermophilic, anaerobic sulfur-metabolizing archaeon, strain MP^T (T = type strain), was isolated from a hydrothermal vent site (Snakepit) on the Mid-Atlantic Ridge (depth, 3550 m). Enrichments and isolation were done under 40 MPa hydrostatic pressure at 95 °C. Strain MP^T was barophilic at 75, 80, 85, 90, 95 and 98 °C, and was an obligate barophile between 95 and 100 °C (T_{max}). For growth above 95 °C, a pressure of 15–17.5 MPa was required. The strain grew at 48–95 °C under atmospheric pressure. The optimal temperature for growth was 85 °C at both high (40 MPa) and low (0.3 MPa) pressures. The growth rate was twofold higher at 85 °C under *in situ* hydrostatic pressure compared to at low pressure. Strain MP^T cells were motile, coccoid, 0.8–2.0 µm in diameter and covered by a hexagonal S-layer lattice. The optimum pH and NaCl concentration for growth at low pressure were 7.0 and 20–30 g l⁻¹, respectively. The new isolate was an obligate heterotroph and utilized yeast extract, beef extract and peptone for growth. Growth was optimal in the presence of elemental sulfur. Rifampicin and chloramphenicol inhibited growth. The core lipids consisted of a major archaeol and a complex lipid pattern consisting of a major phospholipid. The DNA G+C content was 37.1 mol%. Sequencing of the 16S rRNA gene revealed that strain MP^T belonged to the genus *Thermococcus* and it is proposed that this isolate should be designated as a new species, *Thermococcus barophilus*.

Keywords: archaea, barophile, *Thermococcus*, hyperthermophile, deep-sea vent

INTRODUCTION

With the discovery that extremely thermophilic microbial communities inhabit deep-sea hydrothermal vents, numerous strains of hyperthermophilic archaea have been isolated from such environments (Prieur *et al.*, 1995). All of these isolates were obtained by selective cultivation at atmospheric pressure (0–101 MPa). Few attempts to enrich for isolates under *in situ* pressure have yielded mixed cultures of hyper-

thermophiles (Reysenbach & Deming, 1991) and, until now, no isolates have been sustainable in pure culture.

The response of deep-sea psychrophilic bacteria to hydrostatic pressure has been well-documented by Yayanos (1986), while much less information exists on the response of thermophiles to pressure. Pressure can have a stimulating effect on the growth of one isolate and virtually no effect on another. Barophilic psychrophiles tend to be found at or below 2000 m (Yayanos, 1986), while the upper layers are dominated by barotolerant species (Jannasch & Wirsén, 1984). A depth of 6350 m appears to be the shallowest depth for isolation of obligate barophiles (Yayanos, 1986).

Several studies of hyperthermophilic isolates grown under elevated pressure have been reported (Erauso *et*

[†] **Present address:** Department of Biotechnology, Technological Institute of Iceland, Keldnaholt, IS-112 Reykjavik, Iceland.

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al., 1995; Holden & Baross, 1995; Jannasch *et al.*, 1992; Marteinsson *et al.*, 1997; Miller *et al.*, 1988; Nelson *et al.*, 1991, 1992; Pledger *et al.*, 1994; Reysenbach & Deming, 1991). However, not all of these hyperthermophilic strains tested under high-pressure conditions showed a barophilic response at their *in situ* pressures. Barotolerant (Reysenbach & Deming, 1991) and barosensitive (Jannasch *et al.*, 1992) strains have been reported and, in some cases, the pressure response was temperature dependent (Deming & Baross, 1993; Jannasch *et al.*, 1992).

The presently known barophilic hyperthermophiles were all obtained from hydrothermal vent sites, located at a depth of at least 2000 m. These micro-organisms grew better under high pressure or near their *in situ* pressure and their optimal and maximal temperatures were shifted by several degrees under these conditions. During the French-American 'MAR93' cruise in June 1993 on the Mid-Atlantic Ridge, a new hydrothermal site located deeper than most other hydrothermal vent sites was explored. In the present study, a new hyperthermophilic archaeon was isolated near its *in situ* pressure and the effect of pressure on its temperature range for growth was investigated.

METHODS

Reference strains. '*Thermococcus litoralis*' DSM 5474 and *Thermotoga maritima* DSM 3109^T (T=type strain) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). '*Pyrococcus abyssi*' GE5^T was obtained from our laboratory in Roscoff, France.

Collection of samples and storage. Samples were collected during the oceanographic cruise 'MAR93' in June 1993, organized by Institut National des Sciences de l'Univers (INSU) and Woods Hole Oceanographic Institution (WHOI). Active and non-active chimney rocks and hot fluids were collected on the Mid-Atlantic Ridge at a depth of 3550 m from Snakepit (23°22'41.18 N, 44°56'49.84 W) by the American submersible *Alvin*. Aboard the ship R.V. *Jean Charcot*, solid samples were immediately transferred into an anaerobic chamber and then into 10–30 ml sterile glass syringes (Ultrafit; Heinke-Sass-Wolf) that were sealed by plunging needles into rubber stoppers. The syringes were filled with sterile, reduced artificial seawater (Marteinsson *et al.*, 1995) before the cut pistons were put in place and the gas phase was expelled prior to tightening the seal on each syringe. Syringes were transferred into high-pressure vessels and pressurized with cold water up to 40 MPa hydrostatic pressure. Samples were kept under pressure and at 4 °C until enrichment.

Enrichments and isolation under *in situ* hydrostatic pressure. All culturing manipulations preceding pressure experiments were performed in an anaerobic chamber. Samples, stored at 4 °C under 40 MPa hydrostatic pressure, were depressurized and 0.5 ml rock suspensions and hydrothermal fluids were transferred into 10 ml syringes. Each syringe contained either YPS medium (Marteinsson *et al.*, 1995) buffered with PIPES buffer (Sigma; 7.0 g l⁻¹ at pH 6.8) (designated hereafter as YPS-20) or modified *Thermotoga* medium (Huber *et al.*, 1986) which had the following composition (l⁻¹ distilled water): 0.96 g PIPES buffer, 2.4 g NaCl, 0.16 g

MgSO₄·7H₂O, 0.016 g NH₄Cl, 0.04 g CaCl₂·2H₂O, 0.16 g yeast extract (Difco) and 10 mM glucose. The pH of the medium was adjusted to 6.8. Sulfur (0.1 g) was added to the syringes, which had been sealed by plunging needles into rubber stoppers, before media and samples were dispensed in the syringes. Finally, the pistons were put in place and the gas phase was expelled prior to tightening the seal on each syringe. The syringes were then transferred into the high pressure and temperature incubation system, custom-built by Top Industrie (Industrial zone 'Le Plateau de Bière', Dammarie-les-Lys, France), pressurized to 40 MPa and heated to 95 °C (Marteinsson *et al.*, 1997). The isolate was purified under 40 MPa by six serial dilutions at 95 °C. Low pressure enrichments were performed in serum bottles at 95 °C.

Cellular protein extraction and PAGE. Whole-cell proteins were extracted as described by Marteinsson *et al.* (1995). Equal protein concentrations from samples were loaded on 5–20% (w/v) gradient polyacrylamide-SDS gels (5%, w/v, stacking gel) (Laemmli, 1970).

Determination of growth parameters under hydrostatic pressure. To determine growth rates at different temperatures under high and low pressures, cells were grown in 10 ml syringes containing 10 ml YPS-20 medium. The syringes were loaded anaerobically in an anaerobic chamber with 10 ml medium and inoculated with 0.2 ml culture in late-exponential phase grown under 40 MPa pressure at 85 °C. Samples were pressurized to the test pressure before heating the vessel ovens. Heating started at 22 °C and it took approximately 30 min to obtain stable test temperatures (75, 80, 85, 90, 95 and 98 °C). Each temperature was tested at high (40 MPa) and low pressure (0.3 MPa) in parallel experiments. Growth curves at the different temperatures were generated by cooling the pressurized vessels in a continuous flow of cool water for 2 min to approximately 50 °C. The pressure decreased progressively with decreased temperature and the remaining pressure was released gently through a valve. Samples (approx. 0.4 ml) were immediately collected anaerobically and injected into venoject blood-collecting tubes (Terumo) before repressurizing and reheating the syringes to the test temperature (approx. 30 min). Each sample was processed in duplicate and was fixed immediately in 4% (v/v) formaldehyde. At each time-point, analysis was performed in duplicate and, in some cases, the experiment was repeated.

Determination of maximum temperature for growth of the new isolate under hydrostatic pressure was performed in triplicate at 40 and 0.3 MPa. The minimal hydrostatic pressure required for growth above 95 °C was estimated at 0.25 MPa intervals. This experiment was performed in duplicate and repeated three times. In all cases, cells grown at high pressure were used as the inoculum.

Determination of growth parameters. Anaerobic procedures were performed as described by Balch & Wolfe (1976). Standard YPS medium was used in all cultures unless otherwise stated. To determine the optimum pH and NaCl concentration, cells were grown in Hungate tubes (27 ml; Bellco) containing 7 ml YPS medium and N₂ at a pressure of 100 kPa in the headspace. The YPS medium was modified with the following buffers (Sigma), all at 10 mM: pH 3.5–4.5, no buffer; pH 5.0–6.0, MES buffer; pH 6.5–7.0, PIPES buffer; pH 7.5–8.5, HEPES; pH 9.0–10, no buffer. To determine the optimal salt requirement, YPS medium was prepared either without NaCl or supplemented with NaCl. The effects of pH and salinity were determined in duplicate

at 85 °C. Minimal growth temperature experiments were performed in 27 ml Hungate tubes containing 5 ml YPS medium with N₂ in the headspace at 100 kPa and incubated in aluminium heating blocks (Bioblock). The temperatures were monitored with temperature probes placed in control tubes. Cultures on plates were obtained on YPS medium solidified with Gelrite (Erauso *et al.*, 1995). Plates were incubated in an anaerobic jar at 85 °C (gas phase N₂/H₂/CO₂ 90:5:5; 100 kPa) for 3 d.

Determination of cell numbers. Growth was measured by either flow cytometry or microscopy. For flow cytometry, samples were fixed with 1 % (v/v) glutaraldehyde for 20 min at ambient temperature before storage at -80 °C. Cell DNA was stained with PicoGreen (Molecular Probes) at 37 °C for 1 h with a mixture of RNase in TE buffer (Marie *et al.*, 1996). The DNA-specific stain was used at a final concentration of 1:1000 of the commercial solution. Cells were enumerated by a FACsort flow cytometer equipped with a 488 nm laser, and samples were delivered at a calibrated rate of 28 µl for 1–4 min. Forward and right-angle light scatter, as well as DNA fluorescence, were collected as LISTMODE files and processed with custom-designed software CYTOWIN (D. Vaultot, unpublished data).

For microscopy, cells were counted in a Petroff–Hausser chamber (depth, 0.02 mm) using a light microscope (model BH2; Olympus) equipped with a phase-contrast oil immersion objective (×100 magnification). A good correlation was found between microscopic and flow cytometric counts. Growth rates were calculated using linear regression analysis from three to five points along the logarithmic portions of the resulting growth curves.

Substrate utilization and culture conditions. Substrate utilization analysis was done in 27 ml Hungate tubes containing 10 ml medium. The tubes were inoculated with an exponentially growing culture at a final concentration of 5 × 10⁵ cells ml⁻¹ and incubated at 85 °C. All tests were performed in duplicate. Uninoculated medium was used as a negative control for each substrate and inoculated YPS medium (containing yeast extract and peptone) was used as a positive control. Growth was recorded after 2 and 7 d. The ability of the MP^T isolate to use single carbon sources for growth was tested on YPS medium without yeast extract and peptone. This basal medium was supplemented with one of the following carbon sources: starch, maltose, glycogen, sucrose and glucose to a final concentration of 0.5 % (w/v), while yeast extract, peptone, tryptone, pyruvate, casein hydrolysate, casein non-hydrolysate, beef extract, Casamino acids, succinate, propionate and acetate were present at a final concentration of 0.2 % (w/v). A filter-sterilized solution of vitamins (10 ml l⁻¹) (Canganella *et al.*, 1997) was added and N₂ gas was used as the headspace gas. Nitrogen sources were tested in the absence of NH₄Cl with pyruvate (40 mM) added as the carbon and energy source in the mineral basal medium. Urea, thiourea, glutamate and gelatin were all tested at 0.2 % (w/v), while NH₄Cl, KNO₃ and KNO₂ were added at 20 mM to the basal medium. The headspace contained H₂/CO₂ (20:80).

Autotrophic growth under H₂/CO₂ (80:20) was tested in the basal medium. To determine the ability of the isolate to grow in the absence of elemental sulfur (S⁰), cells were cultured in YPS medium from which sulfur compounds had been omitted. The headspace gas was N₂ or H₂/CO₂ (20:80) in these experiments and titanium (III) citrate (1 mM final concentration) was used as reducing agent instead of Na₂S. 9H₂O. Growth was determined by direct cell counts in

a Petroff–Hausser chamber (depth, 0.02 mm) with a phase-contrast microscope.

Antibiotic susceptibility. The sensitivity to antibiotics was tested at 150 µg ml⁻¹ in YPS medium. Cells were inoculated at 80 °C in Hungate tubes. Controls were performed with an antibiotic-sensitive bacterium, *Thermotoga maritima*, cultivated under the same conditions to demonstrate the efficiency of the antibiotic at 80 °C.

Electron microscopy. Preparation of cells for freeze-fracturing was carried out as described previously (Sleytr *et al.*, 1988). Micrographs were taken with a model CM100 electron microscope (Philips) at an acceleration voltage of 80 kV.

Lipid analysis. The lipids were analysed in cultures grown at 85 °C in 11 serum flasks at atmospheric pressure and incubated in ovens (Mettmert). Lipids were extracted from dried cells according to De Rosa *et al.* (1983) and analysed by TLC. One-dimensional TLC was performed by eluting with chloroform/methanol/H₂O (65/25/4 by vol.). Two-dimensional TLC was developed with chloroform/methanol/H₂O (65/25/4 by vol.) followed by chloroform/methanol/ethanol/H₂O (80/12/15/4 by vol.) (De Rosa & Gambacorta, 1994), and compared to lipid standards obtained from other archaea (Pley *et al.*, 1991). The total lipid extract was hydrolysed in 1 M methanolic HCl to cleave the polar head groups. The core lipids were identified by TLC as previously described (Trincon *et al.*, 1988; Huber *et al.*, 1995) using the following solvents: n-hexane/ethyl acetate, 78/22 (v/v); and n-hexane/ethyl acetate, 75/15 (v/v).

DNA base composition. DNA was extracted as described by Charbonnier *et al.* (1992). RNase (20 µg ml⁻¹) was added to the DNA for 1 h at 37 °C, and the DNA was dialysed overnight at 4 °C in TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0). The G+C content (mol %) of the DNA was determined from the melting point in 0.1 × SSC according to Marmur & Doty (1962) using *Escherichia coli* DNA (57 mol % G+C; Sigma) as standard.

16S rRNA gene sequence analysis. The 16S rRNA gene was amplified by PCR as previously described (Reysenbach & Pace, 1995). The double-stranded PCR products were sequenced by cycle sequencing and an ABI 373 automated sequencer. The 16S rRNA sequence was manually aligned with a subset of sequences from the Ribosomal Database Project (RDP) (Maidak *et al.*, 1996), recent GenBank sequence releases and unpublished sequences. The secondary structure was used as a guide so that only homologous sequences were compared and to check for chimeric structures. The phylogenetic trees were constructed using either the evolutionary distance matrix with the algorithm of DeSoete (1983) or by maximum-likelihood analysis with FASTDNAML (Olsen *et al.*, 1994). Bootstrap values were determined for the maximum-likelihood trees with 100 replicates.

DNA–DNA hybridization. The S1 nuclease method (Grimont *et al.*, 1980) of quantitative DNA–DNA hybridizations was performed as described by Popoff & Coynault (1980) on type DE81 filters (Whatman). DNA concentration and purity were estimated spectrophotometrically with Genequant II (Pharmacia). The DNA probes were labelled using a Nick Translation kit (Amersham). The hybridizations were performed at 65 °C (25 °C below melting temperature = optimal conditions) for 24 h in 0.42 M NaCl. Levels of DNA–DNA homology were also determined by the DSMZ

by measuring the rates of reassociation of pairs of single-stranded sheared DNA samples.

RESULTS

Enrichment and purification

One sample of an external layer of a vent chimney resulted in growth in both enrichment media after 45 h incubation under 40 MPa hydrostatic pressure at 95 °C. Six other samples showed no growth after at least 1 week of incubation. Positive enrichments consisted of dense populations of coccoid cells. Samples incubated under atmospheric pressure in serum bottles at 95 °C did not grow. Subcultures grown on *Thermatoga* medium and under 40 MPa pressure could not be successfully maintained. Enrichments on YPS-20 medium were successfully subcultured at 95 °C and purified by a series of dilutions to extinction. Subcultures did not grow at 95 °C at atmospheric pressures. The purified isolate (MP^T) was stored at 4 °C under 35 MPa hydrostatic pressure.

The isolate was diluted on plates containing YPS solid medium containing 2% (w/v) Gelrite. Colonies were observed after 3 d incubation at 85 °C. One colony was subcultured into liquid culture to obtain biomass for protein extraction. Protein profiles of cell extracts from this culture and the MP^T isolate were compared to verify if the purity of the strain isolated under high pressure was successful. Both cultures showed identical protein patterns (data not shown) indicating that purification was successful.

Fig. 1. Micrograph of a freeze-etched and metal shadowed cell of *Thermococcus barophilus* strain MP^T showing the hexagonal S-layer lattice. Cells were grown in YPS medium at 85 °C under atmospheric pressure. Bar, 100 nm.

Fig. 2. (a) Growth of strain MP^T at 85 °C with 40 MPa hydrostatic pressure. Four syringes were used for each time-point and interrupted once for sampling. Growth rates were calculated from linear regression analysis along the logarithmic portion of the growth curves with error bars at 95% confidence. (b) Effect of temperature and hydrostatic pressure on the specific growth rate of strain MP^T. The cells were grown in YPS-20 medium under 0.3 MPa (●) and 40 MPa (○) hydrostatic pressure as described in the Methods. Growth rates were calculated by performing linear regression analysis along the logarithmic part of the growth curves. Error bars (95% confidence interval) of specific growth rates are shown when results of more than two separate experiments were available.

Enrichment attempts for micro-organisms under 40 MPa hydrostatic pressure at 110 °C were unsuccessful.

Morphology

Electron microscopy and phase-contrast microscopy (data not shown) revealed that cells were regular to slightly irregular motile cocci and occurred singly, in pairs or in small aggregates. The cell size ranged from 0.8 to 2.0 µm. Occasional atypical sizes and shapes and giant cells, sometimes more than 5 µm in diameter, were observed. Cells appeared to divide by constriction. Freeze-fracturing (Fig. 1) of intact cells showed that the organisms were completely covered with a hexagonally arranged S-layer lattice (for a review see Sleytr *et al.*, 1996) with centre-to-centre spacings

Fig. 3. pH and NaCl concentration optima for growth of isolate MP^T on YPS medium at 85 °C under atmospheric pressure. Specific growth rates were plotted as a function of (a) pH (in the presence of 2.3% w/v NaCl) and (b) salinity (pH 6.8). Growth rates were calculated from linear regression analysis along the logarithmic part of the growth curves.

between the morphological units of approximately 28.4 nm.

Growth at different temperatures and pressures

Strain MP^T grew up to 100 °C under high hydrostatic pressure, while the upper temperature limit was 95 °C under both low hydrostatic and atmospheric pressure conditions. The isolate required hydrostatic pressure (15.0–17.5 MPa) to grow above 95 °C. The growth rate increased at all temperatures tested when cells grew under high-pressure conditions. The optimum growth temperature was 85 °C under both high- and low-pressure conditions (Fig. 2a, b), but the growth rate was at least double under high pressure than under low pressure (doubling times approx. 28 and 63 min, respectively). The minimal temperature for growth at atmospheric pressure was 48 °C, but that limit was not estimated under high-pressure conditions.

Determination of growth parameters

Growth was observed at pH 4.5–9.5 and the optimum pH was about 7.0. No growth was observed at pH 4.0 and 10.0 (Fig. 3a). The strain required NaCl for

growth and growth was observed at salt concentrations of 1–4% (w/v) NaCl. The optimum salt concentration for growth was between 2 and 3% (w/v) and the maximal salt concentration was less than 5% (w/v) (Fig. 3b).

Determination of growth requirements

Strain MP^T appeared to be an obligate heterotroph. No growth was detected in minimal medium amended with vitamins and with a H₂/CO₂ (80:20) headspace. Optimal growth requirements were determined in the presence of S⁰, although S⁰ was not required for growth and could be replaced by cystine. Yeast extract and peptone could be replaced by several complex substrates, such as tryptone, beef extract, casein, Casamino acids, yeast extract or peptone. Pyruvate and propionate supported poor growth. Other substrates tested, such as starch, maltose, glycogen, sucrose, glucose and acetate, did not give any growth. The strain grew best with NH₄Cl as a nitrogen source, but some growth occurred with thiourea, glutamate, KNO₃ and KNO₂.

Antibiotic susceptibility

The new isolate was insensitive to penicillin and streptomycin at a concentration of 150 µg ml⁻¹, but it was sensitive to chloramphenicol and rifampicin at that concentration. The control bacterium *T. maritima* exhibited the expected pattern of antibiotic susceptibility at 80 °C, demonstrating that the antibiotics were not inactivated by heat.

Lipid composition

The complex lipid pattern of isolate MP^T was analysed by TLC (data not shown). One major phospholipid (*R_F* 0.34) was detected. A minor phospholipid, which was slightly α-naphthol-positive suggesting the possible presence of a glycolipid, was also detected. TLC analysis of the hydrolysed complex lipids showed a major archaeol component, but no caldarchaeol was detected. The archaeol had *R_F* values of 0.74 and 0.80 in n-hexane/ethyl acetate (78/22) and n-hexane/ethyl acetate (75/15), respectively.

DNA base composition

The G+C content of the DNA of isolate MP^T determined by the thermal denaturation method was 37.1 mol%.

16S rRNA sequence analysis

Fig. 4 shows the phylogenetic relationship of strain MP^T with other members of the *Thermococcales*. The levels of similarity (%) between the 16S rRNA of MP^T and the 16S rRNAs of other *Thermococcus* species (accession numbers of the sequences used in this analysis are given in parentheses) are as follows: *Thermococcus* sp. strain ES1 (U82238), 98.6; *Thermo-*

Fig. 4. Phylogenetic relationship of *T. barophilus* based on maximum-likelihood analysis of the 16S rRNA sequences. The bar represents the expected number of changes per sequence position.

coccus hydrothermalis (Z70244), 97.5; *Thermococcus celer* (M21529), 97.9; *Thermococcus* sp. GE3 (Z70245), 98.1; *Pyrococcus* sp. strain KOD1 (D38650), 97.7; *Thermococcus peptonophilus* SM2 (D37983), 97.3; *T. peptonophilus* OG1 (D37982), 97.8; *Thermococcus* sp. GE20 (Z70251), 97.0; *Thermococcus* sp. Str554 (Z70249), 97.3; *Thermococcus fumicolans* (Z70250), 95.5; *T. litoralis* (Z70252), 97.3; *Pyrococcus abyssi* (L19921), 96.7; and *Pyrococcus furiosus* (U20163), 96.7. Maximum-likelihood analysis and phylogenetic analysis using a distance matrix gave similar results. The new strain, designated *Thermococcus barophilus*, is closely related to the strain ES1. Bootstrap analysis confirmed this relationship.

DNA–DNA hybridization

The phylogenetic analysis of the 16S rRNA genes sequences (above) revealed that the strain belongs to the *Thermococcales*. Strain MP^T exhibited DNA–DNA homologies of 68.9% for *T. litoralis* as determined by the filter method and 68% as determined by the spectrophotometric method performed by the DSMZ. The MP^T isolate had 48% DNA hybridization to DNA from *P. abyssi*.

DISCUSSION

The presence of ether lipids confirmed the archaeal nature of the novel barophilic, hyperthermophilic strain MP^T and, based on composition of 16S rRNA sequence, strain MP^T belongs to the genus *Thermococcus* (Zillig *et al.*, 1987). This classification is consistent with the morphological and physiological characteristics of strain MP^T, which are similar to those of this genus. The different physiological characteristics of some *Thermococcus* species, which have similar G+C contents to strain MP^T, are summarized in Table 1.

The members of the genus *Thermococcus* can be divided into two groups based on their G+C content. One group contains at least seven described species with high G+C values (50–60 mol %), and the other group contains three described species, *T. litoralis*, *Thermococcus alcaliphilus* and *Thermococcus chitonophagus* (G+C contents of 38, 42.4 and 46.5 mol %, respectively). On the basis of its low G+C content (37.1 mol %), the new strain could be related to the latter group. However, there are some physiological characteristics, phenotypic patterns and phylogenetic relationships which distinguish the new isolate from the members of this group. DNA–DNA hybridization experiments showed low similarity of strain MP^T to *T. litoralis*. Moreover, similarity between the 16S rRNA of strain MP^T and the 16S rRNA of other *Thermococcus* species used in the phylogenetic relationship was less than 98 %, except for *Thermococcus* strain ES1, whose 16S rRNA was 98.6% similar to that of MP^T. Nevertheless, MP^T could be distinguished from strain ES1 by several parameters (Table 1). The G+C content of the two strains differed by more than 21 %, which is indicative of different species (Johnson, 1994). Strain MP^T grew optimally at about 85 °C, pH 7.0 and with 25 g l⁻¹ NaCl in the medium, whereas ES1 grew optimally at about 82 °C, pH 6.5 and at 6–20 g l⁻¹ NaCl (Pledger *et al.*, 1994). Strains MP^T and ES1 have different growth responses to pressure. MP^T was barophilic at all temperatures tested, while ES1 was not. ES1 only grew at 93 °C if pressurized at 22 MPa, and exhibited a barophilic response at 91 °C under 22 and 44 MPa. Moreover, ES1 was baro-sensitive at 85 °C. However, MP^T was barophilic at all temperatures and was an obligate barophile at 95–100 °C. Furthermore, the new isolate showed the highest growth ratio (twofold difference) between high and low hydrostatic pressure that has been reported for thermophilic organisms. The optimum growth temperature for MP^T did not change with pressure (Fig. 2b).

Most of the deep-sea thermophiles isolated to date have been obtained at depths of 2000–3000 m and do not show barophilic responses under hydrostatic pressures at all temperatures tested, with the exception of *P. abyssi* (Erauso *et al.*, 1993). Psychrophilic isolates obtained at 3584 m and deeper are barophilic at all temperatures in their growth range (Yayanos, 1986). However, strain MP^T was obtained from a depth of 3550 m and showed a similar pressure response to those reported for psychrophilic barophiles. Moreover, MP^T was an obligate barophile above the temperature permitting growth at atmospheric pressure (Fig. 2b).

On the basis of the complex lipid pattern and core lipids, strain MP^T resembles *T. celer* more than any other *Thermococcus* species. De Rosa *et al.* (1987) reported that the phospho-*myo*-inositol derivative of archaeol is the major component in *T. celer* and that caldarchaeol was not found. However, other *Thermococcus* species appear to synthesize caldarchaeol in

Table 1. Characteristics of *Thermococcus* species with low G + C content, strain ES1 and the new isolate MP^T

Strain	G + C content (mol %)	Origin	pH optimum	Growth temperature (°C)		NaCl (g l ⁻¹)		Barophilic response	Reference
				Range	Optimum	Range	Optimum		
<i>Thermococcus litoralis</i> DSM 5474	38.0	Sediment of shallow marine solfatara, Italy	7.2	65–95	88	18–65	25	ND	Neuner <i>et al.</i> (1990)
<i>Thermococcus chitonophagus</i> DSM 10152 ^T	46.5	Smoker fragments, collected at Guaymas Basin (2000 m)	6.7	60–93	85	8–80	20	ND	Huber <i>et al.</i> (1995)
<i>Thermococcus alcaliphilus</i> DSM 10322 ^T	42.4	Shallow marine hydrothermal system, Italy	9.0	56–90	85	10–60	20–30	ND	Keller <i>et al.</i> (1995)
<i>Thermococcus</i> sp. strain ES1	58.6	Polychaete worm, Juan de Fuca Ridge (2200 m)	6.5	54–91	82	6–40	6–20	+	Pledger & Baross (1991)
<i>Thermococcus</i> sp. strain MP ^T	37.1	Chimney wall, Snakepit (3550 m)	7.0	48–100	85	10–40	20–30	+	This study

ND, Not determined.

addition to archaeol (Antoine *et al.*, 1995; Canganella *et al.*, 1997; Huber *et al.*, 1995). *T. alcaliphilus* and *T. chitonophagus* both produce tetraethers (cold-archaeol), but *T. chitonophagus* has one cyclopentane ring as well (Huber *et al.*, 1995; Keller *et al.*, 1995). The archaeols from *T. chitonophagus* were reported at R_F values of 0.75 and 0.62 (Huber *et al.*, 1995), while in MP^T the archaeols were at R_F values of 0.74 and 0.80. Moreover, the presence of glycolipids in strain MP^T is unusual in *Thermococcus* species and has not been observed before in this genus. The possible presence of glycolipids must be confirmed by spectroscopic analyses of a pure compound.

The cell envelope of *T. barophilus* has an unusual S-layer lattice constant of 28.4 nm which is much higher than other values found within *Thermococcus* species. For instance, both *T. alcaliphilus* and *T. chitonophagus* have a much lower lattice constant of around 15 nm (Huber *et al.*, 1995; Keller *et al.*, 1995).

On the basis of its physiological characteristics and phylogenetic relationships, the new isolate represents a new species of *Thermococcus*. This species is named *Thermococcus barophilus* because it grows best under high hydrostatic pressures.

Description of *Thermococcus barophilus* Marteinsson, Birrien and Prieur sp. nov.

Thermococcus barophilus (bar.o'phi.lus. Gr. neutr. n. bar weight; Gr. adj. philos loving; M.L. adj. barophilus weight lover, referring to the weight of the water column).

Cells are regular to irregular cocci and variable in size (usually 0.8–2.0 µm). The cell envelope consists of a hexagonal S-layer lattice (spacing 28.4 nm). Cell division occurs by constriction. Obligate anaerobe. Grows optimally at 2–3% (w/v) NaCl and pH 7.0. At atmospheric pressure, growth occurs at 48–95 °C, with optimum growth temperature at 85 °C under 0.3 MPa hydrostatic pressure. High hydrostatic pressure (40 MPa) stimulates growth at 75, 80, 85, 90 and 95 °C with an optimum growth temperature of 85 °C. Obligately barophilic between 95 and 100 °C and requires 15.0–17.5 MPa to grow at these temperatures.

Obligately chemo-organotrophic. Grows on complex organic compounds. Sulfur is not necessary for growth, but greatly enhances growth and can be replaced by cystine. Membrane lipids are composed of one major phospholipid and an archaeol component. The G + C content is 37.1 mol %. 16S rRNA sequence comparisons place *Thermococcus barophilus* in the *Thermococcales*. Isolated from a chimney wall, under 40 MPa hydrostatic pressure at 95 °C, from the Mid-Atlantic Ridge, Snakepit at depth of 3550 m. The type strain is *Thermococcus barophilus* MP^T [CNCM I-1946 (Collection Nationale de Cultures de Micro-organismes, Institut Pasteur, Paris, France)].

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