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ORIGINAL PAPER

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Extremophiles, Thermophily section, species description *Thermococcus atlanticus* sp. nov., a hyperthermophilic Archaeon isolated from a deep-sea hydrothermal vent in the Mid-Atlantic Ridge

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Abstract An extremely thermophilic archaeon, strain MA898, was isolated from a deep-sea hydrothermal vent on the Mid-Atlantic Ridge. This strain is a strictly anaerobic coccus of approximately 0.7–1.2 μm in diameter. Optimal temperature, pH, and NaCl concentration for growth are around 85 °C, pH 7, and 3%, respectively. Strain MA898 grows preferentially in the presence of elemental sulfur, polysulfur, cystine, or L-cysteine. The microorganism requires rich proteinaceous substrates. BHI-S medium supports rapid growth, with a final concentration of more than 1.2×10^9 cells ml^{-1}, but strain MA898 exhibits poor growth on 2216S medium (yeast/peptone) and poor growth on starch. Growth is inhibited by rifampicin and chloramphenicol at a concentration of 100 μg/ml. The DNA G + C content is 50 mol%. Sequencing of the 16S rRNA gene indicates that strain MA898 belongs to the *Thermococcus* genus, and from DNA/DNA hybridization data it is proposed as a new species: *Thermococcus atlanticus*. The deposition numbers are CIP-107420T and DSM15226.

Keywords Hydrothermal · Thermophiles · *Thermococcus* · Mid-Atlantic Ridge · Proteolysis

Introduction

The *Thermococcales* family is widely distributed in thermal environments (Takai and Sako 1999). It is now represented by three genera, *Pyrococcus* (Fiala and Stetter 1986), *Thermococcus* (Zillig et al. 1983), and the newly described *Paleococcus* (Takai et al. 2000). These organisms are all obligate heterotrophs growing preferentially on proteinaceous substrates, although some of them are also able to degrade carbohydrate substrates such as starch (Godfroy et al. 1997), chitin (Huber et al. 1995), and xylan (Ronimus et al. 1997). Because of these properties, they have been intensively studied for biotechnological purposes (Ciaramella et al. 1995). Their thermophily and their easy cultivation procedures make them good candidates for the development of molecular biology tools such as DNA polymerases (Cambon-Bonavita et al. 2000; Perler et al. 1996; Takagi et al. 1997) or conjugative plasmids (Charbonnier et al. 1992; Noll and Vargas 1997).

The normal approach for isolation of new species requiring purification and identification often reveals isolates that belong to known species. For this reason, we used qualitative dot-blot hybridization as a tool prior to purification, using known *Thermococcales* species as controls. This approach led to the isolation of a new hyperthermophilic, heterotrophic archaeal species from chimney wall fragment. The description of this new species is presented in this paper.

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Materials and methods

Reference strains

Thermococcus aggregans (DSM 10597^T), *Thermococcus stetteri* (DSM 5262), *Thermococcus celer* (DSM 2476^T), *Thermococcus litoralis* (DSM5474^T), *Pyrococcus woesi* (DSM3773^T), and *Thermotoga maritima* (DSM 3109^T) were obtained from the Deutsche Sammlung von Mikroorganismen, (Braunschweig-Stöckeim, Germany). *Thermococcus barophilus* (CNCM I-1946^T) was obtained from the CNCM (Institut Pasteur, Paris, France). *Thermococcus profundus* (DT5432^T) was provided by Tetsuo Kobayashi (RIKEN, Wako, Japan). *Pyrococcus abyssi* strain ST549 (CNCM I 1318), *Pyrococcus glycovorans* (CNCM I-2120^T), *Thermococcus hydrothermalis* (CNCM I-1319^T), *Thermococcus fumicolans* (CIP 104690^T), and *Thermococcus* sp strain ST554 were isolated in our laboratory. *Thermococcus* sp. strains GE3, GE8, and GE20 were provided by Gael Erauso (IUEM, Brest, France) (Marteinsson et al. 1995).

Culture conditions

BHI-S medium consists (per liter) of brain-heart infusion (Difco), 9 g; NaCl, 23 g (instead of sea salt from Sigma, to prevent precipitations); PIPES, 6.05 g; sulfur, 10 g; and resazurin, 1 mg. 2216-S medium (Belkin and Jannasch 1985) consists (per liter) of peptone (Difco), 2 g; yeast extract (Difco), 0.5 g; sea salt, 30 g; PIPES, 6.05 g; sulfur, 10 g; and resazurin, 1 mg. 20AA-S medium consists (per liter) of sea salt, 30 g; PIPES, 6.05 g; sulfur, 10 g; resazurin, 1 mg; 10 ml mineral solution (Balch et al. 1979); 10 ml vitamin solution (Balch et al. 1979); and a solution of 20 amino acids each at 0.1 g/l. A stock solution containing each amino acid (from Sigma) at a concentration of 1 g/l was sterilized separately by filtration (Nalgene filterware, 0.22 μm). Unless otherwise indicated, the pH was adjusted to 7.0 with 5M-NaOH. The medium was sterilized by steaming twice at 100 °C for 30 min on two successive days, transferred into an anaerobic chamber containing $N_2/H_2/CO_2$ (90:5:5), reduced by addition of sodium sulfide to a final concentration of 0.5 g/l, and distributed in Hungate tubes or in 50-ml serum vials with butyl rubber stoppers. Unless otherwise indicated, cultures were incubated at atmospheric pressure under the anaerobic chamber gas mixture at 80 °C.

Sampling

Sampling of hydrothermal chimneys was conducted by the submersible Alvin during the American-French MAR'93 cruise in June 1993 at the hydrothermal TAG (26° N) and Snake Pit (23° N) sites on the Mid-Atlantic Ridge. Whenever possible, samples were brought to the surface in insulated boxes. On board, chimney fragments were aseptically subsampled according to their mineral zonation. Each subsample was crushed in 40 ml sterile seawater in an anaerobic chamber and stored at room temperature in serum vials under anaerobic conditions (N_2 headspace and 0.5 g/l sodium sulfide).

Enrichment cultures and purification

Enrichment cultures were performed on a BHI-S medium into 100-ml serum vials containing 80 ml of the medium. Each sample was used as inoculum at three different dilutions (1/1, 1/10, and 1/100). Cultures were performed in an oven at 80 and 95 °C. Positive cultures were identified by turbidity and microscopic observation. Strains were purified by streaking on BHI-S medium, solidified with Gelrite (Scott Laboratories, Long Island, USA), and incubated in anaerobic jars at 80 °C (Erauso et al. 1995). Purified isolates were stored at 4 °C and could serve as inoculum for at least 1 year.

Determination of cell numbers

Cell numbers in liquid cultures were determined by direct visual counting of cells using a Thoma chamber with an Olympus BH-2 microscope. Optical density (600 nm) was also measured with a spectrophotometer (Spectronic 301 instrument, Milton Roy, Rochester, USA) for determination of optimum growth conditions, and the correlation between turbidity and cell number was determined.

Determination of growth parameters

For optimal temperature determination, cells were grown in Hungate tubes containing 6 ml of BHI-S medium. The headspace was 100 kPa of $N_2/CO_2/H_2$ (90:5:5). Cultures were incubated in aluminum heating blocks (Bioblock, Illkirch, France) and monitored with temperature probes placed in control tubes. For determination of growth rates at different pH values, BHI-S medium was modified using the following buffers (Sigma, St. Louis, USA) each at 20 mM: pH 3 and 4 – no buffer; pH 5 – MES buffer; pH 6 – PIPES buffer; pH 7 – HEPES buffer; and pH 8 and 9 – AMPSO buffer. After addition of sodium sulfide, the pH was adjusted with 1M-HCl. To determine the salt requirement, BHI-S medium was prepared with different dilutions of NaCl (0, 15.6, 23, 30.6, and 46 g/l). The effects of pH and salinity were determined at 80 °C. Three replicates were performed simultaneously at each temperature, pH, or salinity.

Determination of growth requirements

The ability of the isolate to consume different carbon sources was tested in medium where the yeast extract and peptone of the 2216-S medium were replaced by 0.4 g/l ammonium chloride, 10 ml mineral solution (Balch et al. 1979), 10 ml vitamin solution (Balch et al. 1979), and a variety of carbon sources. Tests were performed in tubes containing no nitrogen or carbon substrates to serve as controls for growth on substrates carried over in the inoculum. Most of the carbon sources (peptone, yeast, tryptone, meat, maltose, glucose, cellobiose, xylose, lactose, fructose, saccharose, malt, casein, casamino acids, xylan, and dextrane) were tested at 5 g/l. Starch, cellulose, and chitin were tested at 10 g/l. Formate, acetate, propionate, pyruvate, succinate, and lactate were tested at 2 g/l, and ethanol, glycerol, and methanol were tested at 5 ml/l. Growth was also tested on BHI-S medium (9 g/l) and on 20AA-S medium. Autotrophic growth under a headspace of H_2/CO_2 (80:20) as sole carbon source was tested. To test the ability of the isolate to grow in the absence of elemental sulfur, cells were cultured on BHI-S medium lacking sulfur compounds under 100 kPa headspace of N_2, N_2/CO_2 (80:20), and H_2/CO_2 (80:20). In this case the medium was reduced by titanium nitrilotriacetate (Moensch and Zeikus 1983) at a concentration of 200 μM. To test the ability of the isolate to use a range of electron acceptors, elemental sulfur was replaced by potential electron acceptors (nitrate, nitrite, sulfite, thiosulfate, sulfate) at a concentration of 20 mM and by iron ($FeCl_3$) at 5 mM. Growth on cystine (10 g/l), HCl-cysteine (20 mM), and polysulfide (10 mM) (Blumentals et al. 1990) was also investigated.

Influence of the gas headspace

To test the influence of the gas composition of the headspace, cells were cultured on BHI-S media with various headspace gases: N_2, N_2/CO_2 (80:20), H_2/CO_2 (80:20), and $N_2/H_2/CO_2$ (90:5:5). Production of H_2S was examined using lead acetate paper (Whatman). To test the growth under aerobic conditions, BHI-S medium was prepared under ambient air, without being reduced by addition of sodium sulfide.

Antibiotic sensitivity

Sensitivity to vancomycin, penicillin, kanamycin, streptomycin, rifampicin, and chloramphenicol at final concentrations of 100 μg/ml was tested under the standard growth conditions (BHI-S, pH 7, 80 °C). The bacterium *Thermotoga maritima* was used as control to establish the effectiveness of the antibiotic at 80 °C.

Scanning electron microscopy

Cells were harvested at the end of log phase. For scanning electron microscopy, cells were fixed with formalin 10% (v/v) for 1 h, and then harvested by centrifugation for 20 min at 2,000 *g*. Pellets were resuspended in 23 g/l NaCl, displayed on 0.22 μm filters (Nucleopore), and dried overnight at room temperature. Samples were then coated with gold (SCD040, Balzers) and examined with a scanning electron microscope XL 30 LaB6 (Philips).

Analysis of amino acids, organic acids, and aromatic acids

Each sample was centrifuged 30 min at 8,000 *g* to remove the cells. Half of the supernatant was transferred to an ultrafree-CL-PLGC

10,000 NMWL unit (Millipore) and centrifuged at 4,500 *g* for several hours. Then 20 µl of the filtrate was mixed with 30 µl of a solution of ethanol/water/triethylamine (2/2/1) and vacuum dried. Next, 20 µl of a derivatizing solution containing ethanol/water/triethylamine/ phenylisothiocyanate (7/1/1/1) was added, and after 10 min at room temperature, the sample was vacuum dried. Analysis of amino acids by HPLC (Alliance 2690, Waters) was then performed using the Waters PicoTag method (WAT007360, Waters). Proteins were precipitated in the other portion of the supernatant, with a solution of 5-sulfo-salicylic acid at 2% (w/v) for one night at 4 °C and removed by centrifugation at 8,000 *g* for 10 min. The supernatant was then used for HPLC analysis of glucose, aromatic acids, and linear organic acids. For glucose and linear organic acids analysis, 20 µl of supernatant was eluted on a H^+ exclusion column (polyspher OAKC 1.51270, Merck) at 60 °C, by a 9 mM H_2SO_4 solution with a flow of 0.35 ml min^{-1}, and detected using a differential refractometer (refractometer 410, Waters). For aromatic acids analysis, 20 µl of supernatant were eluted on a H^+ exclusion column (column 28352, Chrompack) at 65 °C, by a 4.5 mM H_2SO_4 solution with a flow of 0.5 ml min^{-1}, and detected at 210 nm (UV detector 486; Waters).

DNA extraction, G+C content, PCR, sequencing, and phylogenetic analyses

Cells were cultured in 1 l on BHI-S medium at 80 °C and harvested at the end of the exponential phase of growth. Genomic DNA was extracted as reported by Godfroy et al. (1996). The DNA was purified by cesium chloride gradient centrifugation as previously described (Juniper et al. 2001). The G+C content of the DNA was determined by thermal denaturation (Marmur and Doty 1962) under the conditions reported by Raguénès et al. (1997). A calibration curve was obtained by using ultrapure DNA from *Escherichia coli* strain B (50 mol% G+C), *Clostridium perfringens* (26.5 mol% G+C), and *Micrococcus luteus* (72 mol% G+C) as standard (Sigma).

The 16S rDNA was PCR amplified from purified genomic DNA as previously described (Wery et al. 2001), except that archaea-specific primers were used (Godfroy et al. 1997).

Previously amplified products of different strains were digested with *Hha1* enzyme (Amersham) for 2 h at 37 °C according to the manufacturer's instructions.

The DNA sequence was determined by using the dideoxy chain termination method (Sanger et al. 1977) by Euro Sequence Gene Service (ESGS, France). We double sequenced 1,416 positions of the 16S rDNA. The sequence was then compared to others available in Genbank using the BLAST program (Altschul et al. 1990). Alignments and similarity levels were obtained by the CLUSTALW method with weighted residues (Thompson et al. 1994). Final alignment was then manually performed using the multiple sequence alignment editor SEAVIEW, and phylogenetic reconstruction was produced using PHYLO_WIN (Galtier et al. 1996) with the following setup: Jukes-Cantor distance matrix, and successively the neighbor-joining (Saitou and Nei 1987), maximum of parsimony (Lake 1987), and maximum likelihood (Felsentein 1981) methods. Bootstrap values were determined according to Felsentein (1985).

Nucleotide sequence accession number

The nucleotide sequence of the 16S rRNA of strain MA898 ^{T} has been deposited at the EMBL database under the accession number AJ310754.

Dot-blot qualitative hybridization and DNA/DNA quantitative hybridization (dot blots)

DNA samples were dialyzed against TE buffer on Millipore VS0 0.25 µm filters, and concentration and purity were determined by absorption at 260 and 280 nm. Chemical denaturation of 0.3 µg of each DNA was performed by addition of 5 µl of 1 M NaOH. It was then neutralized by 7.5 µl of 1 M NaH_2PO_4, diluted by addition of SSC buffer (20×) up to 125 µl, then blotted on a Hybond nylon membrane. The membrane was fixed for 25 s under UV light. DNA probes were labeled using ECL direct nucleic acid labeling and detection system kit from Amersham (Amersham Place, UK). At the time of the experiment, seven strains have been used as probes: *T. hydrothermalis*, *T. fumicolans*, *P. glycovorans*, *P. woesei*, and *Thermococcus* sp. strains ST 554, GE3, and GE8. Hybridization was done at 42 °C during 18 h. For quantitative hybridization, cesium chloride-purified DNA (300 ng) from reference strains (*T. aggregans, T. stetteri, T. profundus, T. hydrothermalis, T. celer*, and *T. barophilus*) and strain MA898 was applied on Hybond nylon membranes as describe above. Herring sperm DNA was used as a negative control. MA898 and *T. celer* purified DNA was used as a probe and labeled with fluorescein as indicated in the ECL kit (RPN 3040 ECL Kit, Amersham, UK). Hybridization was done following the kit's instructions, at 60 °C for 18 h in a hybridization oven (Appligene, France). Luminescence was registered using the FluorS Multimager with the Multi-Analyst software (Biorad).

Results

Enrichment cultures, dot-blot hybridization, and purification

Of the 104 enrichment cultures made on BHI-S medium at 80 and 95 °C, 41 were turbid after one night to 3 days' incubation. Cultures that showed no turbidity after 3 days remained without turbidity after 6 days. Strains used as references for hybridization were chosen for their ability to grow in the same conditions. A qualitative scale of four hybridization levels was determined depending on the blot intensity. DNA from 25 enrichment cultures showed no hybridization or very low hybridization with all *Thermococcales* used as references and therefore contained different species from previously published ones. Six strains were then successfully purified by streaking on Gelrite medium. Their GC contents ranged from 49% to 55%. According to ARDRA profiles (data not shown), three isolates were shown to be closely related to *Thermococcus celer* and two were related to *Thermococcus* sp. GE8 (Marteinsson et al. 1995). One strain, MA898, exhibited an ARDRA pattern different from all previously known for *Thermococcus* strains, suggesting that it might represent a new species (Meunier 1994; Antoine et al. 1995).

Morphology and growth

The cells of strain MA898 were coccoid and occurred as single cells (Fig. 1). Cell size ranged from 0.7 to 1.2 µm. Cells most likely divided by constriction. Motility was not observed.

Isolate MA898 was resistant to vancomycin, penicillin, kanamycin, and streptomycin, at concentrations up to 100 µg/ml, but was sensitive to rifampicin and chloramphenicol at a concentration of 100 µg/ml.

A correlation line between OD_{600} and cell number was determined showing that 1OD corresponded to

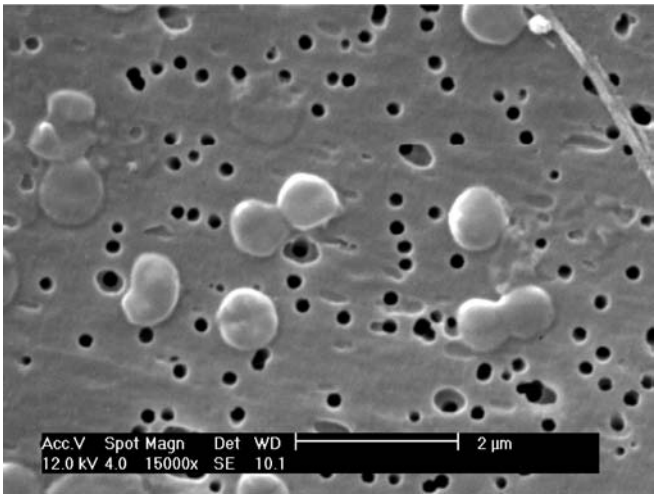

Fig. 1 Scanning electron micrograph of strain MA898. Scale represents 2 µm

3×10^9 cell/ml (estimated value) with a high correlation factor ($r^2 = 0.9948$). Isolate MA898 grew between 70 °C and 90 °C (Fig. 2A). Estimated growth rates were not significantly different between 75 °C and 90 °C, but a maximal cell density was obtained at 85 °C, with up to 6.1×10^8 cell/ml in Hungate tubes. Growth was observed only over a pH range of 5 to 8, with the highest growth rate at pH 6, but the maximum cell density was obtained near pH 7 (up to 6.5×10^8 cell/ml). At pH 6, growth stopped at 4×10^8 cell/ml. No growth was observed at pH 4 or pH 9 (Fig. 2B). Growth was observed at NaCl concentrations ranging from 15.3 g/l to 46 g/l, with an optimum between 23 g/l and 30.6 g/l and a cell concentration reaching 8.4×10^8 cell/ml; poor growth was detected below or above those concentrations (Fig. 2C).

BHI-S medium supported rapid growth, with a final concentration of more than 1.2×10^9 cells/ml in closed vessels after 7 h. On 2216S medium, growth was lower and the culture reached a maximum of 4×10^8 cells/ml. BHI could be replaced by several proteinaceous substrates including peptone, yeast extract, and meat extract at 5 g/l (up to 1×10^9 cell/ml). Cells could be maintained on tryptone, but the cellular concentration reached only 6×10^7 cell/ml. Poor growth was observed on starch after 1 day, reaching a maximum of 8×10^7 cell/ml. None of the other tested substrates (sugars, alcohols, carboxylic acids, mixture of 20 amino acids, or polysaccharides) supported growth of strain MA898. Lower growth was observed in the absence of elemental sulfur (up to 1.6×10^8 cell/ml). Cystine, cysteine, and polysulfide supported rapid growth of strain MA898, but none of the other electron acceptors tested supported growth. When elemental sulfur was present in the culture medium, growth of strain MA898 was accompanied by the production of H_2S. Under these conditions, H_2S was not significantly produced in the non-inoculated control. Mineral medium complemented with vitamins, sulfur, and a headspace of H_2/CO_2 (80:20) did not support autotrophic growth, and headspace of H_2/CO_2 reduced the growth of MA898 on BHI-S by twofold. No growth was observed under aerobic conditions.

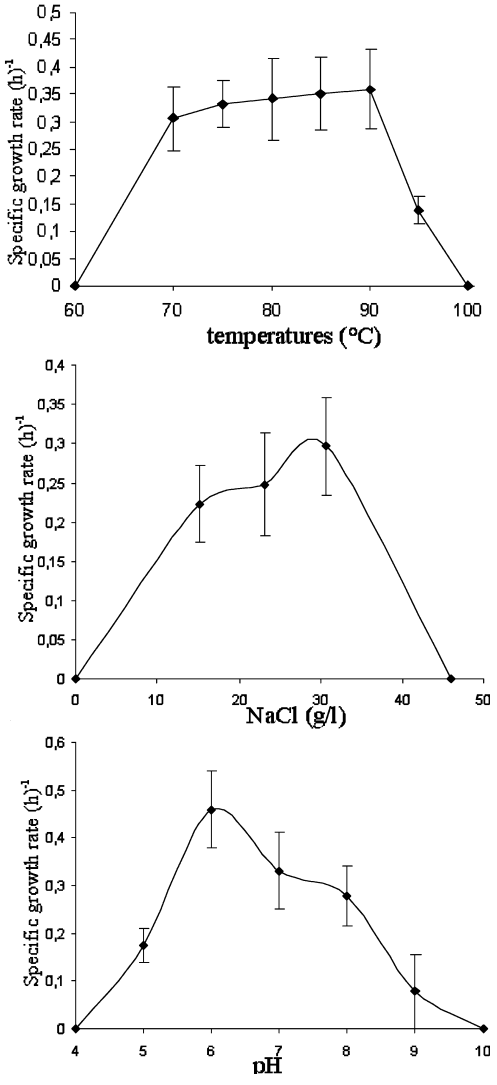

Fig. 2 Temperature, pH, and NaCl optima for growth of isolate MA898 grown on BHI-S medium: specific growth rate as a function of temperature (23 g/l NaCl, pH 7) (*upper*), pH (80 °C, 23 g/l NaCl) (*middle*), and salinity (80 °C, pH 7) (*lower*). Growth rates were calculated using linear regression analysis along the logarithmic part of the resulting growth curves. When enough data were available, information about precision of the growth rate is given (regression coefficient ±2×standard error)

Analysis of metabolic products

Amino acids in BHI-S medium were determined before incubation and after 24 h at 80 °C in a non-incubated medium and were found to be stable. Two amino acids, glutamine and cysteine, were absent, probably due to their thermolability. Strain MA 898 consumed all other amino acids to extinction after 24 h, except proline, which increased 4.5-fold. At the end of the exponential growth, only aspartic acid, alanine, tyrosine,

methionine, isoleucine, tryptophan, and lysine were partially consumed (30% to 50%). Leucine and phenylalanine were consumed preferentially (4.7- and 3.7-fold decrease, respectively).

On BHI-S medium, after 8 h or 24 h, respectively, formate (0.8 and 3 mM), acetate (1.6 and 6.5 mM), phenyl acetate (0.65 and 1.00 mM), propionate (0 and 2.00 mM), and isovalerate (1.8 and 3.3 mM) were formed as major fermentation products. Isobutyrate (0.3 and 1.4 mM), indole acetate (0.05 and 0.13 mM), and *p*-hydroxy-phenylacetate (0.17 and 0.51 mM) were also present in lower amounts.

DNA base composition and 16S rRNA sequence

The mol% G+C content of the DNA of isolate MA898 determined by the thermal denaturation method was 50%. The 16S rRNA gene sequence of strain MA898 was determined, and BLAST results clearly indicated that it was affiliated with *Thermococcus* species (Fig. 3). Similarity values between the 16S rRNA gene of MA898 and other *Thermococcus* species are about 98% with the closest relatives (*T. stetteri, T. profundus, T. celer*, and *T. hydrothermalis*) and between 96% and 97% with the next group (*T. pacificus*, *T. zilligii*, and "*T. acidaminovorans*"). This analysis clearly located strain MA898 in the *Thermococcus* genus, but DNA/DNA hybridization was required to confirm that MA898 represents a new species of this genus (Stackebrandt and Goebel 1994).

DNA/DNA homologies

Strain MA898 exhibited only weak similarities with previously described *Thermococcales*. Considering the phylogenetic tree based on 16S rRNA gene sequences, the levels of similarities were quantified with *T. stetteri, T. profundus, T. celer*, and *T. hydrothermalis*, which exhibited, respectively, an average of 35%, 32%, 22%, and 38% homology with strain MA898. *T. barophilus*, more distantly related, exhibited an average of 17% homology with strain MA898.

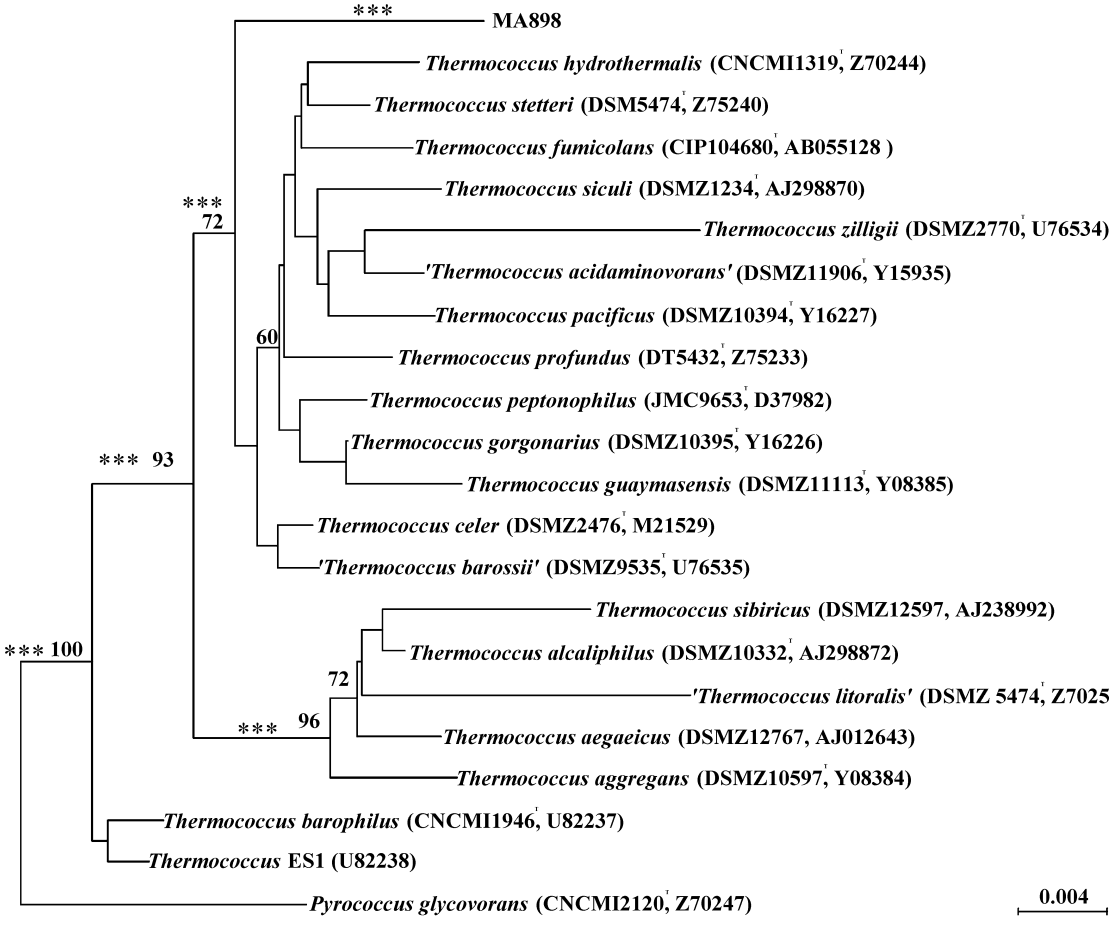

Fig. 3 Phylogenetic relationships based on 16S rRNA sequences. The topology shown is a consensus tree obtained with the three methods (neighbor joining, maximum parsimony and maximum likelihood). The branches retrieved with these methods are indicated with *asterisks* (***). 16S rRNA gene sequences were all obtained from GenBank. The accession numbers for the organisms used in this analysis are indicated on the figure. For this study, 22 species were used and 1,131 sites have been retained for the analysis. The Jukes and Cantor distance was used with 500 bootstraps replicates

Table 1 Characteristics of *Thermococcus* species and of isolate MA898

Species	G + C contents (mol%)	Growth temperature (°C)		pH		NaCl concentration (g/l)		Carbon sources	Growth on amino acids	Sulfur effect[a]	Rifampicin resistance[b]
		Range	Optimum	Range	Optimum	Range	Optimum				
T. celer DSMZ 2476^T	57	Up to 93	88	–	5.8	–	40	Peptides stimulated by sucrose	Yes	E	R
"*T. litoralis*" DSMZ 5474^T	38	65–95	88	6.2–8.5	7.2	18–65	25	Peptides, pyruvate	No	E	S
T. stetteri DSMZ 5262^T	50	60–85	75	5.7–7.2	6.5	10–40	25	Peptides, starch, pectin	No	R	S
T. profundus DT 5432^T	52.2	50–90	80	4.5–8.5	7.5	10–60	20	Peptides, pyruvate, starch, maltose	nd	R	S
T. peptonophilus JCM 9653^T	52	60–100	85	4–8	6	10–50	30	Peptides	nd	E	S
T. aggregans DSMZ 10597	42	60–94	88	5.6–7.9	7		20	Peptides, casein, dextrose, maltose	nd	E	nd
T. pacificus DSMZ 10394	53.3	70–95	80/88	6–8	6.5	1–5%	2/3.5%	Peptides, starch	nd	E	nd
T. guaymasiensis DSMZ 11113	46	56–90	88	5.6–8.5	7.2	1–5%	2/3.5%	Peptides, casein, dextrose, maltose	nd	E	nd
T. gorgonarius DSMZ 10395	50.6	68–95	80/88	3.5–9	7		30	Proteins, peptides	nd	E	nd
T. hydrothermalis CNCM I1319	58	53–100	85	2–10	7	20–100 Sea salt	40	Peptides, maltose	Yes	E	R
T. zilligii DSMZ 2770	46.2		75		7.4		25	Peptides, casein	nd	E	R
T. acidaminovorans DSMZ 11906	49	56–93	85	5–9.5	9	1–6%	2–3%	Peptides	Yes	E	nd
T. aegaeicus DSMZ 12767	45.5	50–90	88	4–9	6	0.5–6.5%	2.7%	Peptides, starch	No	E	nd
T. barophilus CNCM I1946	37	75–95	85	4.5–9.5	7	1–4%	3%	Peptides		E	S
T. barossi DSMZ9535	60	60–92	82.5	3–9	6.5/7.5	10–40	20	Peptides, maltose		R	
T. fumicolans CIP 104690	54–55	73–103	90	4.5–9.5	8.5	6–40	13–26	Peptides, pyruvate	Yes	E	S
T. sibiricus DSMZ 12597	38.4	40–88	78	5.8–9	7.5	5–70	18–20	Peptides	nd	E	nd
MA 898	49.8	70–95	85	4–9	7	15.3–46	30	Peptides	No	E	S

[a] *E* Elemental sulfur stimulates growth, *R* elemental sulfur is required for growth

[b] *R* Rifampicin resistant, *S* rifampicin sensitive

References are: *T. celer* (Zillig et al. 1983), *T. litoralis* (Neuner et al. 1990), *T. stetteri* (Miroshnichenko et al. 1989), *T. profundus* (Kobayashi et al. 1994), *T. peptonophilus* (Gonzalez et al. 1995), *T. aggregans* (Canganella et al. 1998), *T. pacificus* (Miroshnichenko et al. 1998), *T. guaymasiensis* (Canganella et al. 1998), *T. gorgonarius* (Miroshnichenko et al. 1998), *T. hydrothermalis* (Godfroy et al. 1997), *T. zilligii* (Ronimus et al. 1997), *T. acidaminovorans* (Dirmeier et al. 1998), *T. aegaeicus* (Arab et al. 2000), *T. barophilus* (Marteinsson et al. 1999), *T. barossii* (Duffaud et al. 1998), *T. fumicolans* (Godfroy et al. 1996) and *T. sibiricus* (Miroshnichenko et al. *2001*)

Discussion

The marine hyperthermophilic strain MA898 belongs to the archaeal domain on the basis of its 16S rRNA sequence. From the 16S rRNA gene sequence and the DNA/DNA hybridization, strain MA898 clearly belongs to the *Thermococcus* genus and is a new species. Strain MA898 and *T. gorgonarius* have similar conditions for optimal growth, but their morphological traits clearly differentiate the two strains. Strain MA898 is also close to "*T. acidaminovorans*," except for its pH optimum, and to *T. barophilus*, except for its GC content. Strain MA898 exhibited very rapid growth only on rich proteinaceous substrates, consuming all amino acids except proline. Proline is probably an end product of proteolytic activity due to prolidase (Willingham et al. 2001), one of the first key enzymes in the peptidolytic pathway in *P. furiosus*. Organic acids such as formate, acetate, phenyl acetate, propionate, or isovalerate were produced. These organic acids are end products of amino acid degradation, as described for *P. furiosus* (Adams et al. 2001) and other *Thermococcus* species (Godfroy, personal communication). Strain MA898 was not able to grow on tryptone at 5 g/l, which could be due to a low amount of alanine (measured at 0.013 g/l in tryptone solution). However, it consumes the 0.35 g/l available on the BHI-S medium to reach 10^9 cell/ml. Strain MA898 differs from the other previously described *Thermococcus* species in its nutritional requirements, as it is not able to grow with most sugars or alcohols or a mixture of the 20 amino acids (characteristics of *Thermococcus* species are summarized in Table 1). Poor growth was observed on starch, but the cell number was rapidly limited at 8×10^7 cell/ml. Growth of strain MA898 is not strictly dependent upon elemental sulfur, but elemental sulfur greatly enhances its growth and can be replaced by cystine or polysulfide with a reduced efficiency. In the absence of elemental sulfur, under an $N_2/H_2/CO_2$ atmosphere, growth yields are low. Under H_2/CO_2, growth is inhibited in the absence of sulfur. This is apparently due to inhibition by H_2 produced as an end product, as reported for most of the *Thermococcales*. On the basis of its physiological characteristics, from 16S rRNA sequences, and from DNA/DNA hybridization, the new isolate MA898 represents a new species of *Thermococcus,* named *Thermococcus atlanticus* due to its Mid-Atlantic Ridge origin.

Description of *Thermococcus atlanticus* sp. Nov.

Thermococcus atlanticus sp. nov. Cambon-Bonavita, Godfroy, and Barbier. (a. tlan.ti'cus, L. masc. Adj. for Atlantic Ocean, from the Mid-Atlantic Ridge from which the organism was isolated). Cells are spherical cocci (from 0.7 to 1.2 μm in diameter), and division occurs by constriction. Not motile. Obligate anaerobes. Grows optimally on rich proteinaceous medium, BHI-S, at 23–30.6 g/l NaCl and at pH around 7. The temperature range for growth is 70–95 °C, and the temperature optimum is around 85 °C. Obligate chemoorganotroph, grows only on proteic products. Poor growth is observed on starch; no growth is observed on other carbohydrates. Sulfur is not necessary for, but greatly enhances, growth. 16S rDNA gene sequence comparisons affiliate *T. atlanticus* to the *Thermococcus* genera. EMBL accession number for the 16S rRNA sequence is AJ310754. The G+C content is 50%. Source of isolate: active chimney wall fragment recovered from a hydrothermal site in the Mid-Atlantic Ridge. Type strain is *T. atlanticus*, MA898, and has been deposited at the CIP under the number CIP-107420T and at the DSMZ (DSM15226).

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